Effects of household indoor air quality and environment on respiratory health of Maltese families.

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Abstract

There is no doubt that the prevalence of asthma has been increasing worldwide with westernised countries faring worse than less developed ones. The ISAAC study in Malta, which was the largest local epidemiological study on asthma and allergies in childhood, has already established a high prevalence of asthma in Maltese children, with 27.2% reporting wheezing sometime in their life. It also revealed an extremely high prevalence of allergic rhinitis with Maltese adolescents (13–14-year olds) ranking as having the second highest prevalence worldwide.

This study recruited children who participated in the Respira project, were adolescents (12-15-year olds), were studied in a case control design. Sixty-six cases were children who exhibited symptoms of uncontrolled asthma (namely wheezing in the past 12 months, exercise induced wheeze and nocturnal cough in the absence of a cold). The sixty-three controls were children who never had any symptoms suggestive of asthma, and who never had any manifestations of other allergic conditions. These children were equally distributed throughout the northern, central and southern areas of the island. These children together with their fathers and mothers underwent spirometry, exhaled nitric oxide testing, skin prick testing, acoustic rhinometry. Their houses were visited, and dust was collected for the presence of allergens and endotoxin.

Adolescents who had uncontrolled asthma symptoms were found to have a higher lifetime exposure to tobacco smoke. 30.3% of cases were exposed to second-hand smoking (SHS) in their first year of life, while 40.9% had some current exposure to tobacco smoke. This contrasted to what was reported in the control group, where only 6.6% were exposed to SHS in the first-year old life, and 18.3% had current exposure in this group.

Familial allergic conditions were extremely important factors in predicting whether a child was likely to be included in the uncontrolled asthmatic group. Having a sibling who had a history of asthma, was the strongest of these predictors, with an Odds Ratio of 6.1. This was followed by a father who had a history of asthma (OR 5.338) and/or a mother who had a history of nasal allergies (OR 3.038). Hence, one can observe the importance of a genetic predisposition, but also remember that these relatives would usually share the same dwelling and subsequently the same indoor environment which could lead to factors which could precipitate allergic conditions.

Adolescents who had uncontrolled respiratory symptoms tended to live closer to roads with busy traffic (48.8% lived within 50m of such a road) when compared to children who had no symptoms (27.8%, $p = 0.049$). 36.5% of cases were reported be living in a house which had a noticeable smell of mould, while only 8.5% of the controls lived in a household with such a smell ($p < 0.001$). Also, there were more visible signs of mould in the bedrooms of case children (25%) when compared to control children (9.8%, $p = 0.034$). These conditions point to humid indoor environments, possibly promoting fungal growth and the presence of spores, which could have been triggering allergic conditions.

Clinical testing in children confirmed that the most likely cause of uncontrolled symptoms in the case group was of an allergic aetiology, as in this group both FeNO ($p <0.001$) and serum total IgE ($p = 0.027$) was much higher when compared to the control group. Also, nasal patency by acoustic rhinometry revealed that in the left nostrils were smaller in the case group when compared to the control group ($p = 0.011$). The children who participated in this study were

mostly atopic to house dust mite, followed by cat dander and olive tree pollen, with asthmatic adolescents being more likely to be atopic to house dust mite when compared to control children.

Fathers who participated in this study were mostly atopic to house dust mite, followed by *Parietaria* pollen and grass mix pollen. Mothers were also mostly atopic to house dust mite, followed by *Parietaria* pollen but in contradistinction to fathers, the third most common aeroallergen they were atopic to was olive tree pollen.

Case children were more likely to have a higher total serum IgE level in autumn when compared to spring ($p = 0.015$), while their fathers and mothers also had higher total mean serum IgE level in autumn when compared to spring ($p = 0.006$, $p = 0.009$). While there was no significant seasonal serum total IgE variability for doctor-diagnosed asthmatic fathers, doctor-diagnosed asthmatic mothers had a significantly higher mean serum total IgE level in winter when compared to autumn ($p = 0.036$).

We also identified groups of individuals in both children and parents who were characterised by extremely high total IgE levels and higher airway inflammation, as evidenced by higher FeNO levels, and lower FEV_1 and FEV_1/FVC. Case children and parents who were classified in these groups were also more likely to have a higher serum specific HDM IgE level.

Mean endotoxin levels were higher in houses in which mould growth had been reported, and in which mould growth was reported in the child's bedroom ($p = 0.024$, $p = 0.011$). While there were no significant differences in the dust allergen levels collected from the houses of case and control children, mean HDM allergen levels in houses of case children who had a very high

IgE level and high FeNO had higher HDM levels were detected in the house dust ($p = 0.017$). These findings were reproduced when the data from these adolescents were analysed together with the corresponding adolescents who participated in the same project, and who lived in Sicily, confirming higher mean house HDM allergen in the houses with the adolescent phenotype having a serum high total IgE ($p = 0.007$), and a strong correlation between these children's specific HDM IgE and the presence of this allergen in the house dust ($p = 0.019$).

This study confirmed that HDM as an indoor allergen in Maltese houses was a strong predictor for asthma in Maltese adolescents. It also identified phenotypes of both adolescents and adults being affected to a greater extent by this allergen. On the other hand, this study did not explain why the more common case phenotype who had lower serum IgE levels and airway inflammation had uncontrolled symptoms. Certain home characteristics such as houses which were located closer to busy roads and having the presence of indoor mould increased the likelihood of a child having asthma. Perhaps, further studies designed around first identifying phenotypes and then focusing on how various indoor environmental factors affect the particular phenotype of interest could be the answer to identifying which indoor environmental factors are instigating the expression of a particular asthma phenotype.

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List of Abbreviations

Alt A1	Alternaria allergen
AMCA L	Minimum nasal cross-sectional area left nostril
AMCA R	Minimum nasal cross-sectional area right nostril
ATS	American Thoracic Society
DEP	Diesel exhaust particles
Der p1	House dust mite allergen; Dermaphagoides pteronyssinus
Der f1	House dust mite allergen; Dermaphagoides farinae
eCO	Exhaled carbon monoxide
ECRHS	European Community Respiratory Health Survey
ELISA	Enzyme-linked immunosorbent assay
eNO	Exhaled nitric oxide
ERS	European Respiratory Society
ETS	Environmental tobacco smoke
Feld d 1	Cat dander allergen
FEV_1	Forced expiratory volume in one second
FEV_1/FVC	FEV_1/FVC ration
FVC	Forced vital capacity
HDM	House dust mite
IgE	Immunoglobulin E
ISAAC	International Study of Asthma and Allergies in Childhood
Phl p5	Timothy grass allergen
SHS	Second hand smoke
WHO	World Health Organisation

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1.1 Introduction

1.1.1 Asthma

Asthma is one of the most recognised and prevalent respiratory diseases. It has been a major public health problem since at least the early 1970s. As of 2013, around 235 million individuals have been diagnosed with asthma and it is the commonest non-communicable disease in children[1]. It has its highest prevalence in the early years of life, with its prevalence rate decreasing in older age groups.

Now while asthma is a well-recognised condition, giving it a precise definition has proved to be a challenge over the years. In fact, different entities such as the American Thoracic Society (ATS), the British Thoracic Society (BTS) and the Global Initiative for Asthma (GINA) have updated their definition of asthma multiple times over the years. Asthma has been more often described rather than defined.

GINA has described asthma as a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation. (GINA, 2014)[2,3].

On the other hand, the BTS did not attempt to give asthma a definition, by stating that there is no gold standard definition[4]. This society then describes its diagnosis as being a clinical one with the presence of symptoms such as wheeze, breathlessness, chest tightness and cough, yet how these correlate to actual measures of inflammation and airway hyper-responsiveness remains unclear.

The fact that these well-established societies, who have an immense background of research at hand, have found it difficult to give asthma a precise definition, leads to one conclusion: this is a truly heterogeneous condition, in which various pathways

leading to the symptoms of asthma are triggered or mediated by different environmental and lifestyle factors. Thankfully the multifactorial aetiology of this condition together with is high incidence has triggered further research by the scientific community which has led to a better understanding of asthma and the risk factors associated with it.

The pathophysiology of asthma is complex and mediated by various cells, cytokines and pathways with lead to bronchial hyper-responsiveness (BHR) and airway inflammation. These include cells such as mast cells, eosinophils and T lymphocytes, together with mediators such as cytokines, chemokines, histamine and leukotrienes, which lead to airway inflammation resulting in the typical symptoms of asthma.

There is no doubt that the prevalence of asthma has been increasing worldwide with westernised countries faring worse than less developed ones[5-6]. This has been particularly evidenced in the International Study of Asthma and Allergies in Childhood (ISAAC) study. In this study which has been the largest study to document the worldwide prevalence on asthma, Malta has been one of the 56 countries participating in this study.

The standardised questionnaire which was used, was completed by the parents of the recruited children (6000 between June 1996 and November 1997). The students studied were subdivided into two age groups: 5 to 7-year-old and 12 to 15-year-old children. Around 3000 students participated in the 5-7-year-old group, while another 3000 participated in the 12-15-year-old group. The children's parents completed the 5-7-year old questionnaires, while the 12-15-year-old children completed the

questionnaires themselves. The results clearly illustrated a high prevalence of asthma and associated symptoms in developed countries such as the UK, Australia and New Zealand which exceeded 30%. On the other hand, countries such as Albania, China and India had much lower rates of around 4%. One should note that Malta had a relatively higher prevalence of almost 15%, which increased when the study was repeated in 2001-2002. This was a closer rate to the more developed countries such as Finland.

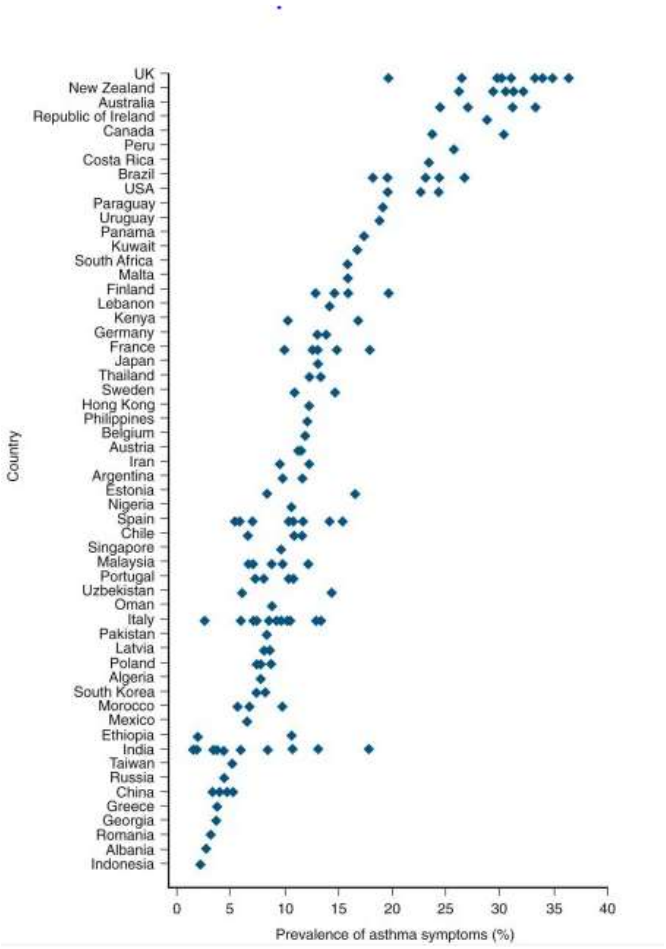


Figure 1. Prevalence of asthma symptoms in 13-14 year olds as reported by written questionnaire-*from The International Study of Asthma and Allergies in Childhood [ISAAC] Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema. ISAAC. Lancet 351:1225–1232, 1998*

Such findings led to the second phase of the ISAAC study (ISAAC II) this time concentrating in finding the relationships between atopic sensitisation and the

variation between different countries. Here the atopic sensitization was not found to be correlated with current wheeze. However, there was a correlation between wheezing and atopy in countries with higher gross national income (typically in the developed world)[7-8].

Such findings and studies have thus highlighted the possibility that western style living and associated environmental factors must have an impact on the genetics and physiology of asthma, and thus resulting in a higher prevalence of asthma in these countries. This has led to scientists postulating theories which could explain the mechanism of such a possible phenomenon. David Strachan was the pioneer of the "Hygiene Hypothesis", when he first published this in the British Medical Journal in 1989. This theory was developed to explain the phenomenon leading to the increase in atopic and allergic conditions reported in data collected from several epidemiological studies involving cohorts collected between 1961 and 1991 from the United Kingdom, Europe and other worldwide cohorts. This has been possibly attributed to a decrease in rate of infections, which has been thought to be associated with a great incidence of asthma.

Strachan proposed that the change in lifestyle which the more affluent families could afford, associated with smaller families (less children per family unit), cleaner households and earlier treatment of infections led to a higher prevalence of hay fever, atopy and eczema in these some families. He suggested that children having less siblings may have a higher tendency to develop these atopic conditions due to being exposed to fewer infections in their earlier years of life[10].

The explanation which was given for this phenomenon was that infections in the earlier years of life, which would be transmitted between siblings, or from other children in day-care centres, would have led to the development of a predominately

T-helper (Th)1-mediated immune system which would lead to a down regulation of the Th2-mediated system. With the cleaner environment which was being inhabited by these young children, they were not contracting frequent infections, and therefore their immunity tended to be more Th2-mediated. The cytokines associated with this pathway led to the atopic symptoms which they developed later in life, and thus consequentially led to the increased prevalence of asthma which was being observed[9].

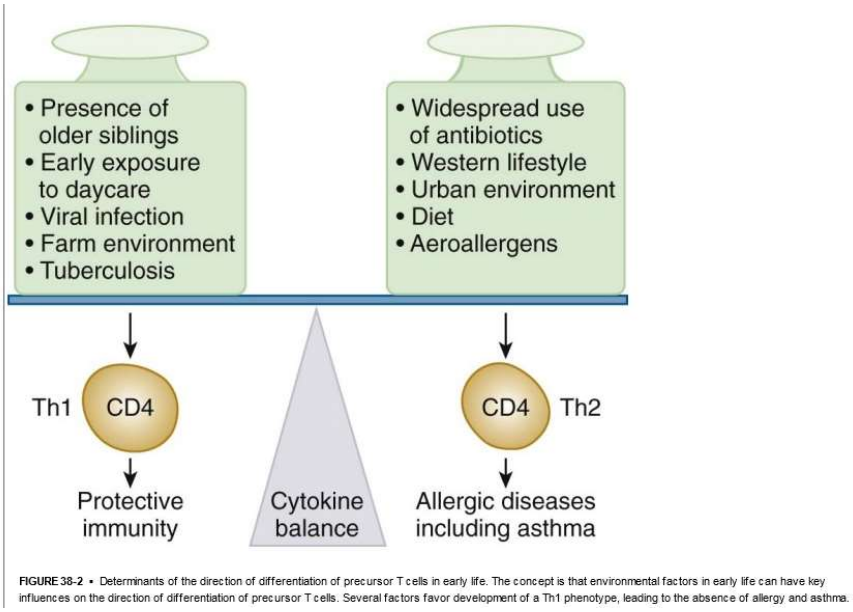


FIGURE 38-2 • Determinants of the direction of differentiation of precursor T cells in early life. The concept is that environmental factors in early life can have key influences on the direction of differentiation of precursor T cells. Several factors favor development of a Th1 phenotype, leading to the absence of allergy and asthma.

Figure 2: Determinants of the direction of differentiation of precursor T cells in early life.

The hygiene hypothesis thus gave importance to the environment and lifestyle which individuals were being exposed to, and which change was leading to a greater prevalence of asthma. This gave rise to further research on the subject, which led to conflicting results, some confirming that this theory could in fact confirm the rising prevalence of atopy and some disproving it, and making it unlikely that the hygiene

hypothesis alone can explain the surge in asthma[11-12]. For example, endotoxins from gram negative bacteria were thought to be protective in the hygiene hypothesis[13-16], particularly in the context of a farming environment. Other studies such as those conducted by Bolte et al[17] and Thorne et al[18] actually found that the presence of endotoxin was related to a greater prevalence of atopy and asthma in their cohorts.

1.1.2 **Allergic Rhinitis**

Allergic rhinitis has been defined by the American Academy of Allergy (2008) as being characterised by paroxysms of sneezing, rhinorrhoea, and nasal obstruction, often accompanied by itching of the eyes, nose, and palate[19]. It is a common condition, affecting 10% to 30% of children, depending on the country or region studied and tends to be commoner in industrialised countries[20-22]. The Phase 1 ISAAC study, reported that the prevalence of allergic rhinoconjunctivis was globally 14.6% in the 13-14 year group, with Maltese children reporting the second highest prevalence in this worldwide group.

The risk factors which have been reported for allergic rhinitis are a family history of atopy, male sex, birth during the pollen season, firstborn status, early use of antibiotics, smoking exposure in the first year of life, exposure to indoor allergens, Serum immunoglobulin E (IgE) >100 int. units/mL before the age of six (6) and the presence of allergen-specific IgE[24-25].

1.2.1 Aeroallergens

As allergy has long been recognised as a primary cause for asthma symptoms[26], the importance of aeroallergens which can be inhaled and thus introduced to the

respiratory system, and which can cause allergic symptoms is consequentially very plausible.

These aeroallergens can be divided into perennial allergens (which are present throughout the year, most often are present indoor and are not affected by seasons) and seasonal allergens (which as the name implies depend on the season and weather conditions for their presence, e.g. pollens).

Perennial allergens tend to cause persistent asthma symptoms, while seasonal allergens will cause spikes in symptoms during months where their concentrations in the environment are higher.

The presence of allergens will vary from country to country. This will be related to a number of variables, which include geographic position and climate, socioeconomic situations and housing, and cultural differences. For example, a warm Mediterranean climate does not favour the growth of Birch trees, which produce a common allergen in colder northern European countries. On the other hand, while dogs are seen as ritually unclean by most Sunni and Shi'a Muslim jurists[27], it is a common belief amongst Hindus that caring for a dog will pave their way to heaven[28]. Therefore, we can expect to find a higher presence of dog-related allergens in households inhabited by Hindus, when compared to Muslim homes.

No studies have been carried out to date in the Maltese Islands, to explore the presence of allergens in Maltese homes, their concentrations, and the sensitisation of Maltese people. One can therefore only speculate that it is likely that the seasonal

allergens present in Malta are similar to other southern Mediterranean countries, and that indoor allergens in homes are comparable to those in other westernised countries.

Common inhaled allergens which can be attributed to asthma-related symptoms in our climate can include the following:

- Animal allergens (both pets and pests: cats, dogs, rodents)
- House dust mites
- Cockroaches
- Indoor and outdoor fungi (Cladosporidium herbarum, Alternaria alternata)
- Outdoor plant allergens (tree pollen, grass pollen, timothy grass, pellitory)

1.2.2 Dog and Cat ownership

The effect of pet ownership on atopy and asthma has long been a challenge in research. This is due to different results from various epidemiological studies. While some cohorts reported an odds ratio for asthma related with sensitisation to dogs and/or cats to range from 3 to 9.2%[29,30], other studies suggest early exposure to these mammals in a child's life is actually potentially protective, and decreases the risk of wheezing in these individuals [31-34]. For both cat (Fel d 1) and dog (Can f 1) allergen, levels of more than 8000 to 10,000 and >80,000 nanograms per gram of fine dust, respectively, have been identified as targets above which there is an increased risk for allergic sensitization and symptoms. It is likely that what relates to the development of asthma-like symptoms in the presence of these pets is related to

whether the exposed person develops an IgE-mediated reaction to its allergen, or in other words, there is sensitisation, though this might not necessarily be related to exposure [35]. On the other hand it has been postulated that the presence of a cat results in extremely high concentrations of Fel d 1 (the protein found in cat saliva and skin which is thought to be the cause of IgE sensitisation in humans), which in early life could have a similar effect to immunotherapy in the treatment of allergic conditions[36,37].

Dog exposure has been shown to be rather protective against developing asthma and other allergic conditions[38,39]. It has been postulated that the presence of dogs changes the microbial diversity in the household which could have an impact on human health. An interesting study carried out in Atlanta, Georgia, USA, where there is a low ownership of cats in African-American communities, did not show a significant association between cat ownership and asthma[40]. It will be interesting to understand whether the opposite is true in a Maltese cohort, where cat ownership is very common.

1.2.3 House Dust Mite

House dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) are arthropods which can colonize soft furnishings such as bedding, sofas, and carpets. They absorb humidity from the environment and therefore cannot thrive in arid atmospheres. An element of atmospheric humidity is needed for their survival, ideally ranging between 55% and 75% [41]. They feed on residual organic material, which in a household may include human and mammal derived debris[42] (e.g. skin, hair). This feeding is known as dermatophagy. House dust mite tend to have a symbiotic relationship with fungi. The presence of fungi helps convert part of the

organic material in dust, and this is a sort of pre-digestion of the skin scale lipids which would facilitate the dust mites' process of digestion. On the other hand, house dust mites do not digest fungal spores during this process. We need to note that humid environments promote the life cycle of both house dust mites and mould, and it is not surprising that they developed such a relationship, especially considering that the indoor environment may promote both their growth. In fact a study performed in French dwellings showed close relationships of moulds and housedust mite[42]. The main proteins which have been identified as allergens in dust mites are Der p 1 and Der f 1[43], and mite concentrations of >2µg of dust have been identified as imposing an increased risk level for developing mite sensitisation and asthma-related symptoms[44,45]. What has also been recently shown in work by Vroman et al, is that chronic house dust mite exposure induced a pronounced eosinophilic allergic reaction as revealed in brochoalveolar lavage and lung tissue[46].

1.2.4 Moulds

The role of fungi on human health was poorly recognised until its importance became more prominent in recent years. Fungal disorders can span from local irritation, to hypersensitivity and worse still, infections, some of which can have devastating effects.

For the purpose of this review, the focus will be on the role of mould on allergy. Common indoor fungi include *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus*. Indoor levels of spores tend to be lower than those found outdoors[47]. When this is not the case, one has to suspect that there is an indoor source, such as a fungal infestation due to dampness, which is causing the high indoor spore levels.

Several disorders have been reported as being related to fungal hypersensitivity. This tends to be due to an individual developing an IgE-mediated immune response [48,49]. Type 2 innate lymphoid cells (ICL-2s) have also been implicated in this process [50]. This lineage has been recognised to secrete interleukins 5 and 13 (IL-5, IL-13), which have both been implicated in eosinophilic inflammation. This is especially mediated through IL-5 which recruits these cells from the bone marrow, activates them, and elicits their migration to sites of allergic inflammation such as the bronchial mucosa and the nasal mucosa. Thus interleukins have been reported extensively for their role in asthma and allergic rhinitis[51].

The role of fungal allergy in asthma has also been well established, especially when related to outdoor fungi such as *Alternaria*[52]. The role of fungal sensitisation and its close link to severe asthma has been reported by one of the leading UK Asthma research groups in Manchester, who proposed giving this condition a new term – Severe asthma with fungal sensitisation (SAFS)[53].

Further to the importance of the presence of moulds, several studies have confirmed the relationship between the perceived dampness of a dwelling to asthma symptoms [54-56].

Allergic rhinitis, has also been thought to be commoner in cases where indoor mould growth has been noted[57]. Children who have been sensitised to *Alternaria* have a higher prevalence of allergic rhinitis without necessarily developing asthma [58].

Other conditions which have been found in conjunction with the presence of fungal hypersensitivity are Hypersensitivity pneumonitis, Allergic bronchopulmonary aspergillosis and Allergic fungal rhinosinusitis.

1.2.5 Pollen

Pollen has long been implicated as a cause for asthma-related symptoms and rhinitis, especially when these symptoms have a seasonal pattern. Pollen types and concentration vary from climate to climate and country to country. Logically, one cannot study all the pollen types present in a country, as even in a small country such as Malta, these would amount to hundreds. On the other hand, certain types of pollens have been typically associated, in the literature, with sensitisation and atopy. These tend to be related to wind pollination, a process known as anemophily. Almost all gymnosperms (e.g. pine tree, fir) are anemophilous, and so are many plants in the order Poales, such as grasses [59].

Specific pollens have been historically associated with allergic conditions, such as Birch tree pollen in northern European countries. One will not expect such pollens to be present in a Mediterranean country such as Malta, which has a mild winter and a hot dry summer. There have been no studies locally which looked into the prevalence of specific pollen allergy. On the other hand, two studies by D'Amato (in Naples, Italy)[61] and Bousquet (in Montpellier, France)[60] look into this in areas which have similar climates to Malta. These can be used as a reference, as a similar trend could be present locally.

Table 1: Presence of airborne pollen and skin positivity adapted from D'Amato et al [61]

Montepellier (Bousquet et al)					Naples (D'Amato)			
Airborne presence	%	Skin test positivity	%		Airborne presence	%	Skin test positivity	%
Cupressaceae	15	Poaceae	86		Urticaceae	42	*Parietaria*	82
Poaceae	14	*Plantago*	36		Poaceae	12	Poaceae	38
Quercus	13	*Parietaria*	27		*Olea*	10	*Olea*	23
Plantago	8	Cupressaceae	15		Corylaceae	7	*Artemisia vulgaris*	17
Pinus	7	Oleaceae	15		*Alnus*	6	*Plantago*	7
Platanus	6	*Plantanus*	13		*Platanus*	5	*Chenopodium*	3
Oleaceae	4	*Trifolium*	13		*Artemisia*	4	*Platanus*	2
Urticaceae	3	*Medicago*	13		Cupressaceae	3	*Cupressaceae*	2
Artemisiae	1	*Artemisia vulgaris*	9		Chenopodiaceae	2	*Corylus*	2

Bousquet et al found that pollens from the Poaceae family (which include graminaceae – grasses) to be the second most prevalent airborne pollen in Montepellier, and the one causing most atopy (SPT positive in 86% of his sample). Pollen from the Poaceae family were again the second most common in the analyses by D'Amato, but only the second most important in causing SPT positivity (38%). The pollen in Naples which was causing most sensitisation was Parietaria (82%). These results show the differences in two southern Mediterranean cities, with Naples being further south and closer to Malta. Unfortunately, no similar studies were published locally.

D'Amato describes the following pollen seasons in the Mediterranean cities [62]:

a) A low winter pollen season (from December to the end of March) marked by the presence of pollens from trees such as Cupressaceae (Cupressus and Juniperus), Acaciae (Mimosa).
b) A high spring-summer pollen season (April-July), which is of marked allergological interest, due to high concentrations of grass pollens (e.g. gramineae), Parietaria and Olea (Olive).
c) A summer-autumn season (August-October) marked by a less pronounced peak of Parietaria and sometimes of Gramineae and pollens of herbaceous plants.

Interestingly several studies[63,64] have suggested an increased frequency of pollen-related respiratory symptoms in subjects who live in urban areas. This would initially seem illogical, as one would expect higher pollen concentrations in rural areas. However, it is thought that air pollution which is secondary to vehicular traffic such as particulate matter, sulphur dioxide and ozone has been associated with an inflammatory effect of airways, increasing their permeability which leads to an easier triggering of immune pathways by aeroallergens. Also, air pollution particles could act as a carrier of allergens, depositing them more effectively into the small airways[65].

Locally, Montefort et al[72] reported local trends in respect to allergic rhinitis in Maltese children aged 13-15 years-old. 52.7% of schoolchildren who had participated in this study reported having nasal problems. The vast majority of these (84.5%) manifested seasonal variation in their symptoms. They reported having most

symptoms in the winter months (February/March/April). They complained of less symptoms during the summer months.

1.2.6 Endotoxin

Endotoxin, also known as lipopolysaccharides (LPS), are found in the outer membrane of Gram-negative bacteria. They can elicit strong immune responses, and they are abundant in the indoor environment [66], They have been associated with the presence of mammals, pests and organic waste[67,68], and in humans are thought to be involved in the triggering of an immune response resulting in lung inflammation[69,70]. One of the most important studies associating the presence of high endotoxin levels with an increased asthma prevalence was carried out in the United States and spanned throughout 831 homes. Thorne et al reported significant relationships between increasing endotoxin levels and diagnosed asthma, asthma symptoms in the past year, current use of asthma medications and current wheeze[71].

1.3.1 Allergic conditions in Malta – The ISAAC study

The International Study of Asthma and Allergies in Childhood (ISAAC) was the first worldwide study, carried out using standardised questionnaires and defined most of what we know on worldwide childhood asthma and allergy distribution. The Maltese Islands were included from the study's first phases, and this has to date been the largest local study on asthma. ISAAC phase one, which commenced in 1993, used a core written questionnaire, focusing on two age groups: 6-7 years and 13 – 14 years. Phase two began in 1998, and included core questionnaires, skin prick tests for atopy, bronchial responsiveness to hypertonic saline, blood samples for serum IgE, genetic analysis and a risk factor questionnaire module. Phase 3 was a repeat of the phase

one study, about seven years after this had been undertaken. Malta participated in Phase I (1995) and Phase III (2001-2002) of this study.

In the Maltese phase 1 campaign, the older age group recruited was expanded to include 13-15-year-old children. 4184 children participated in the study, and the data was collected between January and July 1995, and later continued between October and November 1995. Montefort et al[72] reported that 27.9% of these participants had experienced wheezing sometime in their lives, while 16% had wheezed in the previous 12 months of answering the questionnaire. Equally high rates of exercise-induced wheezing and nocturnal cough were reported at 20.6% and 31.8% respectively. 52.7% reported having a nasal problem in their lifetime, and 47.4% had a nasal problem in the preceding 12 months (this was the second highest in the worldwide ISAAC cohort). 32.3% had been diagnosed with allergic rhinitis, while 28.9% had associated itchy eyes. These parameters were quite similar between male and female groups, with females having slightly higher prevalences. 11.1% of the participating children had been given a diagnosis of asthma by their doctor. 76.5% of children who had wheezed in their lifetime had an atopic relative, and these tended to be more prone to exercise-induced symptoms. Some children were found to be smokers (13.4%), and these tended to attend state schools rather than private schools. They also had more persistent wheezing, exercise-induced wheeze and nocturnal symptoms. 57.1% were passive smokers, and these experienced more exercise-induced wheezing and nocturnal cough than their counterparts who were not exposed to environmental tabacco smoke.

Children who had wheezed in the past, were less likely to have blankets in their bedroom or household pets. Children who lived close to a road with busy traffic were more likely to have reported wheezing sometime in their lifetime. Fsadni et al also reported a higher prevalence of children reporting a nocturnal cough in the absence of a cold in children of all age group who lived near roads used by heavy vehicles[104].

As regards rhinitis, this was commoner in females, who also tended to have persistent symptoms. Also, children who had rhinitis were more likely to have an atopic relative. There were no associations between pets and rhinitis in this cohort. Rhinitis was reported mostly in the period spanning from January to April and peaked in May, with a smaller peak being reported between September and October.

The authors of this report concluded that allergic conditions were a common problem in the Maltese Islands, and this was higher than the mean reported in the ISAAC worldwide cohort. They also noticed that children suffering from asthma could had not been receiving adequate treatment as many of these were still quite symptomatic and at risk of life-threatening asthmatic attacks. They noted that parents of asthmatics were taking precautions to decrease HDM exposure by avoiding soft toys, thick carpets and blankets in their children's bedrooms. Family history of atopy, personal and passive smoking, and busy roads were a strong influence on the presence of allergic conditions.

As mentioned earlier on in this section, the ISAAC study was repeated locally in 2002 as part of ISAAC Phase 3. This was again reported by Montefort et al[73]. Similar number of subjects in the 13 to 15-years old group were involved in this study

(4184). In this age group, similar prevalence rates of wheezing were reported when compared to the 1995 study, yet there was a higher proportion of these children who had been given a diagnosis of asthma (14.1%) and hay fever (40.7%). Interestingly the rate of diagnosed asthma was almost identical to the prevalence of current wheezing in this study (14.6%). The authors reported that again girls seemed to be more likely to be current wheezers when compared to boys ($p < 0.01$) and that the girls also reported nocturnal symptoms more than boys ($p < 0.001$). The Phase 3 children reported better symptom control, with the proportion of children reporting more than 12 wheezing attack in the last 12 months decreasing form 19.7% in 1995 to 15.2% in 2002, and those having 4-12 wheezing attacks decreasing from 19.7% to 15.2% ($p<0.01$). The authors felt that this was probably due to the greater rate of asthma diagnosis, which led to the children receiving the required medication to control their symptoms.

1.3.2 Geographical distribution of wheezing in ISAAC Malta

Fsadni et al[104] looked into the geographical distribution of wheezing in children, as reported in both ISAAC 1 and ISAAC 3. They noted that there was an increase in the reporting of wheezing when comparing ISAAC phase 1 and phase 3, with the largest increases being reported in the Central East, Grand Harbour area, East and Central Northern regions in the 5-8-years old group. More importantly a significant increase ($p < 0.0001$) was noted in the central south area (18.1% in Phase 1 vs 29.3% in Phase 3). A significant decline in wheezing was noticed in Gozo (30.9% in Phase 1 vs 19.6% in Phase 3) and the South (27.1% in Phase 1 vs 16.7% in Phase 3) in the 13-15-year old age group. The authors postulate that the higher vehicular traffic in the

areas which reported an increased prevalence of wheezing could have been the contributing factor for this observation. In fact, further adding value to this hypothesis, they noted an increased prevalence of nocturnal cough in children of all age groups when these lived close to roads used by heavy vehicles ($p = 0.005$).

1.4 **Aims of this study**

The ISAAC study was certainly a landmark in the local knowledge of asthma prevalence and other allergy-related conditions in schoolchildren, and continues to be a reference to all local research on the subject. Unfortunately, there have been no similar local studies on prevalence of similar conditions in adults. Also, ISAAC was an observational study which no actual sampling of domiciliary environmental factors, such as the effect of households, home indoor allergens and their influence on local respiratory health.

The study which was presented in this thesis had been designed with all of the above in mind. This was part of the Respira Study, within the Cross-Border Program Italy-Malta 2007-2013, a cross-sectional survey was performed in Sicily and Malta, two islands in the Mediterranean Sea. In Sicily, 1,325 schoolchildren were randomly selected from the four communities of Gela, Niscemi, Mazzarino, and Butera in the Gela Health District, located in the south Mediterranean coast of Sicily; 1,075 were recruited from 6 schools in Malta.

On the Sicilian side, a petrochemical industry, operating since 1965, was active and located close to the urban area of Gela, while the towns of Niscemi, Mazzarino, and Butera were in a mainly rural area. On the Maltese side, during the time of the

study, two power stations located in the south of the island were still running on heavy fuel oil.

In Sicily, the study involved all 12 lower secondary schools: six schools in Gela, 77,000 inhabitants, representing the industrial area, three schools in Niscemi, 26,400 inhabitants, two in Mazzarino, 11,800 inhabitants, and one in Butera, 4,900 inhabitants, in rural areas located about 15, 27, and 16 km from Gela, respectively. In Malta, eight schools were involved: four in Hamrun (9,397 inhabitants), one in Cospicua (5,589 inhabitants), one in Zejtun (11,521 inhabitants), two in Mosta (20,241 inhabitants). Children selected in Cospicua and Zejtun were analyzed together because the two communities are in close proximity (Figure 1).

The study was approved by the local Ethical Committees. All parents of the children signed an informed consent. The respect of individual privacy concerning clinical data was granted.

Through this cohort which participated in the Respira study, a detailed case control study was carried out in order to understand which household indoor factors could have been the cause for such relatively high prevalence of allergy-related conditions in Maltese children. The homes in which these individuals dwelled were also visited, in order to look into the effect of these households on the adults which inhabited them. It was important to study whether there were factors in these households affecting the respiratory health of the adults in a similar way as to how they could have been affecting the children, or whether the children more sensitive to these factors due to early exposure and sensitisation. The allergens were investigated to look into which had been causing most atopy locally. There were many questions on

what was affecting respiratory health in Maltese individuals locally which needed answering, and this was an attempt at starting to obtain these answers.

1.5 Hypothesis of this study

The **hypotheses** which this thesis aims to answer are two. That there are differences between the characteristics of families who have a child who experiences uncontrolled respiratory symptoms and others who do not have a symptomatic child. Secondly that there are differences in the indoor household characteristics of families who have a member who has respiratory symptoms and those who do not have members having such problems.

2. **Methodology:**

2.1 Study Design

The present research study was part of the Respira Project, which was co-financed by the European Commission and the Maltese Ministry of Health. The project was powered to recruit 600 children in order to be representative of the Maltese population. Over 900 students, aged between 12 and 15 years-old were invited from six Maltese secondary education schools. Consent forms were sent to these children's parents, following an information campaign which was organised during parents meeting which were held prior to the beginning of the scholastic year. Children whose parents had accepted to allow them to participate in the study were then given a questionnaire which was to be completed by the children themselves, with the assistance of an investigator. They were then given a parent questionnaire, which was to be completed by their parents at home. This included more detail about the child's respiratory health, perinatal information and environmental information. These questionnaires were based on and mainly used elements from the ISAAC study [19].

Children, together with their families (father, mother, sibling) were then selected to participate in the case control study being presented in this thesis. The children who were selected as "cases", had symptoms of uncontrolled asthma. An equal number of "control" families were also selected. The study protocol envisaged recruiting thirty three (33) cases and thirty three control children. The criteria to choose the above groups were based on data from questionnaires which the children's parents had answered.

The children who were selected to be included in the “case” group, were selected according to a positive response to two or more of the following criteria: current wheeze, recent exercise-induced wheeze and a current nocturnal cough in the absence of a cold (“current” meant over the previous 12 months), which their parents would have reported in the above-mentioned questionnaires. Children selected to participate as part of the control group had no symptoms of uncontrolled asthma, history of asthma and/or rhinitis and no history of atopy (which included dust, pet or pollen allergy).

The table below depicts the case and control group symptom characteristics. An equivalent number of families (the protocol was set for 33 members for each group, but these selection was maximised during the study to collect more data and increase the opportunity for more data analysis) were chosen from each couple (at the time of the study there was no mixed education, and boys and girls were separated in different schools for each area) of secondary schools in the northern, central and southern regions of our country. The cases and controls were matched by age, as only students from the second and third year classes were selected, and sex by choose equal numbers from each school (schools were single gender, and there were three boy schools and three girl schools).

Table 2: Case and Control criteria

Case Group (at least 2 positive answers)	**Control Group**
Wheezing in the last 12 months	No wheezing in the last 12 months
Exercise induced wheezing in the last 12 months	No exercise induced wheezing in last 12 months
Nocturnal cough in the last 12 months	No nocturnal cough in the last 12 months
	No doctor diagnosed asthma
(above symptoms were not to be in the context of a respiratory infection)	No history of rhinitis
	No allergic rhinitis to pollen
	No allergic rhinitis to other allergens
	No cat allergy
	No dog allergy
	No pollen allergy
	No food allergy

These families were then invited to attend clinics held at Mater Dei Hospital. The families from the central part of the island were invited during the period between April and June 2012. Those from the southern region were invited between October and December 2012, and finally the northern group were brought in between January and March 2013. These groups were analysed separately to assess the possibility of a seasonal effect on their symptoms, and their numbers were proportionally equal in each phase of the study, which were separated according to different seasons as described later on in this section.

The parents were asked to complete a questionnaire on their own respiratory health (father and mother answered separate questionnaires), based on a number of

validated questionnaires. These questionnaires were previously used in IMCA project[74], another EU-funded study.

All the subjects (adults and children) were then examined clinically, and the following tests were performed:

- Peripheral Blood Pressure
- Peripheral Oxygen Saturation
- Spirometry to assess FEV_1, FVC, FEV_1/FVC, FEF25-75, FEF50
- Exhaled Nitric Oxide as a measure of airway inflammation
- Acoustic Rhinometry to assess nasal patency
- Exhaled Carbon Monoxide to assess recent smokers/passive smokers and exposure to vehicular carbon monoxide
- Skin Prick Testing to assess atopy/sensitization to:
    a. House dust mite mix
    b. Cockroach
    c. Olive tree pollen
    d. Grass mix
    e. Parietaria
    f. *Alternaria* mix
    g. Cat Epithelium
    h. Dog Epithelium
    i. *Cynodon dactylon* - Dog's tooth grass
    j. Histamine (positive control)

k. 0.9% saline (negative control)

- Blood letting for Total IgE and Specific IgE to:

a. T9 - Olive pollen
b. W12 – Golden rod pollen
c. W19 – Spreading pellitory pollen
d. E1 – Cat epithelium/hair
e. E5 – Dog epithelium/hair
f. M2 – *Cladosporidium herbarum*
g. M6 – *Alternaria tenuis*
h. D1 – *Dermatophagoides pteronyssinus*

These families were then invited to have environmental tests performed in their households. The households were visited individually and information regarding the house was documented. This would include the type of house, GPS co-ordinates, materials used in the finishing of the house, furnishings and detergents used for general house cleaning. The families were asked to avoid house cleaning for the prior two days to sampling, to avoid finding insufficient dust for sampling, or potentially collecting disproportionately low allergen concentrations due to recent house-cleaning.

The aim was to have the households analysed within 15-28 days from the day the family was invited for the clinical tests. This would ensure that the clinical results

would reflect the recent exposure to the household indoor air environment present in same period of time.

2.1.1 School Selection

In the Maltese islands one finds that secondary schools were run either by the government (state), the church or private educational entities. It was decided *a priori* that the schools to be selected were to be government (state) schools. Government secondary schools accepted children from specific geographical regions on the island. This was not the case for church schools and private schools at the time when the study was carried out. Therefore, selecting a specific government school would mean that the participating children all lived in the same area of the island, which meant that later the homes of the same specific area could be studied once the families were recruited. Church and privately-run schools did not have definite catchment areas, meaning that such a geographical analysis would not be possible.

During the time period in which the study was carried out, male and female students were schooled in different buildings altogether, and mixed schooling had not yet been introduced. Thus, for each area to be studied (North, Central, South) a male school and a female school were selected. An effort was made to select boys' and girls' schools which were as geographically close to each other as possible to limit this possible but inevitable bias.

The following schools were selected:

Central Malta Schools

a. Liceo Hamrun boys school (today known as San Gorg Preca College Hamrun Secondary School) – found in Triq Wenzu Mallia, HMR 1241.
b. Maria Regina Junior Lyceum girls school (today known as San Gorg Preca College Middle School, Blata l-Bajda) – found in Mountbatten Street, Hamrun, HMR 1572.

These two schools were located within meters of each other, in the town of Hamrun, which is a busy town full of businesses and commercial premises present in this area. It schools children who lived in the central part of the island, from villages bordering the northern part of the island (Dingli, Rabat, Bahrija, Mgarr, Mtarfa), towns which are considered to be central (Hamrun, Qormi, Zebbug, Siggiewi, Sta Venera, Valletta, Pieta, Msida, Birkirkara) and those bordering the southern part of the island (Marsa, Paola). Most of these towns are busy with vehicular traffic.

Southern Malta Schools

a. Verdala Boys School (today known as St Margaret's College Boy's Secondary School) – Found in St Nicholas Road, Cospicua, BML 9035
b. Zejtun Girls Secondary School (today known as St Thomas More College, Secondary School, Triq Luqa Briffa, Zejtun. ZTN 2719

These two schools were both located in the southwestern part of the island and were 4km away from each other. They accepted children from the towns and villages located around the port area (Cospicua, Zabbar, Senglea, Vittoriosa), the areas around the Delimara Power Station (Marsascala, Marsaxlokk, Kalkara) and the towns and villages situated between these two areas, which include Fgura, Zejtun, Zabbar and Xghajra. These areas were of particular interest from an environmental point of view, due to the presence of the Port, which is potentially a source of pollution from maritime activity, and the exhaust fumes from the Delimara power station, which at that point in time was run on heavy fuel oil.

Northern Malta Schools

a. Zokrija Boys secondary school (today known as Maria Regina College, Mosta Secondary School, Zokrija) – Found in Triq il-Biedja, Mosta MST 1633
b. Lilly of the Valley, Girls secondary school (today known as Maria Regina College, Mosta Secondary School) – Found in Triq Wied il-Ghasel, Mosta. MST 2142

The Mosta schools are also located within walking distance from each other. These schools accept children who live in northern and more rural towns and villages. These include Mosta itself, and Gharghur, Naxxar, Iklin, Mellieha. School children from Qawra, Bugibba and St Paul's Bay also attended these schools. These localities are located close to the northern coastal area of the island, and had at the time become popular with foreign immigrants, but also with families seeking cheaper housing, as

properties in these areas demanded lower premiums in comparison to other areas in the island.

2.1.2 Questionnaires

2.1.2.1 Student questionnaire

The student questionnaire was distributed to the pupils during one of their lessons and completed by themselves with the assistance of the investigators. It was adapted from the SINPHONIE project, and was translated to Maltese[81]. Validation was confirmed through a professional back translation. The full questionnaire can be read in **appendix 2**. The different relevant sections found in this questionnaire are described here:

Questions C1-C34: Questions on respiratory health including recent wheezing. The students were asked about recent symptoms (past 7 days), which could be related to allergic conditions. They were also asked to state whether they experienced these symptoms at home, school or in another location.

Questions C35-C38. The students were asked to state whether they were exposed to passive smoking, the length of time in which they were exposed, and the place where this happened.

Questions C39 – C46. The students were asked to describe their perception of their school's general environment.

2.1.2.2 Parent questionnaire

The second questionnaire was distributed to the pupils to be given to their parents and completed by one of these adults. It was also adapted from the SINPHONIE project and was translated to Maltese. Validation was confirmed through a professional back translation. The full questionnaire can be read in **appendix 2**. The different relevant sections found in this questionnaire are described here:

Questions PQ9 – PQ15. These questions related to general information about the child, including date of birth, age, sex, ethnicity and height and weight.

Questions PA1 – PA10. These were questions about the child's perinatal period and were aimed at gathering information such as birth weight, premature birth, maternal or paternal smoking during pregnancy and the early years of life and any respiratory related problems during the child's early years of life.

Questions PB1 – PB22. These questions focused on asthma-related symptoms, most particularly wheezing. The timespans investigated in these questions were divided into "ever" and past 12 months. As regards questions on asthma-related medications, these questions also tackled their use over the preceding 3 months.

Questions PB23 - PB 46. These questions investigated symptoms of other atopic conditions such as allergic rhinitis and/or eczema. Again, these questions asked whether the child ever had these symptoms and whether they occurred over the past 12 months prior to the completion of the questionnaire. Seasonality was also investigated in question PB26, which required the parent to state during which months they had noticed the child to be experiencing sneezing or a blocked nose not in conjunction with a cold or the flu.

The parents were also asked to report whether there was a history of allergic disorders in the family, and state who suffered from these, and whether these were due to asthma, allergic nasal symptoms and/or eczema.

Questions PC1-PC19. The parents were asked to report which symptoms related to allergy (such as itching, runny nose, cough etc) were experienced by their children over the previous 3 months. They were also asked to describe the frequency of these symptoms, and whether they corresponded to school attendance or being at home.

Questions PD1-PD13: The child's dietary habits were to be reported through these questions.

Questions PE1-PE37: There were questions which related to the home environment, including heating, cooling, mould and dampness and general

home characteristics. This section also asked for information on smoking habits in the household.

Questions PF1-PF4: These questions tackled socioeconomic status, including parental education and employment, and whether there was any dependence on state benefits.

Questions PG1-PG9. The parents were asked to report about the child's perception of their school's general environment.

2.1.2.3 Adult health questionnaire (IMCA)

The IMCA (Indicators for Monitoring COPD and Asthma in the EU) was a study sanctioned by DG Sanco[74], which was developed using several established respiratory questionnaires. Its aim was to collect as much data as possible on the respiratory health of European adult citizens. The core questionnaire using in this study was based on questionnaires which had been previously used and validated. Below the different sections found in this questionnaire are described:

Questions CC: Data on socio-demographic characteristics including age, date of birth, sex, schooling have been collected through these questions.

Questions CD: These questions focus on symptoms of asthma and COPD, and were based on previously validated questionnaires (BOLD[75,76], AIRE[77], ECRHS[78], CORSQ[79]). The MRC (Medical Research Council) Dyspnoea scale and family history was also requested.

Questions CE: Co-morbidity: in order to obtain information on co-existent diseases in a list of conditions included in the Charlson Index[80] : myocardial infarction, congestive heart failure, other vascular disorders, stroke, dementia, rheumatic disorders, ulcer of stomach or duodenum liver diseases, diabetes, neuromuscular problem, renal disease, cancer. Other conditions or health problems included in general health surveys were also included: high blood pressure, disabling backaches, migraine headaches, high cholesterol levels and thyroid problems.

Questions CF: The questions investigated the presence of risk factors which could lead to various conditions, such as smoking and alcohol use.

Questions CG: The adults were asked whether they had been using health services, such as smoking cessation programs, spirometry, and hospitalisations or specialist outpatient clinics.

Question CH: The parents were asked to report on any treatment or drugs which had been recently prescribed to them.

2.1.3 Recruitment

The recruitment period started at the beginning of the scholastic year (September 2012). This involved talks with the children's parents, informing them about the Respira project, its aims and methodology, clinical evaluation and home sampling. This information was delivered through a PowerPoint® presentation, and information and consent sheets were distributed to the parents. These were then either collected during this activity, or through the heads of school who received these documents at a later date.

During the scholastic year, the children's questionnaires were completed during school hours and the parents' questionnaires were distributed, to be completed at home by one of the parents and returned to the school administrators.

The parents' questionnaires were used to subdivide the children into the "case" and "control" groups, as described in section 2.1. The pupils were shortlisted by first identifying the ones who fitted the criteria for "case". Then, an equal number of "control" children were identified using the same methodology, yet with criteria which fitted this group.

Once this shortlisting was completed, the parents were contacted by means of a phone call, and informed that their family was being invited for the study, which would involve clinical and home analysis. They were still free to accept or decline this offer, even if they had given their consent for the study in the first place. The parents were blinded to whether their family was part of the "case" or "control" group. It was made

clear that it was necessary for the child to participate in the clinical evaluation. The participation of the siblings was optional.

During the clinical evaluation, the study was explained again to the family, and they were invited to participate in the home evaluation. They remained free at this stage to decide whether they wanted their homes to be evaluated or not, particularly due to privacy issues.

2.1.4 Ethical Approval

Ethical approval was sought and obtained both from the Maltese Department of Education Research Directorate and the University Research and Ethical Council. These are found in appendix 1, together with the various consent forms in which parents allowed the children to participate in the study, have the questionnaires completed, and for both parents and children to undergo the clinical phase. The ethical approvals were applied for the Respira Project, as a whole. Dr Christopher Zammit, a co-investigator in the Respira Project, applied for ethical approval from UREC, while the approval from the Maltese Department of Education Research Directorate was obtained by myself.

2.1.5 Fieldwork

The questionnaires were distributed according to the following timeframe:

Central Malta Children

The questionnaires were distributed to the boys between the 26th March 2012 and the 29rd of March 2012. They were distributed to the girls between the 30th March 2012

and the 3rd of April 2012. These questionnaires were completed and collected during Spring.

The families were then invited and underwent the clinical phase of the study between the 17th April and the 8th of May. The homes were visited more or less in the same period, between the 24th April and the 27th June 2012.

Southern Malta Children

The questionnaires were distributed to the boys between the 10th October 2012 and the 18th October 2012. They were distributed to the girls between the 25th October 2012 and the 30th October 2012. These questionnaires were completed and collected during Autumn.

The families were then invited and underwent the clinical phase of the study between the 30th October and the 20th November. The homes were visited in the same period, between the 29th October and the 19th December 2012.

Northern Malta Children

The questionnaires were distributed to the boys and girls simultaneously, during the last scholastic week of the 2012 (between the 17th December and 21st December). These questionnaires were completed and collected during the Winter months. This collection was done to accelerate the winter campaign, and avoid any fieldwork during the students' examination period.

The families were then invited and underwent the clinical phase of the study between the 1st January and the 12th February 2013. The homes were visited roughly in the same time span, between the 7th January and 13th March 2013.

2.1.6 Clinics

The families were invited to attend the clinical phase of the study during the time frames described in section 2.1.5.

They were contacted through a telephone call, after they were selected to participate in the study according to the "case" and "control" predetermined requisites which have been described in section 2.1. The families who accepted to participate in this phase, attended clinics at Mater Dei Hospital. These clinics were manned by doctors and nurses who were briefed to follow the study protocol and trained to perform all the necessary investigations.

All the available equipment was calibrated prior to starting the clinic. On arriving at the clinic, the family members were identified and asked to complete a further consent form documenting that they accepted to go through the clinical evaluation, have blood tests taken, undergo spirometry, skin prick testing, rhinometry, exhaled nitric oxide, and that they consented to all these results being used for research purposes, but were assured that these were to remain anonymous.

The standard American Thoracic Society (ATS) guidelines were used to perform spirometry. The participants were seated, and nose clips were used to close the nasal orifices. The participants were coached in deep breathing, with a deep inhalation prior to putting the mouthpieces in the mouth, between their teeth. The subject's lips

needed to seal around the mouthpieces to prevent air leakage, followed by a strong, forced exhalation lasting six (6) seconds. The patient was allowed to rest for several seconds, and repeat the test, as described above. Three reproducible tests needed to be obtained from each participant, and therefore the test would need to be repeated for a number of times. Reproducibility was checked by observing the numerical values of FEV1 and FVC (these needed to be within 5% of each test), and the same flow-volume loop patterns.

All the measurements for each family were documented in a clinic booklet (see **appendix 3**). The participants were then weighed, and their height was measured. They were individually examined by a doctor, as a screening measure for other conditions.

The time taken to carry out the above measures was also important for the participants' bodies to acclimatise to the indoor air present in the clinic. This was essential for the first evaluation, with was the exhaled nitric oxide. It was an important prerequisite established by the manufacturer of the NIOX MINO® device (Aerocrine Ltd) to ensure an accurate exhaled nitric oxide measurement. This instrument used chemiluminescence analysis to determine the concentration in the gase phase by the reaction of NO with ozone to produce nitrogen dioxide in an excited state. The subject exhaled directly into the analyses, keeping a steady flow, which was guided by the instrument, through a visual aid in the form of a cloud on the screen, and auditory by the sound of a continuous beep.

The next test was spirometry (Spirolab III® by MIR), which was performed by each family member according to ERS/ATS guidelines[82] established to standardise this measurement. The room in which this test was performed was constantly air-conditioned, in order to avoid temperature fluctuations which could interfere with air volumes being measured during this test. The tests for all participants were carried out within the same hours of the day (4-6pm) to avoid the effects of diurnal variation on lung function.

The family then underwent acoustic rhinometry (A1 Acoustic Rhinometer by GMI Instruments). The system produced a click sound which travelled in the instrument past the microphone into the tube. An appropriately size nose piece was attached to the instrument to match the individual's nostril and produce a correct seal. The subject was instructed to breath to the nose and confirm that the seal was adequate. The left nostril was measured first. The subject was instructed to take a breath in and hold the breath while taking the measurement. The measurement was then documented, and the test was repeated for the subject's right nostril.

They finally had skin prick testing (using ALK abello inc kits), as long as they had not recently been on drugs which would have affected the results (e.g. antihistamines, oral steroids, doxepin etc) or were pregnant (in the case of mothers, as to avoid rare but possible anaphylactic reactions during skin prick testing in a vulnerable female). The skin over both forearms was cleaned with surgical spirit. A numbered grid was marked with a pen over the forearm with a number next to each area onto which a drop of allergen was going to be placed. The allergens were separately placed onto the skin, and using a standard disposable needle, each allergen was pricked into the

intradermal space. The subject would develop wheals at the site of pricking, which were then measured fifteen (15) minutes after the subject was pricked. The measurements taken were the maximal diameter of the wheal (A) and the diameter 90^{o} to it (B) and the average was measured and documented [(A+B)/2].

Finally, the participants underwent bloodletting for total and specific IgE.

2.1.7 Immunoglobulin E

All blood samples taken during the clinics, were stored in the Immunology Lab at Mater Dei Hospital. They were centrifuged, and then stored in a freezer at -20^{o}C. When all the blood samples were collected from the clinical phase, these were analysed. The samples were thawed to room temperature prior to analysis.
Separate kits were used to analyse Total IgE and Specific IgE (R-Biopharm AG). The specific IgE antibodies to be analysed were the following: Olive pollen, Golden rod pollen, Spreading pellitory pollen, Cat epithelium/hair, Dog epithelium/hair, *Cladosporidium herbarum*, *Alternaria tenuis*, *Dermatophagoides pteronyssinus*.

Analysis was through an ELISA (enzyme-linked immunosorbent assay) technique whereby there was a coated well with the antigen of interest, in which the serum was placed. If this contained the relevant antibody this would bind to the antigen in the well and form a complex. The complex was then detected by first adding the conjugate which had an enzyme tagged to it which upon addition of the substrate would change colour proportionate to the concentration of the complex formed in the first step.

In between steps three wash procedures [using phosphate buffer saline (PBS) as base] were performed, to remove any unbound substances which could have otherwise interfered with the binding in the next step.

The wells for specific IgE analysis were coated with a disk containing the allergen of interest, whilst those for Total IgE were coated with an antigen ready-prepared by the kit manufacturers. The results were then inputted in a standard Excel spreadsheet, to be used for eventual statistical analysis.

The following ranges were used:

Table: Standard ranges according to age for serum Total IgE

Total IgE

Reference Ranges	
WHO 75/50	**U/ml**
Newborn	< 11
Up to 3 mths	< 25
3 – 12 mths	< 37
1 to 5 yrs	< 135
5 to 10 yrs	< 144
Adult	< 188

Table: Standard ranges according to age for serum specific IgE

Specific IgE's

Reference Ranges		
U/ml	**EAST* class**	**Interpretation**
0.00 - 0.34	0 (0.0 - 0.9)	None found or hardly exists
0.35 - 0.69	1 (1.0 - 1.9)	Low
0.70 - 3.49	2 (2.0 - 2.9)	Increased
3.50 - 17.49	3 (3.0 - 3.9)	Significantly increased
17.50 - 49.99	4 (4.0 - 4.9)	High
50.00 - 99.99	5 (5.0 - 5.9)	Very high
>100.00	6	Extremely high
EAST =EnzymeAllergo Absorbance Test		

2.1.8 Home analysis

The homes were visited in the time periods described in section 2.1.5. On visiting the home, all the information was documented in pre-prepared fieldwork booklet check-lists. The type of dwelling was documented, together with its GPS co-ordinates using a GPS location device. If the dwelling was an apartment or a maisonette, the floor level was documented. The number of rooms was noted, together with the number of inhabitants living in the dwelling.

A general description of the dwelling was noted, especially focusing on important features which characterised the house.

The following home characteristics were carefully documented:

- Type of equipment used for air conditioning (heating/cooling)
- Furniture (materials, new furniture, carpets, curtains)
- Description of type of floors, walls, ceiling etc
- Type of apertures (windows, frame used and type of glass)
- Household cleaning products used for floor and windows
- Scheduling of house cleaning
- Description of surrounding area (roads, factories, mobile antennas)
- Photos were taken of the façade (with the owner's consent)

A sketch of the room which was to be vacuumed was made on site, documenting the area of the room, aperture position and size. The method used to collect and store the vacuum samples is described in section 2.1.9.

2.1.9 Dust Samples

Dust sample collection was performed according to a pre-set methodology in the living rooms.

A standard household vacuum cleaner was used, which was powered by local household electricity supply. This was connected to a Dustream® Collector, which consisted of a plastic adaptor. Into this adaptor, a Dustream® Filter, which was a nylon cylindrical filter was inserted. This was designed to collect dust samples which were meant to be analysed for indoor allergens and endotoxin.

New and clean disposable gloves were used while handling the Dustream® Collector (Inbio biotechnologies^{INC}) and samples to avoid contaminating the material which were to be collected. The vacuum cleaner was turned on, and six separate areas measuring one square meter ($1m^2$) on the floor each were sampled for a duration 20 seconds per area. Then, a further six $1m^2$ areas were sampled from above the floor (tables, chairs, sofas, window sills etc). The Dustream® filter was removed from the collector, and placed into a small labelled Ziploc bag. The Dustream® collector was then rinsed first with sterile water and then dried with a clean paper wipe. It was then rinsed again with 70% ethanol to prevent cross-contamination from one sample to the next. The samples were stored in a freezer at -20ºC. At the end of sampling for a set of houses, the samples were sent to a central lab in Palermo (CNR – Consiglo Nazionale di Ricerca, Istituto di Biomedicina e Immunologia Molecolare "A. Monroy") for analysis. During analysis the dust was sieved through a No. 45 mesh screen, having a 355µm diameter (VWR No. 57332146) to remove large particles and fibres. 100 mg (±5mg) fine dust was weighed and placed into a 75mm x 12 mm plastic test tube (Sarstedt No. 55.476). 2.0ml PBS-T (0.05% Tween 20 in phosphate

buffered saline, pH 7.4) were added to this sample. For smaller samples (between 20mg and 100mg) a proportional amount of this substance was added. The amount of dust in mg was multiplied by 20 to give the appropriate volume of buffer in μL needed. Samples <20mg were labelled as "Not Enough Sample" and not processed. Each sample was resuspended in a vortex and later mixed for 2 hours on a laboratory rocker at room temperature. It was then centrifuged for 20 minutes at 2,500 rpm at 4°C. Supernatant (approximately 1.5ml) was then aspirated with a Pasteur pipette, for measurement of antigen. The remaining dust pellet was discarded.

The antigens analysed using Elisa kits from Inbio technologies^{INC} were the following:

a. *Dermatophagoides pteronyssinus* (Der p 1) – House dust mite
b. *Felis domesticus* (Fel d 1) – Cat dander
c. *Phleum pratense* (Phl p 5) – Timothy grass
d. *Alternaria alternata* (Alt a 1) – Mould common on plants

The dust supernatant was also tested for Bacterial endotoxin using endpoint Limulus Amebocyte Lysate (LAL) tests, supplied by Lonza group Ltd.

2.2 Statistical Analysis

2.2.1 Statistical Analysis used for questionnaire analysis

Hypothesis testing was carried out mainly by using the Chi-Square test. This test was used to examine the association between any two categorical variables. For all tests, the null hypothesis specified that there was no association between the categorical variables (row percentages vary marginally) and is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specified that there was a significant association between the two categorical variables (row percentages vary significantly) and was accepted if the p-value is less than the 0.05 criterion.

Modelling was carried out mainly through Logistic regression analysis. Logistic regression models are appropriate to relate a categorical dependent variable to a number of predictors collectively. The advantage of using Logistic regression analysis was that the model will identify the significant predictors and ranks them by their contribution in explaining the total variance. Moreover, for each significant predictor an odds ratio and corresponding 95% confidence interval was computed.

2.2.2 Tests for normality for Clinical Test results

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality assumption of the clinical tests for each case and control group separately. The null hypothesis specified that the score distribution was normal, and was accepted if the p value was exceeding the 0.05 level of significance. The alternative hypothesis specifies that the score distribution is non normal, and is accepted if the p value is less than 0.05 criterion.

The results of the normality tests described above indicated whether a parametric test such as the independent sample t-test or a non-parametric test such as the Mann Whitney U Test was to be used. The results for the normality tests are presented in **appendix 4**).

2.2.3 Tests used to analyse clinical results

Since FEV_1 and FEV_1/FVC were found to have a normal distribution using the tests described above, the Independent samples T-Test (parametric test) was used to compare mean FEV_1 and FEV_1/FVC ratios between two independent groups ("cases" and "control" groups). The null hypothesis specified that mean FEV_1 and FEV_1/FVC ratios varied marginally between the two groups, and was accepted if the p-value exceed 0.05 level of significance. The alternative hypothesis specified that the mean FEV_1 and FEV_1/FVC ratios varied significantly between the two groups, and was accepted if the p-value was less than the 0.05 criterion.

Since FeNO, serum IgE and rhinometric results were found to have a non-normal distribution, using the Shapiro-Wilk test, the Mann Whitney U Test was used to compare mean FeNO , mean total serum IgE and the minimal cross-sectional area between two independent groups ("cases" and "control" groups). This is a non-parametric alternative to the independent samples T-test. The null hypothesis specified that the mean FeNO , the mean serum total IgE or minimal cross sectional area varied marginally between the two groups, and was accepted if the p-value exceed 0.05 level of significance. The alternative hypothesis specified that the mean

FeNO, the mean serum total IgE or minimal cross sectional area varied significantly between the two groups, and is accepted if the p-value is less than the 0.05 criterion.

2.3 Role in Respira Project

The roles of the author of this thesis in the Respira Project is included in the following list:

- Involved in preliminary meetings and study design
- Selection and procurement of equipment needed
- Recruitment and questionnaire collection shared with a co-investigator
- Contacting and meeting all the families involved
- Organisation and carrying out clinical tests
- Training of any health care workers who helped in clinical tests
- Liaison with immunology lab for allergen analysis
- House visits with a focus on allergen sampling
- Transportation of sampling to Palermo CNIR
- All data analysis pertaining to clinics and house allergens

Chapter 3 Results

3.1 Descriptive statistics from the overall Respira cohorts

After the recruitment period, and inputting of all the data from the Respira project, a grand total of 2053 parent questionnaires were collected and inputted in a database from the whole project. This included 862 questionnaires with data on Maltese children, and 1191 questionnaires from parents of Sicilian children.

The following results will depict the information collected from the Maltese population of children who participated in the Respira project.

During the recruitment period, 1075 children were invited to participate in the study. 862 parents accepted to sign the consent form (as the children were under the legal age for autonomous consent) and fill in the parent questionnaire for their children, resulting in 80.2% giving their consent.

Out of these children, 142 were invited to attend clinics (parents were contacted through a telephone call). 14 parents refused to attend the clinics, resulting in a participation of 128 (90.1%) children and their families.

3.1.1 Demographics of the Maltese case and control groups

A total of 128 children were successfully recruited to undergo the local case control study. 66 children were cases (and therefore had two or more symptoms of uncontrolled asthma) and 62 were controls (had no asthmatic or atopic symptoms).

Table 3: Gender between cases and controls

			Group		
			Case	Control	Total
Child Sex	Male	Count	39	28	67
		Percentage	59.1%	45.2%	52.3%
	Female	Count	27	34	61
		Percentage	40.9%	54.8%	47.7%
Total		Count	66	62	128
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 2.487$, $p = 0.115$

52.3% (n=67) were males while 47.7% (n=61) were female. The proportions of male and female children did not vary significantly between the case and control groups ($p = 0.115$). The mean age was 12.65 years (SD 0.727). The mean weight was 50.237kg (SD 13.221) and the mean height was 151.028 cm (SD 18.932).

The proportions of children living in the North, Centre and South of Malta did not vary significantly between the case and control groups (p-value = 0.736).

Table 4: Distribution of cases and controls according to area

			Group		
			Cases	Control	Total
Malta Area	Centre	Count	23	26	49
		Percentage	34.8%	41.3%	38.0%
	South	Count	21	19	40
		Percentage	31.8%	30.2%	31.0%
	North	Count	22	18	40
		Percentage	33.3%	28.6%	31.0%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(2) = 0.614$, $p = 0.736$

3.1.2 Description of the case group

59.4% (n=39/66) were male, while 40.9% (n=27/66) were female. The mean age was 12.67 years (SD 0.664). The mean weight of the children in the case group was 52.52kg (SD 13.96) and the mean height 150.01cm (SD 21.01).

As wheezing in the past 12 months was a selection criterion for being in the case group, this was present in 97% (n=64/66) of respondents. Of note is the fact that 30.3% (n=20/66) reported wheezing in the previous 30 days to completing the questionnaire. 62.1% (n=41/66) had exercise induced wheeze in the past 12 months, while 62.1% (n=47/66) reported nocturnal cough in the absence of a cold, in the past 12 months.

78.1% (n=50/66) had received a diagnosis of asthma from their doctor in the past. Of these, only 26% (n=13/50) were on regular medications for asthma. There was a large variance in whether children actually took their rescue medication regularly as prescribed or not, which was reported by the parents in clinic. Therefore it was not possible to analyse with confidence the effects of inhaled therapy in this study.

97% of the cases (n = 64/66) had wheezing in the past 12 months. 30.3% wheezed in the previous 30 days (n=20/66). Exercise induced wheeze was also prevalent in this group at 62% (n=41/66).

71.2% (n=47/66) had a history of cough over the previous 12 months, while 23.8% of cases (n=15/66) were reported to have a chronic cough. 12.1% (n=8/66) had chronic phlegm production.

The frequency of having a history of rhinitis was 56.1% (n=37/66) in the case group. It seems that most of these reported symptoms in the previous 12 months, as 54.5% (36/66) claimed this.

3.1.3 Description of the control group

44.4% (n=28/63) were male, while 54.0% (n=34/63) were female. The mean age was 12.63 years (SD 0.794). The mean weight of the children in the case group was 47.762kg (SD 12.00) and the mean height 152.16cm (SD 16.50). As wheezing in the past 12 months, wheezing in the past 30 days, exercise-induced wheeze and nocturnal cough were exclusion criteria for being included in the control group, no members of the control group had a positive response to these questions. The same could be said for rhinitis, rhinoconjunctivitis and chronic phlegm production.

3.1.4 Perinatal information

The average birth weight of the children who participated in the study was 3300.94g (SD 647.866). Six (6) children had a low birth weight, defined as <2500g in the ICD-10[93] (10th revision of the International Statistical Classification of Diseases and Related Health Problems). Four (4) of these children were in the case group while only one (1) was in the control group. Although the proportion of underweight born children in the case group (7.5%) exceeds the corresponding proportion in the control group (2.5%), the difference between these two proportions is not statistically significant.

Table 5: Low birth weight cases versus controls

			Group		
			Case	Control	Total
Low Birth Weight	True	Count	4	1	5
		Percentage	7.5%	2.2%	5.1%
	False	Count	49	44	93
		Percentage	92.5%	97.8%	94.9%
Total		Count	53	45	98
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.425$, $p = 0.233$

Although the proportion of prematurely born children in the case group (13.6%) exceeded the corresponding proportion in the control group (11.1%), the difference between these two proportions was not statistically significant.

Table 6: Premature birth cases versus controls

			Group		
			Cases	Control	Total
Born Prematurely	Born Prematurely	Count	9	7	16
		Percentage	13.6%	11.1%	12.4%
	Born at Term	Count	57	56	113
		Percentage	86.4%	88.9%	87.6%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.189$, $p = 0.664$

Question: Was the child- breastfed?

Although the proportion of children who were not breastfed in the case group (50.0%) exceeded the corresponding proportion in the control group (41.7%), the difference between these two groups was not statistically significant.

In this question, children who had been breast-fed were compared to children who had never been breastfed. Although the proportion of children who were breastfed in the control group (58.3%) exceeded the corresponding proportion in the case group (50.0%), the difference between these two proportions was not statistically significant.

Table 7: Breastfeeding cases versus controls

			Group		
			Cases	Control	Total
Breast Fed	True	Count	33	35	68
		Percentage	50.0%	58.3%	54.0%
	False	Count	33	25	58
		Percentage	50.0%	41.7%	46.0%
Total		Count	66	60	126
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.879$, $p = 0.349$

3.1.5 Early life and current exposure to tobacco smoking.

Question: Did the mother smoke during pregnancy?

The first question which was asked regarding tobacco smoke, was whether the mother had smoked during her pregnancy.

Table 8: Mother smoking during pregnancy

			Group		
			Cases	Control	Total
Mother smoked during pregnancy	True	Count	6	3	9
		Percentage	9.1%	5.0%	7.1%
	False	Count	60	57	117
		Percentage	90.9%	95.0%	92.9%
Total		Count	66	60	126
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.793$, $p = 0.373$

Although the proportion of children whose mother smoked tobacco during her pregnancy in the case group (9.1%) exceeded the corresponding proportion in the control group (5.0%), the difference between these two proportions was not statistically significant (p-value = 0.373).

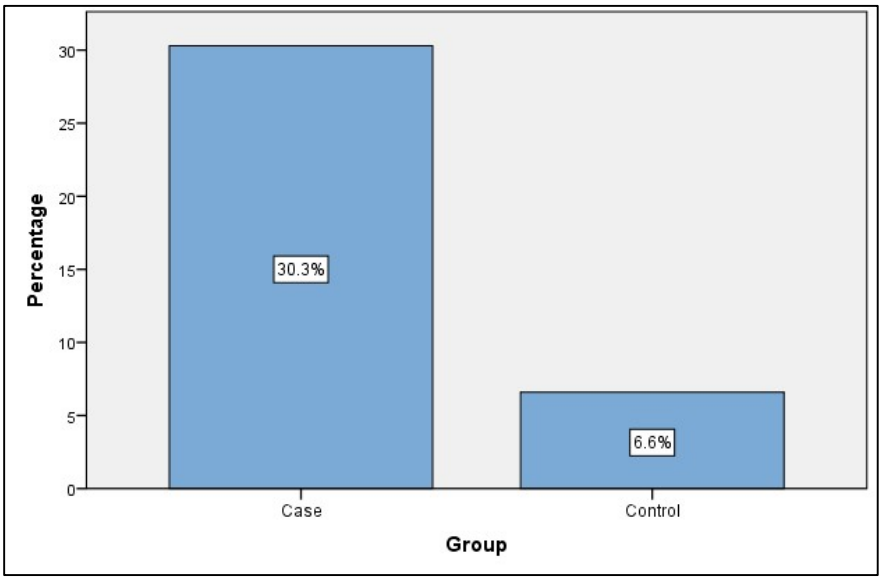
During the first year of life, has been your child exposed to tobacco smoking?

Table 9: SHS first year of life case versus control

			Group		
			Case	Control	Total
Child exposed SHS 1st year of life	True	Count	20	4	24
		Percentage	30.3%	6.6%	18.9%
	False	Count	46	57	103
		Percentage	69.7%	93.4%	81.1%
Total		Count	66	61	127
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 11.663$, p = 0.001

The proportion of children exposed to second hand smoking (SHS) in the first year of life in the case group (30.3%) exceeded the corresponding proportion in the control group (6.6%). The difference between these two proportions is statistically significant (p-value 0.001).



$X^2(1) = 11.663$, p = 0.001

Figure 1: SHS First year of life

Question: Is your child exposed to tobacco smoke in the dwelling.

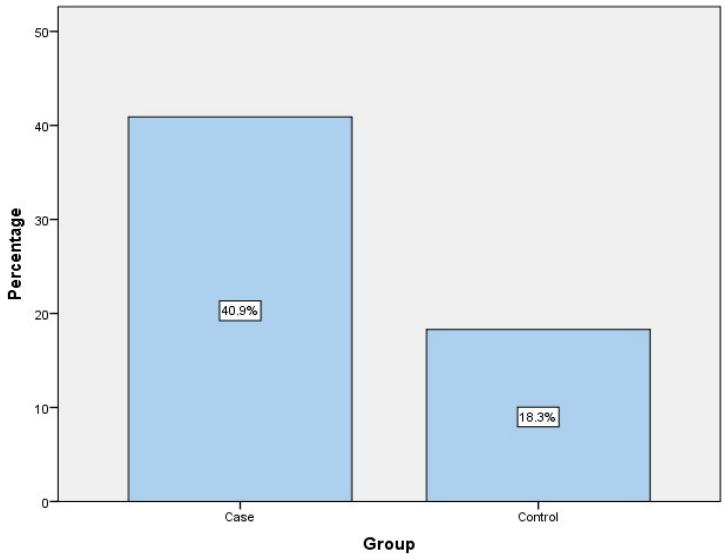
In this question, an answer which included "Yes" (answers 1-3), and therefore related to any exposure to SHS at home, were considered as "True" for SHS exposure, and "No" (answer 4.) was considered as "False" to SHS exposure.

Table 10: Current exposure to SHS cases versus controls

			Group		
			Case	Control	Total
Child exposed SHS 1st year of life	True	Count	27	11	38
		Percentage	40.9%	18.3%	30.2%
	False	Count	39	49	88
		Percentage	59.1%	81.7%	69.8%
Total		Count	66	66	60
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 7.605$, $p = 0.006$

The proportion of children who were currently exposed to secondhand smoking (SHS) in the case group (40.9%) exceeded the corresponding proportion in the control group (18.3%). The difference between these two proportions was statistically significant.



$X^2(1) = 7.605$, $p = 0.006$

Figure 2: Current exposure to SHS

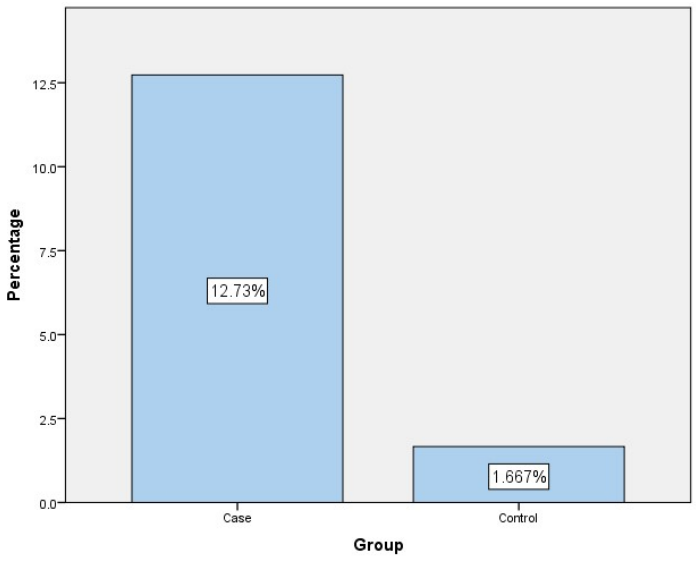
Question: During the first two years of life, did your child suffer from any respiratory infections, such as Bronchitis?

Table 11: Bronchitis in the first two years of life

			Group		
			Case	Control	Total
Bronchitis in first two years of life	True	Count	7	1	8
		Percentage	12.7 %	1.7%	7.0%
	False	Count	48	59	107
		Percentage	87.3%	98.63%	93.0%
Total		Count	55	55	60
		Percentage	100%	100.0%	100.0%

$X^2(1) = 5.424$, p = 0.027

The proportion of children who suffered from bronchitis in the first two years of life in the case group (17.7%) exceeded the corresponding proportion in the control group (1.7%). The difference between these two proportions was statistically significant (p-value 0.027).



$X^2(1) = 5.424$, p = 0.027

Figure 3: Bronchitis first year of life

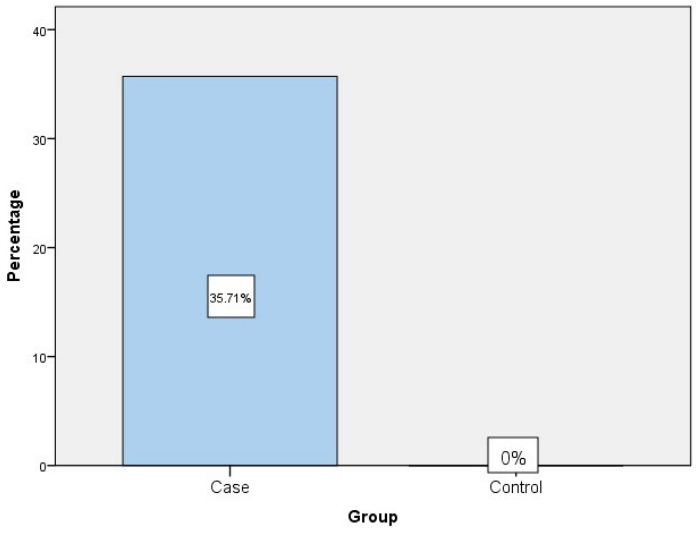
Question: During the first two years of life, did your child suffer from any infections, such as: Asthmatic Bronchitis?

Table 12: Asthmatic Bronchitis in the first two years of life

			Group		
			Case	Control	Total
Asthmatic Bronchitis in first two years of life	True	Count	20	0	20
		Percentage	35.7%	0.0%	17.5%
	False	Count	36	58	94
		Percentage	64.3%	100.0%	52.5%
Total		Count	56	56	58
		Percentage	100%	100.0%	100.0%

$X^2(1) = 25.122$, $p < 0.001$

The proportion of children who suffered from asthmatic bronchitis in two years of life in the case group (35.7%) exceeded the corresponding proportion in the control group (0.0%). The difference between these two proportions was statistically significant (p-value < 0.001).



$X^2(1) = 25.122$, $p < 0.001$

Figure 4: Asthmatic bronchitis first year of life.

The same could not be said for pneumonia in the first two years of life as there were no differences between the case and control group (p-value = 0.468).

As for bronchiolitis, there were more children in the case group (n=3) who had a history of bronchiolitis in the first two years of life, when compared to the control group (n=0), yet this was not statistically significant (p-value = 0.096).

3.1.6 Allergic conditions present in the family

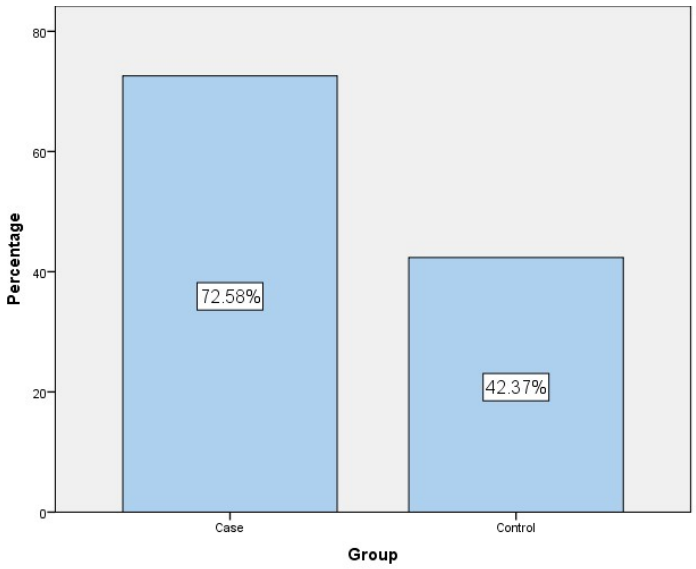
Question: Are there any allergic disorders in the family?

Table 13: Allergies in Family

			Group		
			Case	Control	Total
Allergies in Family	True	Count	45	25	70
		Percentage	72.6%	42.4%	57.9%
	False	Count	17	34	51
		Percentage	27.4%	57.6%	42.1%
Total		Count	62	62	59
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 11.314$, p = 0.001

The proportion of family members of children in the case group who had a history of allergies (72.6%) exceeded the corresponding proportion in the control group (42.4%). (p-value 0.001).



$X^2(1) = 11.314$, p = 0.001

Figure 5: Family History of Allergies

Question: Does the Father of the Child suffer from Asthma?

Table 13: Father suffers from asthma

			Group		
			Case	Control	Total
Father has Asthma	True	Count	14	16	16
		Percentage	21.2%	12.4%	12.4%
	False	Count	52	113	113
		Percentage	78.8%	87.6%	87.6%
Total		Count	62	66	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 9.652$, $p = 0.002$

The proportion of fathers of children in the case group who had a history of asthma (21.2%) exceeded the corresponding proportion in the control group (3.2%). (p-value 0.002).

Question: Does the Mother of the Child suffer from Asthma?

Table 15: Mother suffers from asthma

			Group		
			Case	Control	Total
Mother has Asthma	True	Count	13	3	16
		Percentage	19.7%	4.8%	12.4%
	False	Count	53	60	113
		Percentage	80.3%	95.2%	87.6%
Total		Count	62	66	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 6.617$, $p = 0.009$

The proportion of mothers of children in the case group who had a history of asthma (19.7%) exceeded the corresponding proportion in the control group (4.8%). (p-value 0.002).

Question: Does the Sibling of the Child suffer from Asthma?

Table 16: Sibling suffers from asthma

			Group		
			Case	Control	Total
Sibling has Asthma	True	Count	21	3	24
		Percentage	31.8%	4.8%	18.6%
	False	Count	45	60	105
		Percentage	68.2%	95.2%	81.4%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 15.582$, $p < 0.001$

The proportion of siblings of children in the case group who had a history of asthma (19.7%) exceeded the corresponding proportion in the control group (4.8%). (p-value 0.002).

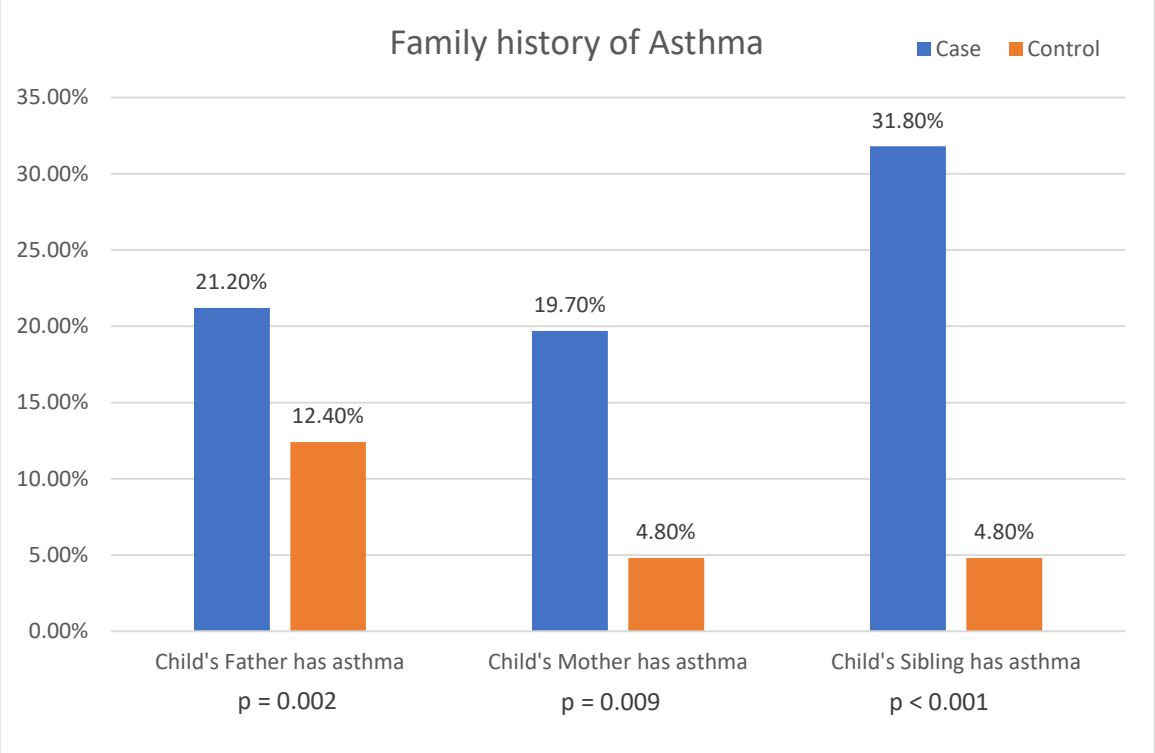


Figure 6: Family history of asthma, for cases and controls

Question: Does the Father of the Child suffer from Allergic nasal symptoms?

Table 16: Father suffers from allergic nasal symptoms

			Group		
			Case	Control	Total
Father has Allergic Nasal symptoms	True	Count	14	8	22
		Percentage	21.2%	12.7%	17.1%
	False	Count	52	55	107
		Percentage	78.8%	87.3%	82.9%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.652$, $p = 0.147$

Although the proportion of children's fathers who suffered from allergic nasal symptoms (21.2%) exceeded the corresponding proportion in the control group (12.7%), the difference between these two proportions was not statistically significant. (p-value 0.147).

Question: Does the Mother of the Child suffer from Allergic nasal symptoms?

Table 18: Mother suffers from allergic nasal symptoms

			Group		
			Case	Control	Total
Mother has allergic nasal symptoms	True	Count	19	6	25
		Percentage	28.8%	9.5%	19.4%
	False	Count	47	57	104
		Percentage	71.2%	90.5%	80.6%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 7.656$, $p = 0.005$

The proportion of mothers of children in the case group who had a history of allergic nasal symptoms (28.8%) exceeded the corresponding proportion in the control group (9.5%). ($p = 0.005$).

Question: Does the Sibling of the Child suffer from Allergic nasal symptoms?

Table 18: Sibling suffers from allergic nasal symptoms

			Group		
			Case	Control	Total
Sibling has allergic nasal symptoms	True	Count	14	5	19
		Percentage	21.2%	7.9%	14.7%
	False	Count	52	58	110
		Percentage	78.8%	92.1%	85.3%
Total		Count	66	66	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 4.523$, $p = 0.029$

The proportion of siblings of children in the case group who had a history of allergic nasal symptoms (21.2%) exceeded the corresponding proportion in the control group (7.9%). ($p = 0.029$).

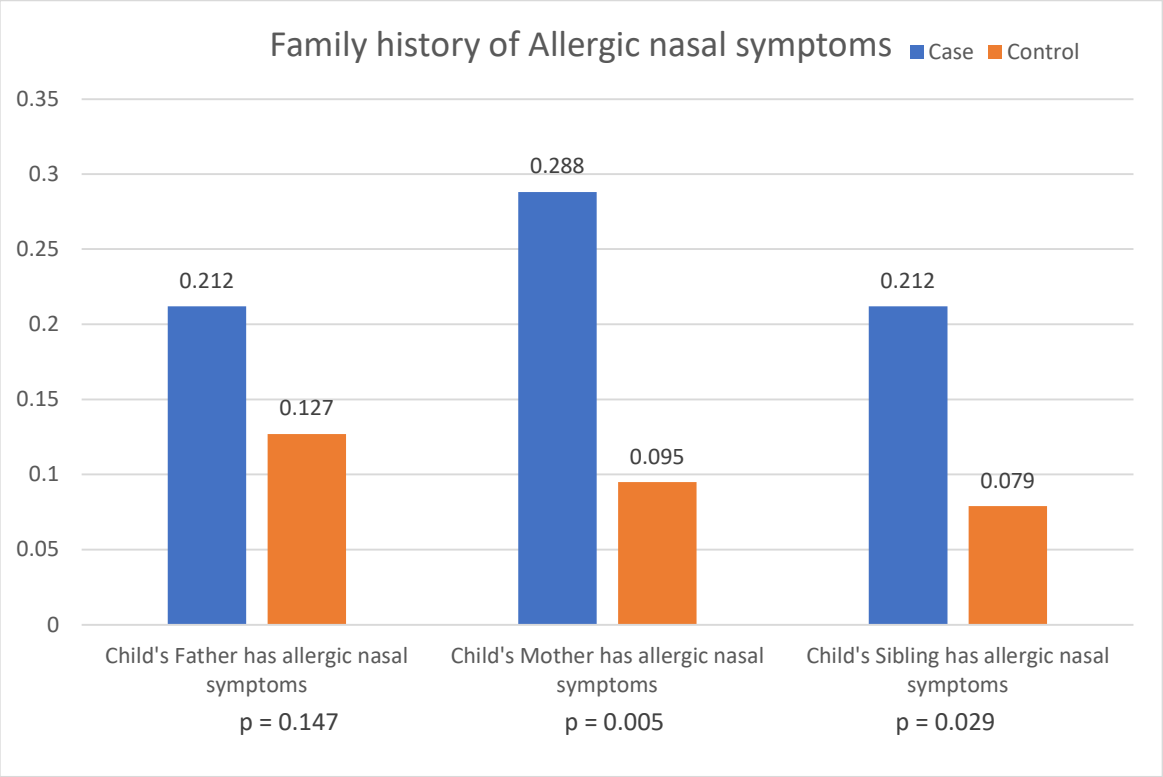


Figure 7: Family history of allergic nasal symptoms, cases versus controls

Question: Does the Father of the Child suffer from Eczema

Table 19: Father suffers from eczema

			Group		
			Case	Control	Total
Father has Eczema	True	Count	3	3	6
		Percentage	4.5%	4.8%	4.7%
	False	Count	63	60	123
		Percentage	95.5%	95.2%	95.3%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.003$, $p = 0.638$

There were no differences in the proportion of children's fathers who suffered from eczema (4.5%) compared to the corresponding proportion in the control group (4.8%) (p=0.638).

Question: Does the Mother of the Child suffer from Eczema

Table 20: Mother suffers from eczema

			Group		
			Case	Control	Total
Mother has Eczema	True	Count	11	3	14
		Percentage	16.7%	4.8%	10.9%
	False	Count	55	60	115
		Percentage	83.3%	95.2%	89.1%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 4.722$, $p = 0.027$

The proportion of mothers of children in the case group who had a history of eczema (16.7%) exceeded the corresponding proportion in the control group (4.8%). (p-value 0.027).

Question: Does the Sibling of the Child suffer from Eczema

Table 22: Sibling suffers from eczema

			Group		
			Case	Control	Total
Sibling has Eczema	True	Count	9	4	13
		Percentage	13.6%	6.3%	10.1%
	False	Count	57	59	116
		Percentage	86.4%	93.7%	89.9%
Total		Count	66	63	129
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.889$, $p = 0.140$

Although the proportion of children's siblings who suffered from eczema (13.6%) exceeded the corresponding proportion in the control group (6.3%), the difference between these two proportions was not statistically significant. (p-value=0.140).

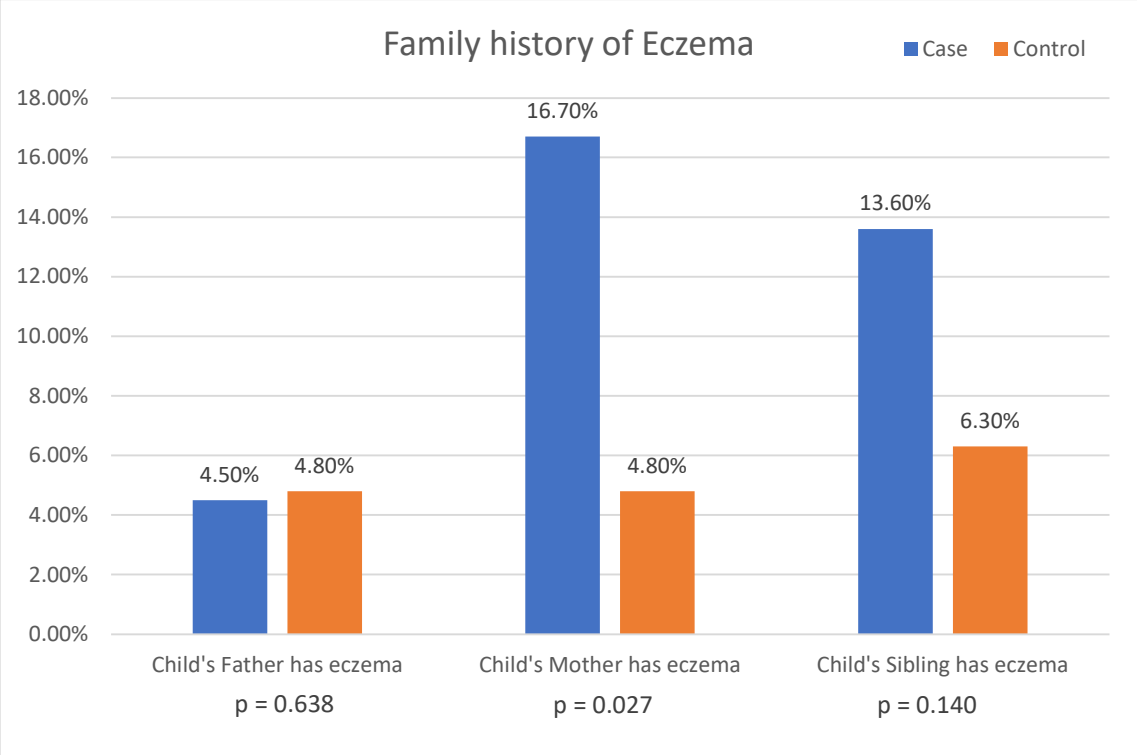


Figure 8: Family history of eczema, cases versus controls

3.1.6.1 Multinomial logistic regression for familial allergies

The various answers given in the questionnaires which focused on the allergic family history were analysed using a multinomial logistic regression. This was done to better understand whether the presence of a family member with a particular allergic condition acted as a predictor to the child to be a member of the case group.

The Pseudo R-square test used for the logistic regression being presented here, predicts that this model will explain total variation for 27.2% of this population (Nagelkerke = 0.272).

Pseudo R-Square

Cox and Snell	0.204
Nagelkerke	**0.272**
McFadden	0.165

Table 23: Logistic regression for familial allergies as predictor for cases

Likelihood Ratio Tests

		Likelihood Ratio Tests		
Effect	-2 Log Likelihood of Reduced Model	Chi-Square	df	P-value
Intercept	53.314	.000	0	.
Father has Asthma	58.544	5.229	1	**0.022**
Mother has Rhinitis	57.661	4.347	1	**0.037**
Sibling hasAsthma	62.147	8.833	1	**0.003**

Group		B	Std. Error	Wald	df	Odds Ratio	95% C.I.for Odds Ratio Lower Bound	Upper Bound
Case	Intercept	-0.622	0.236	6.940	1			
	FatherAsthma=Yes	1.675	0.821	4.165	1	**5.338**	1.069	26.670
	FatherAsthma=No	0	.	.	0	**.**	.	.
	MotherRhinitis=Yes	1.111	0.549	4.097	1	**3.038**	1.036	8.912
	MotherRhinitis=No	0	.	.	0	**.**	.	.
	SiblingAsthma=Yes	1.808	0.679	7.093	1	**6.100**	1.612	23.079
	SiblingAsthma=No	0	.	.	0	**.**	.	.

Since the dependent variable has two categories, then the logistic model assumed a binomial distribution and a logit link function. The Parsimonious model identified three significant predictors were siblings of children who had a history of asthma was the best predictor since it has the lowest p value (p = 0.003). This was followed by having a father who had a history of asthma (p = 0.022) and mother having a history of rhinitis (p = 0.037). This 3-predictor logistic regression model explained 27.2% of the total variation in the responses. The remaining 72.8% of the total variation would have been explained by other predictors not included in this analysis.

For a child, whose father had a history of asthma, the odds that the child is in the case group rather than the control group was **5.338 times** that of a child whose father did not have a history of asthma.

For a child, whose mother had a history of rhinitis, the odds that the child is in the case group rather than the control group was **3.038 times** that of a child whose mother did not have a history of rhinitis.

For a child, whose sibling had a history of asthma, the odds that the child was in the case group rather than the control group was **6.1 times** that of a child whose sibling did not have a history of asthma.

3.1.7 Pet Ownership

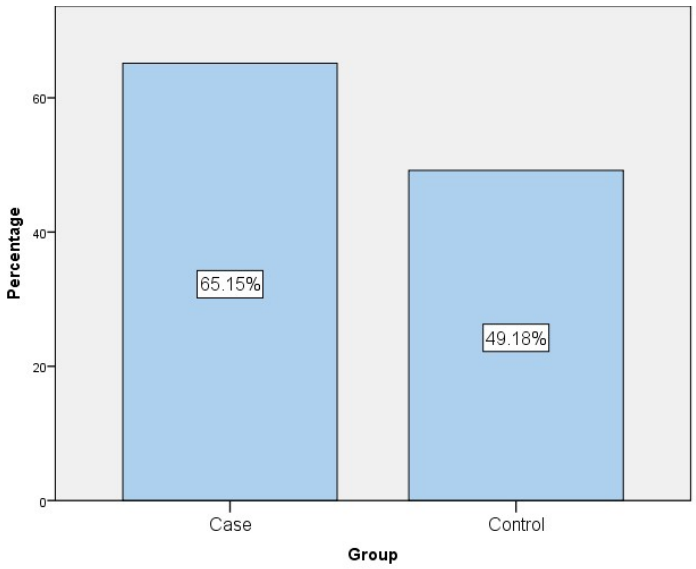
Question: Is there a pet at home?

Table 24: Pets at home

			Group		
			Case	Control	Total
Pets at home	True	Count	43	30	73
		Percentage	65.2%	49.2%	57.5%
	False	Count	23	31	54
		Percentage	34.8%	50.8%	42.5%
Total		Count	66	61	127
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.309$, $p = 0.050$

The proportion of pets present in the children's homes in the case group (65.2%) exceeded the corresponding proportion in the control group (49.2%). (p=0.05).



$X^2(1) = 3.309$, $p = 0.050$

Figure 9: A pet is present at home

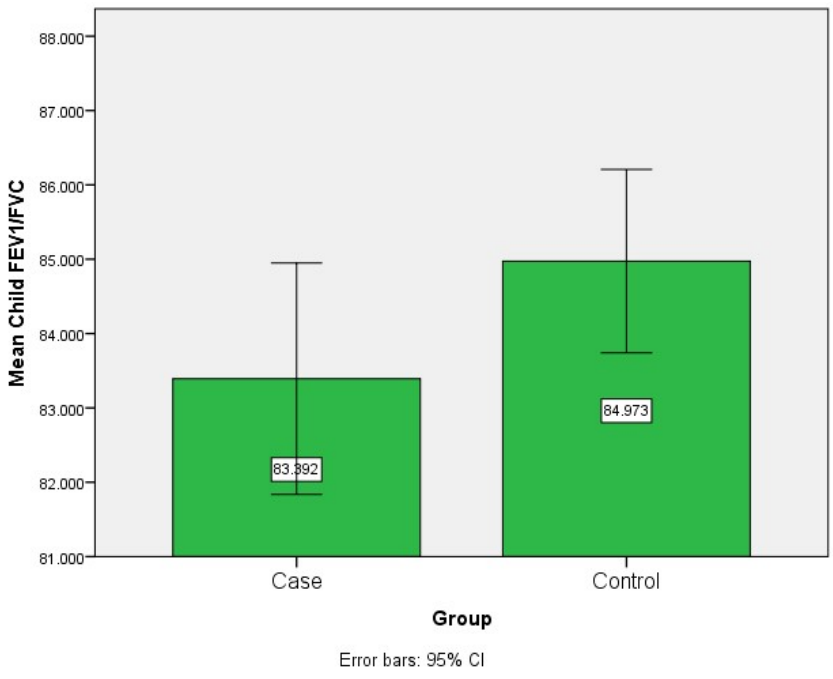
3.2 Comparisons between clinical measures

3.2.1 Child's FEV_1 Case group versus Control group

The mean FEV_1 in the case group was 109.631% predicted (SD 14.322) whilst that in the control group was 109.700% predicted (SD 13.472). There was no statistically significant difference between these two means (p=0.489).

3.2.2 Child's FEV_1/FVC Case group versus Control group

The mean FEV1/FVC ratio in the case group was 83.392% (SD 6.278) whilst that in the control group was 84.973% (SD 4.774). The FEV1/FVC ratio was slightly lower in the case group when compared to the control group, but this did not reach statistical significance (p=0.059).

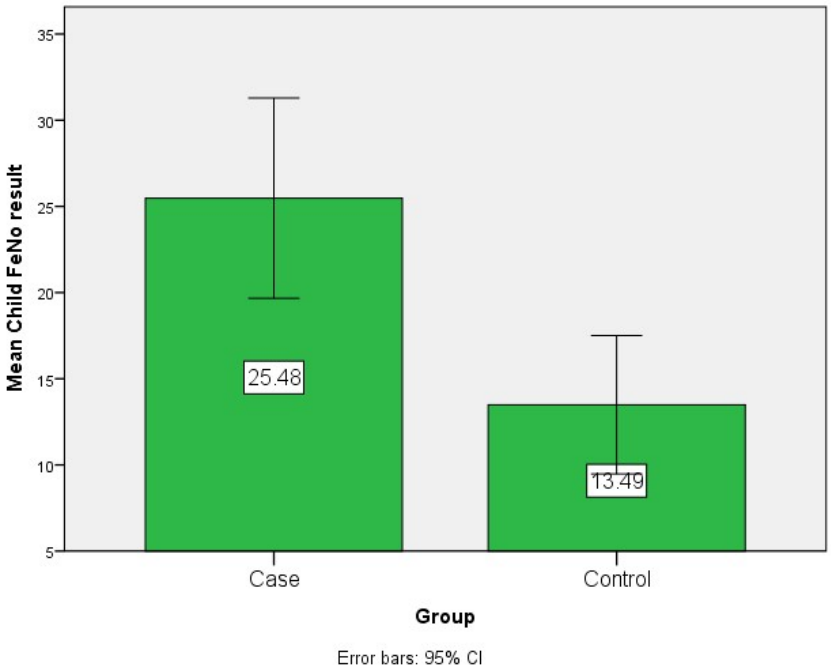


p = 0.059

Figure 10: Child's FEV_1/FVC

3.2.3 Child's FeNO Case group versus Control group

The mean FeNO in the case group was 25.48ppb (SD 23.435) was significantly higher when compared to the mean found in the control group was 13.49ppb (SD 15.133). This difference was strongly significant ($p < 0.0001$).

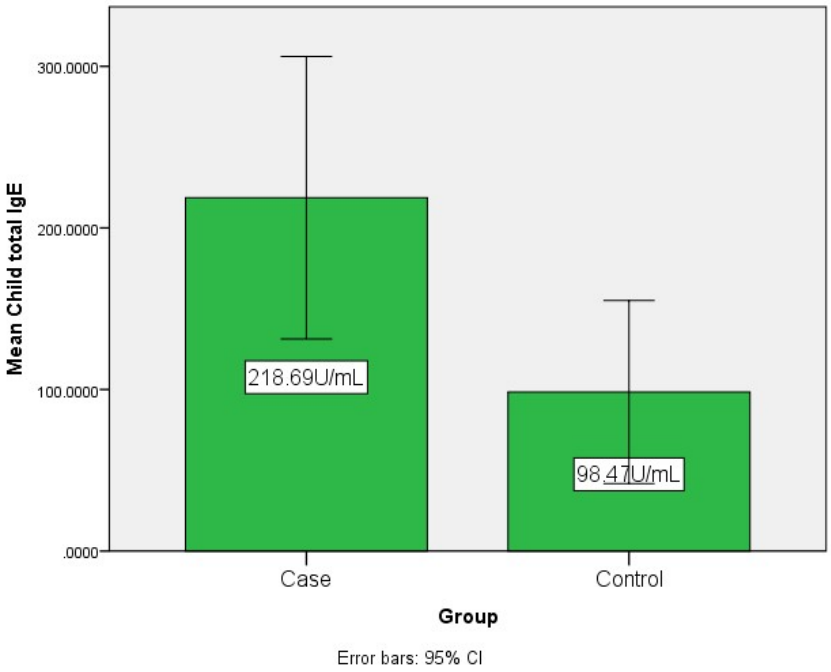


$p < 0.0001$

Figure 11: Child's FeNO

3.2.4 Child's Total IgE Case group versus Control group

The mean serum total IgE in the case group was 218.690U/mL (SD 338.410) was significantly higher when compared to the mean found in the control group was 98.470U/mL (SD 199.357). The p-value was 0.027.

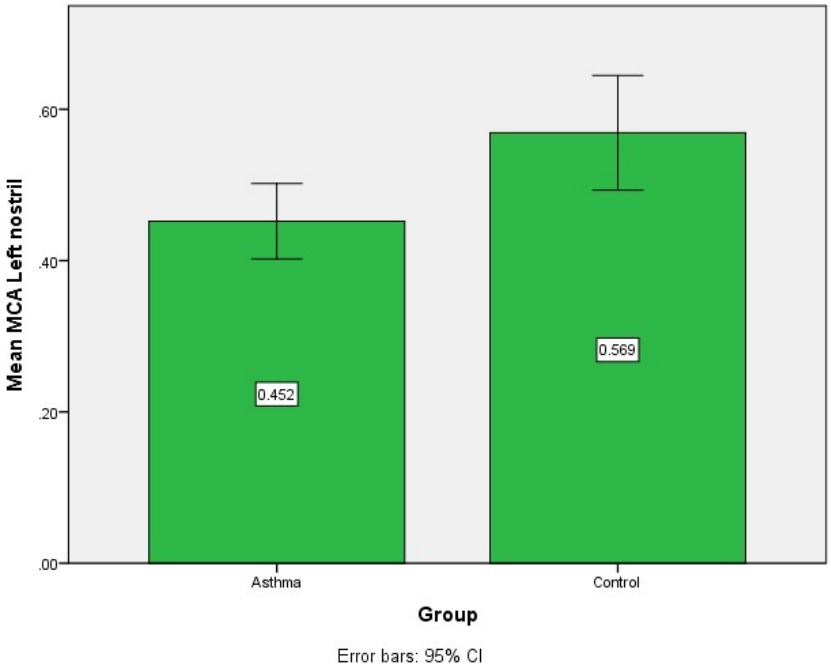


p = 0.027

Figure 12: Child's Total IgE

3.2.5 Child's minimal cross-sectional area (AMCA) per nostril Case group versus Control group

The AMCA as assessed by acoustic rhinometry for the children's left nostril for the case group and the control group was compared. The mean AMCA L for the case group was smaller, 0.452 cm^2 (SD 0.201) than the control group's mean AMCA L, which was 0.569cm^2 (SD 0.294). This difference was statistically significant (p = 0.011).



p = 0.011

Figure 13: Mean Child's AMCA L

The AMCA as assessed by acoustic rhinometry for the children's right nostril for the case group and the control group was compared. The mean AMCA R for the case group was marginally smaller, 0.532 cm^2 (SD 0.480) than that for the control group, which was 0.624 cm^2 (SD 0.586), and did not reach statistical significance (p = 0.742).

3.3 Allergen Analysis

3.3.1 Children's Skin Prick Test Results

All the children who attended clinics (n = 129) were invited to have Skin Prick Testing (SPT) to assess for atopy to specific aeroallergens. Eight (8) children refused to have skin prick testing.

Table 25: SPTs for all Children

Skin Prick Test	Number Positive (n)	Percentage Positive (%)
House Dust Mite	47	38.8
Cat dander	14	11.7
Olive tree pollen	14	11.7
Cockroach	10	8.3
Parietaria pollen	10	8.3
Alternaria	9	7.4
Dog dander	3	2.5
Grass pollen mix	1	0.8
Dog tooth grass	1	0.8
	11 Children had all tests negative	

*Total Children 129. 8 refused SPTs. Total children who had SPTs =121

The commonest atopy in the study population, which was detected through SPTs was that to House Dust Mite (HDM). 38.8% (n=47) of these children tested positive to this allergen. This was followed by olive tree pollen and cat dander, with 14 children (11.7%) testing positive to either of these allergens. Parietaria pollen and cockroach atopy followed with 10 children (8.3%) who had a positive SPT to either of these allergens. 9 children (7.4) were atopic to Alternaria. Dog dander atopy was relatively rare with only 3 children (2.5%). Only one child (0.8%) was atopic to the grass pollen mix allergen or dog tooth grass.

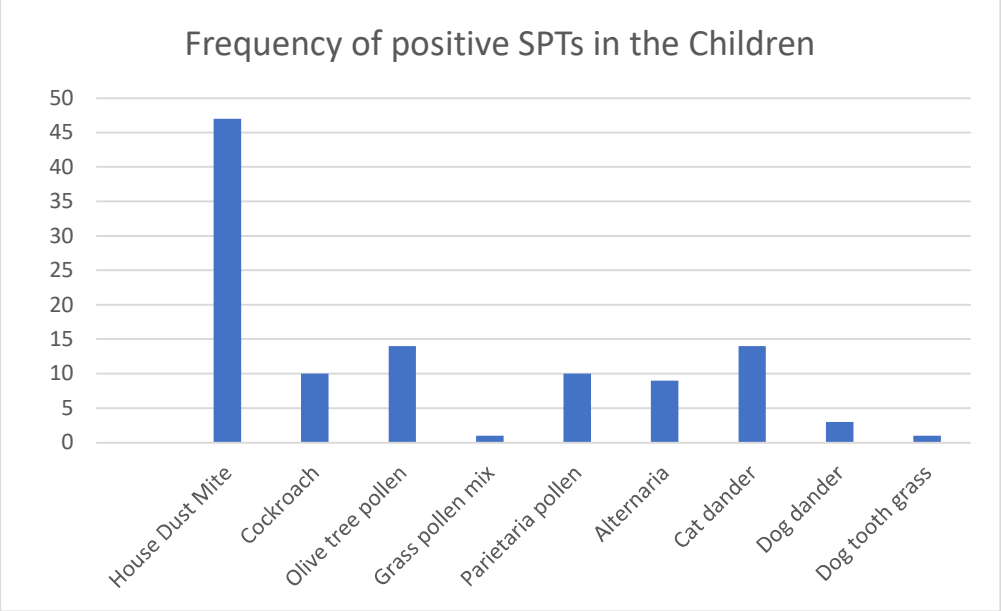


Figure 14: Frequency of positive SPTs in the child group

The SPT results for Case children and Control children were then compared (table 26). The only significant difference was seen in HDM SPT, where thirty-four (34) Case children (54%) had positive SPT to HDM when compared to thirteen (13) Control Children (20.6%), ($p < 0.001$). There was a general trend for Case children to be more likely to have positive SPTs to the other allergens, yet these were not statistically significant. The most notable was that for Parietaria pollen, where eight (8) children (12.7%) had a positive SPT to this weed, when compared to only 2 control children (3.4%, $p = 0.062$).

Table 26: Comparing SPTs for Case Children to Control Children

Skin Prick Test	Cases (n=63)		Controls (n = 58)		p value
	Number Positive (n)	Percentage Positive (%)	Number Positive (n)	Percentage Positive (%)	
House Dust Mite	34	54	13	20.6	**p < 0.001**
Cockroach	6	9.5	4	7	p = 0.437
Olive tree pollen	9	14.5	5	8.6	p = 0.237
Grass pollen mix	0	0	1	1.8	p = 0.475
Parietaria pollen	8	12.7	2	3.4	p = 0.062
Alternaria	7	11.1	2	3.4	p = 0.103
Cat dander	10	15.9	4	6.3	p = 0.103
Dog dander	2	3.2	1	1.7	p = 0.531
Dog tooth grass	0	0	1	1.7	p = 0.479

Total cases with SPTs = 63 (3 refused SPTs). Total controls with SPTs = 58 (5 refused SPTs) – test used Chi Squared

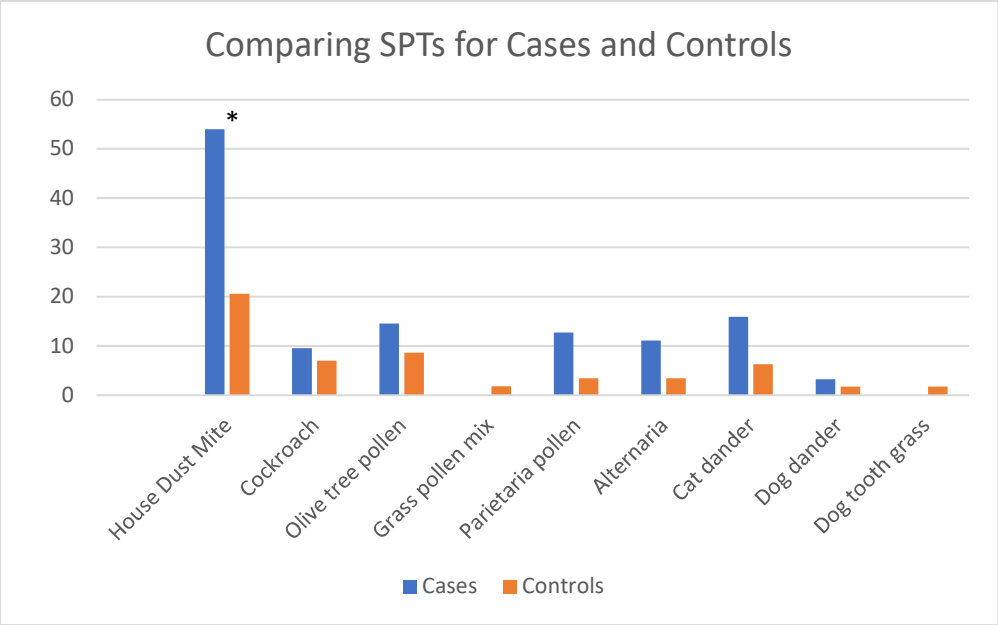


Figure 15: Comparing SPTs for Case Children to Control Children

3.3.2 Fathers' Skin Prick Test Results

A total of eight nine (89) fathers underwent the SPT panel. Similarly, to the children group, the most commonly positive SPT was that to HDM (n=31, 34.8%). On the other hand, grass and weed atopy seemed to be commoner in the fathers. A positive SPT to parietaria pollen was the second commonest atopy (n=18, 20.2%), and this was followed by the grass pollen mix (n = 18, 14.6%). Olive pollen atopy was the 4th commonest result (n=12, 13.6%). Mammal atopy was again relatively low, with

11.4% (n = 10) of fathers have a positive SPT to cat dander, and only 7 fathers (7.9%) were atopic to dog dander.

Table 27: Frequency of positive SPTs in the father group

Skin Prick Test	Number Positive (n)	Percentage Positive (%)
House Dust Mite	31	34.8
Cockroach	10	11.2
Olive tree pollen	12	13.6
Grass pollen mix	13	14.6
Parietaria pollen	18	20.2
Alternaria	8	9
Cat dander	10	11.4
Dog dander	7	7.9
Dog tooth grass	6	6.7

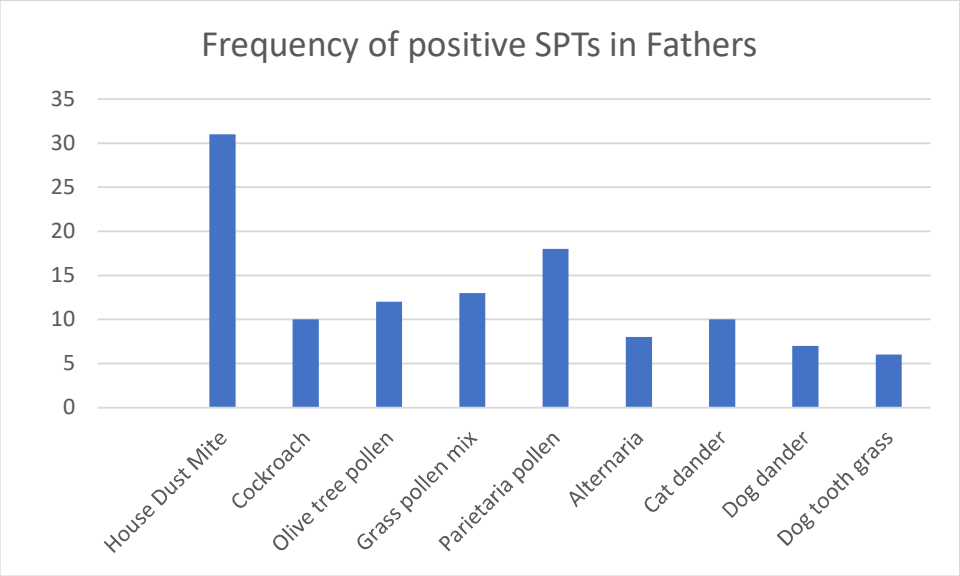


Figure 16: Frequency of positive SPTs in the father group

The SPT results for fathers of Case children and Control children were then compared (table 28). There were no significant differences between these two groups.

Table 28: Comparing SPTs for Fathers of Case Children to Fathers of Control Children

Skin Prick Test	Fathers of Cases (n=41)		Fathers of Controls (n = 48)		p value
	Number Positive (n)	Percentage Positive (%)	Number Positive (n)	Percentage Positive (%)	
House Dust Mite	13	31.7	18	28.6	p = 0.364
Cockroach	3	7.3	7	14.6	p = 0.230
Olive tree pollen	7	17.5	5	10.4	p = 0.257
Grass pollen mix	7	17.1	6	12.5	p = 0.378
Parietaria pollen	9	22.0	9	18.8	p = 0.455
Alternaria	4	9.8	4	8.3	p = 0.551
Cat dander	6	14.6	4	8.3	p = 0.285
Dog dander	3	7.3	4	8.3	p = 0.589
Dog tooth grass	1	2.4	5	10.4	p = 0.142

Chi Squared was used

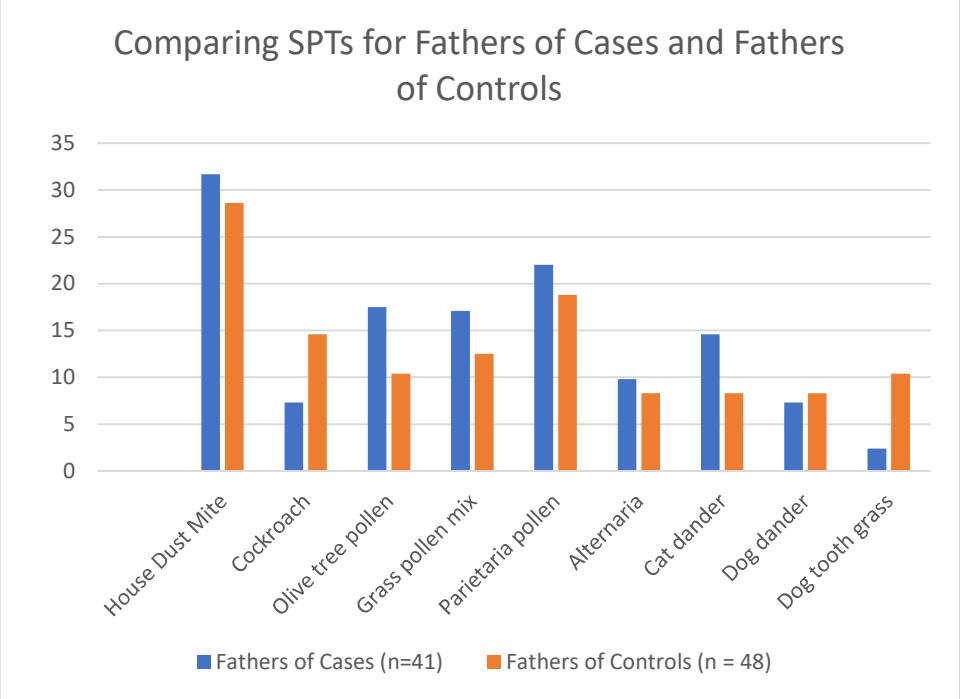


Figure 17: Comparing SPTs for Fathers of Case Children to Fathers of Control Children

3.3.3 Mothers' Skin Prick Test Results

House dust mite remained the most common positive SPT in the mothers, with 22.3% (n = 27) of this group exhibiting this, as was the second commonest allergen, parietaria at 15.7% (n = 19). The third most positive SPT was another pollen, olive tree, at 14.3% (n = 17). 9.9% of mothers (n = 12) had atopy to dogs and/or cats.

Table 29: Frequency of positive SPTs in the mother group

Skin Prick Test	Number Positive (n)	Percentage Positive (%)
House Dust Mite	27	22.3
Cockroach	11	9.2
Olive tree pollen	17	14.3
Grass pollen mix	13	10.7
Parietaria pollen	19	15.7
Alternaria	9	7.6
Cat dander	12	9.9
Dog dander	12	9.9
Dog tooth grass	5	4.1

*Total Mothers 129. 8 Mothers did not have SPTs (some pregnant). Total mothers with SPTs = 121.

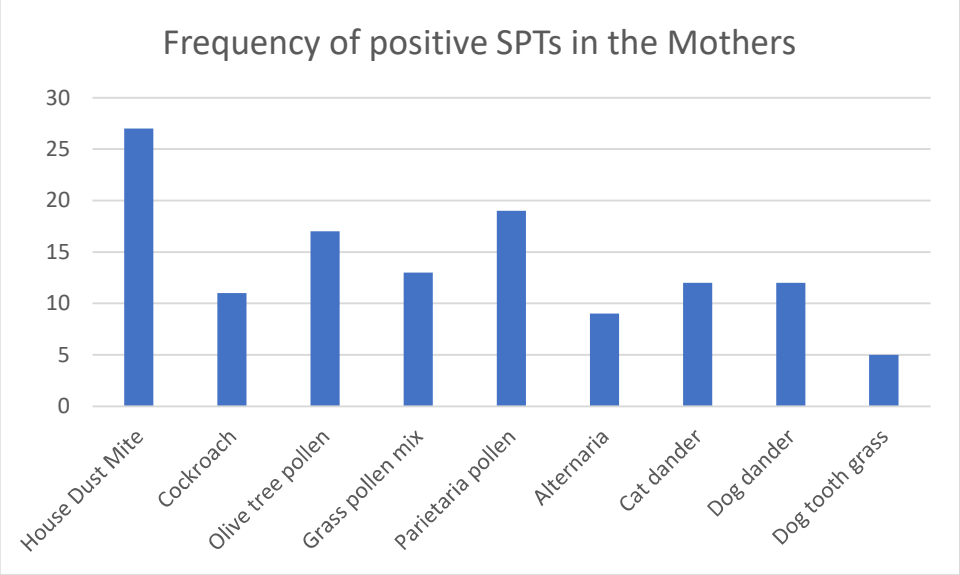


Figure 18: Frequency of positive SPTs in the mother group

In contrast to the father group, the mothers of case children did have a significantly higher tendency to have a positive SPT to HDM (32.3% vs 11.96%, p = 0.006). There were no other differences between these two groups for the rest of the tested allergens.

Table 30: Comparing SPTs for Mothers of Case Children to Mothers of Control Children

Skin Prick Test	Mothers of Cases (n=62)		Mothers of Controls (n = 59)		p value
	Number Positive (n)	Percentage Positive (%)	Number Positive (n)	Percentage Positive (%)	
House Dust Mite	20	32.3	7	11.9	**p = 0.006**
Cockroach	4	6.6	7	11.9	p = 0.245
Olive tree pollen	10	16.4	7	12.1	p = 0.341
Grass pollen mix	6	9.7	7	11.9	p = 0.462
Parietaria pollen	10	16.1	9	15.3	p = 0.547
Alternaria	6	10.0	3	5.2	p = 0.262
Cat dander	8	12.9	4	6.8	p = 0.206
Dog dander	8	12.9	4	6.8	p = 0.215
Dog tooth grass	3	4.8	2	3.4	p = 0.524

Chi-squared was used

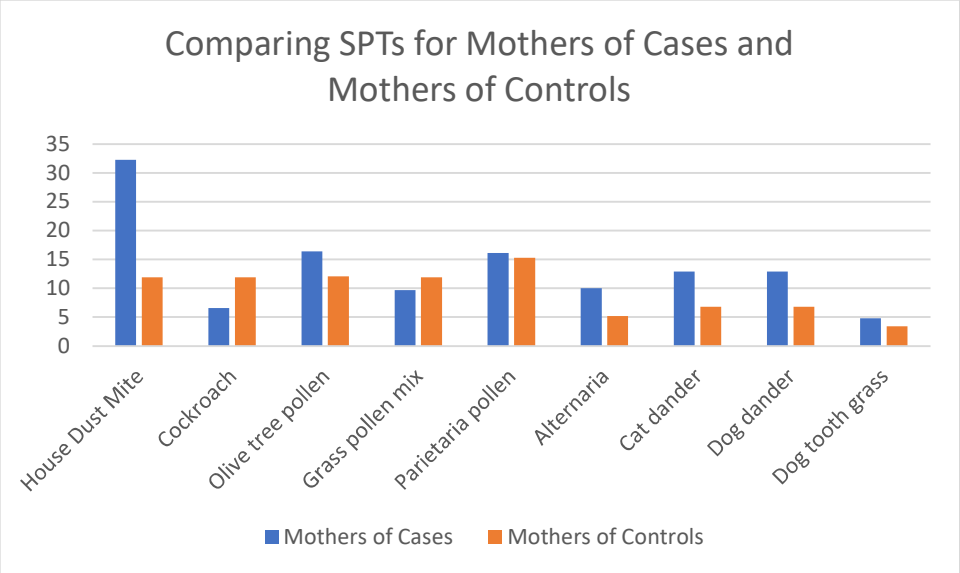


Figure 19: Comparing SPTs for Mothers of Case Children to Mothers of Control Children

3.4.1 Specific IgE Analysis for the children cohort

Out of the 129 children who participated in the study, 103 accepted to have their blood sampled for specific IgE analysis. The reason for this was mainly due to an element of needle phobia. The mean total IgE for all the children who underwent this analysis was 170.501U/mL (SD 302.436). The highest mean level for a specific allergen was measured for HDM (4.919U/mL, SD 12.578). This was five times higher than the allergen with the second highest mean, Parietaria, (0.951U/mL, SD 3.456). (N.B. please refer to section 2.1.7 for all ranges for serum total and specific IgE levels).

Table 31: Mean allergen serum specific IgE for all children

	Mean (U/mL)
Child's Total IgE	170.501
Child Specific Olive IgE	0.471
Child Specific Goldenrod IgE	0.214
Child Specific Parietaria IgE	0.951
Child Specific Cat IgE	0.646
Child Specific Dog IgE	0.298
Child Specific Cladosporidium IgE	0.196
Child Specific Alternaria IgE	0.233
Child Specific HDM IgE	4.919

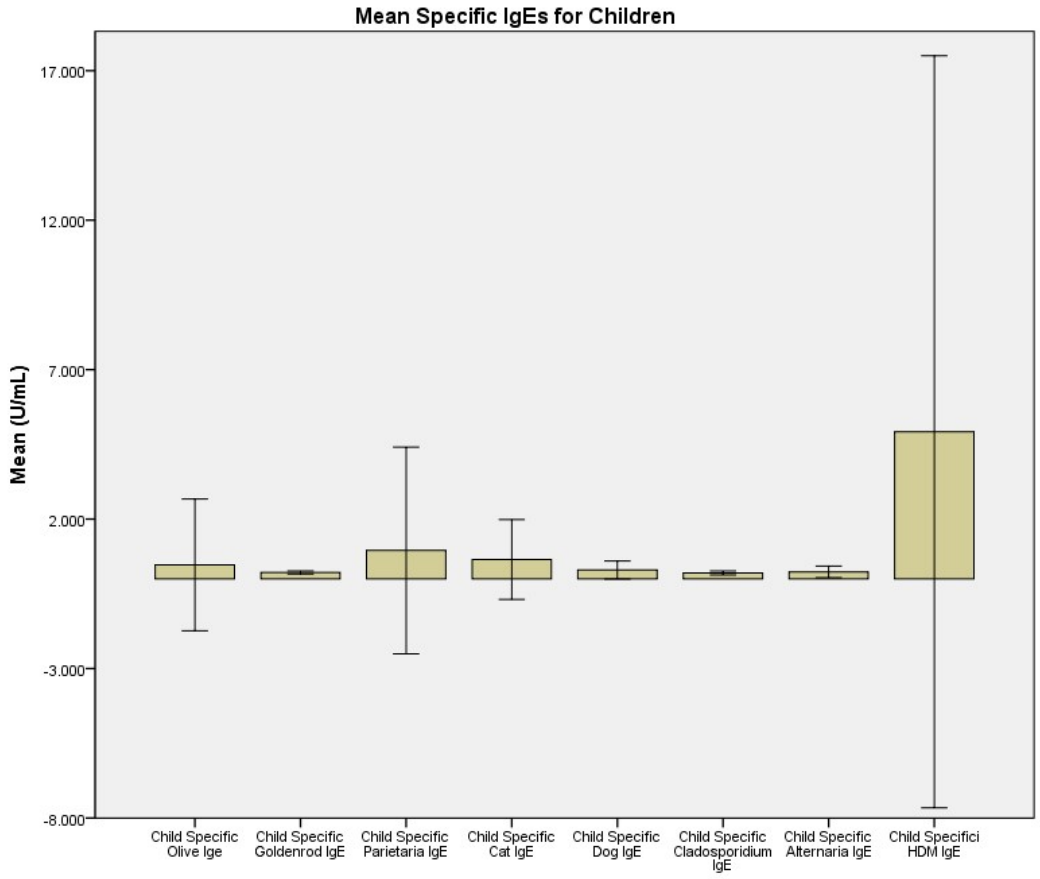


Figure 20: Mean Specific IgEs for Children

The differences between the specific IgE levels for Case Children and Control Children were then analysed (Table 32).

Table 32: Differences in Mean specific IgE levels for Case Children and Control Children

		N	Mean (U/mL)	SD	SE	95% CI Lower Bound	95% CI Upper Bound	P Value
Child Total IgE	Case	63	230.35	356.38	44.90	140.60	320.11	**p = 0.025**
	Control	51	96.55	197.82	27.70	40.92	152.20	
Child Specific Olive IgE	Case	56	0.27	0.21	0.03	0.21	0.32	p = 0.943
	Control	48	0.71	3.22	0.47	0.22	1.65	
Child Specific Goldenrod IgE	Case	56	0.22	0.05	0.01	0.21	0.23	p = 0.796
	Control	47	0.21	0.05	0.01	0.19	0.23	
Child Specific Parietaria IgE	Case	56	1.52	4.62	0.62	0.28	2.76	p = 0.181
	Control	47	0.27	0.32	0.05	0.18	0.36	

Child Specific Cat IgE	Case	56	0.83	1.73	0.23	0.37	1.29	p = 0.432
	Control	47	0.43	0.54	0.18	0.27	0.59	
Child Specific Dog IgE	Case	56	0.30	0.37	0.05	0.21	0.39	p = 0.811
	Control	47	0.30	0.24	0.04	0.23	0.37	
Child Specific Cladosporidium IgE	Case	56	0.19	0.04	0.01	0.19	0.20	p = 0.592
	Control	47	0.20	0.09	0.01	0.17	0.23	
Child Specific Alternaria IgE	Case	56	0.27	0.26	0.03	0.20	0.33	p = 0.194
	Control	47	0.19	0.04	0.01	0.18	0.21	
Child Specific HDM IgE	Case	56	7.77	14.99	2.00	3.75	11.78	**p = 0.001**
	Control	47	1.53	7.78	1.13	0.75	3.81	

Mann-Whitney test was used.

As mentioned earlier, the mean total serum IgE level was significantly higher (table 32) in the case children (230.36U/mL, SD 356.38) when compared to the control group (96.56, SD 197.83) (p = 0.025). The only allergen for which the mean specific IgE was significantly higher in the case group was that for HDM (7.77U/mL vs 1.53U/mL, p = 0.001). Mean specific serum IgE in the case group for Parietaria (1.52U/mL SD 4.62) and Alternaria (0.27U/mL SD 0.19) were higher than the control group (0.27 U/mL SD 4.62 vs 0.19U/mL SD 0.04 respectively), yet this were not significantly so (p = 0.181 and p = 0.194 respectively).

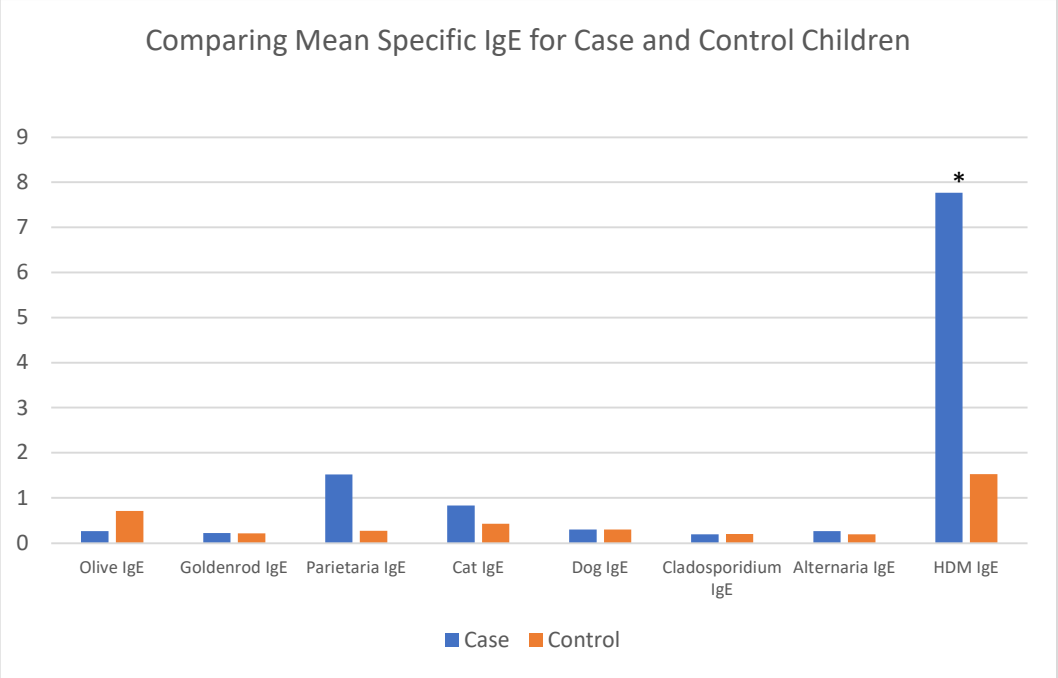


Figure 21: Differences in Mean specific IgE levels for Case Children and Control Children

3.4.2 Specific IgE Analysis for the father cohort

Ninety one (91/91 who attended, 100%) fathers who attended the clinical phase of the study, agreed to have blood letting. Sevently one (71) of these were randomly chosen for their blood testing for specific IgE levels (due to funding limitations). The highest mean specific IgE levels in all the fathers who participated in the study was that for HDM (2.027U/mL SD 4.565). This was followed by specific IgEs to two pollens, parietaria (1.11U/mL SD 3.16) and olive (0.40U/mL SD 0.99).

Table 33: Mean allergen serum specific IgE for all fathers

	Mean (U/mL)	SD
Father Total IgE	92.335	165.814
Father Specific Olive IgE	0.397	0.993
Father Specific Goldenrod IgE	0.220	0.056
Father Specific Parietaria IgE	1.105	3.164
Father Specific Cat IgE	0.358	0.661
Father Specific Dog IgE	0.204	0.066
Father Specific Cladosporidium IgE	0.199	0.071
Father Specific Alternaria IgE	0.200	0.492
Father Specific HDM IgE	2.027	4.565

Figure 22: Mean Specific IgEs for Fathers

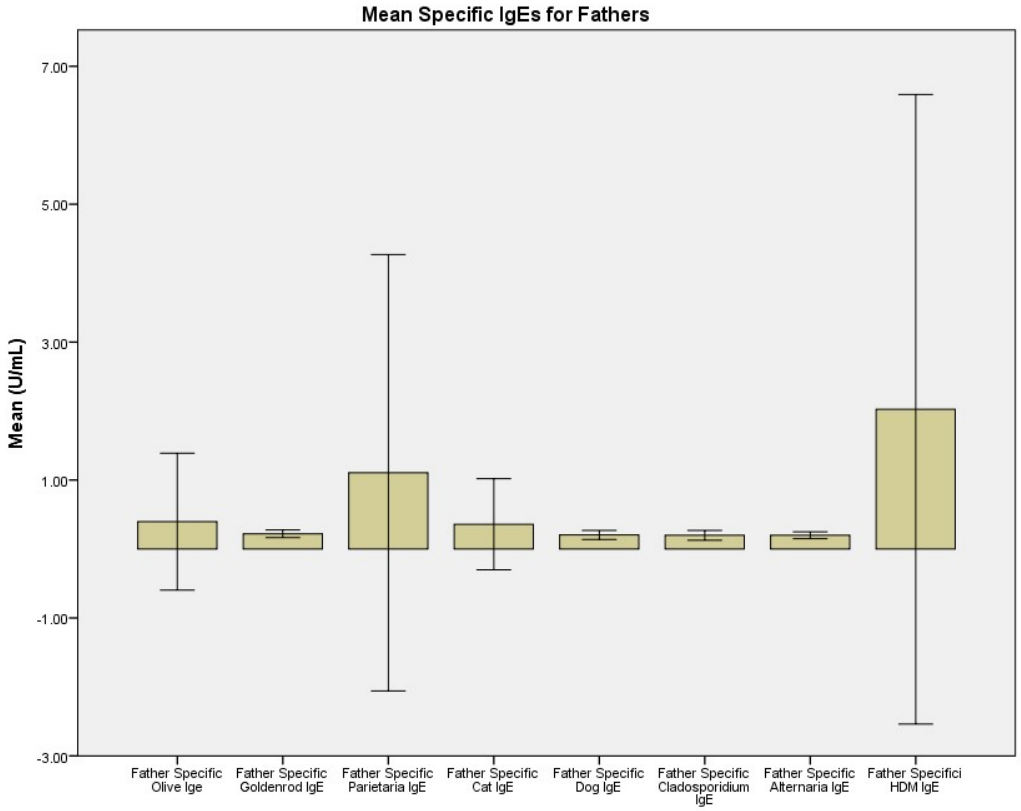


Table 34: Differences in Mean specific IgE levels for Fathers of Case Children and Control Children

			Mean		SE	95% CI		P Value
		N	(U/mL)	SD		Lower Bound	Upper Bound	
Father's Total IgE	Case	44	101.530	184.781	27.86	45.35	157.71	p = 0.965
	Control	47	83.728	147.376	21.50	40.46	127.00	
Father Specific	Case	39	0.483	1.268	0.20	0.07	0.89	**p = 0.018**
Olive IgE	Control	32	0.291	0.484	0.09	0.12	0.47	
Father Specific	Case	39	0.229	0.067	0.01	0.21	0.25	p = 0.351
Goldenrod IgE	Control	32	0.210	0.039	0.01	0.20	0.22	
Father Specific	Case	39	1.210	3.508	0.56	.0726	2.35	**p = 0.040**
Parietaria IgE	Control	32	0.978	2.737	0.48	0.01	1.96	
Father Specific	Case	39	0.468	0.879	0.14	0.18	0.75	p = 0.204
Cat IgE	Control	32	0.224	0.066	0.01	0.20	0.25	
Father Specific	Case	39	0.208	0.082	0.01	0.18	0.23	p = 0.916
Dog IgE	Control	32	0.199	0.039	0.01	0.19	0.21	
Father Specific	Case	39	0.195	0.040	0.01	0.18	0.21	p = 0.430
Cladosporidium IgE	Control	32	0.203	0.097	0.02	0.17	0.24	
Father Specific	Case	39	0.199	0.054	0.01	0.18	0.27	p = 0.963
Alternaria IgE	Control	32	0.200	0.043	0.01	0.18	0.22	
Father Specific	Case	39	1.9672	4.157	0.67	0.62	3.31	p = 0.954
HDM IgE	Control	32	2.0991	5.085	0.90	0.27	3.93	

Mann-Whitney test was used.

When the mean specific IgE levels for fathers of the Case children was compared to those of the Control children, two allergens stood out – Olive and Parietaria. The mean serum IgE levels for olive pollen in the Case fathers (0.483U/mL SD 1.267) was higher than the mean for Control fathers (0.292U/mL SD 0.484) (p = 0.018). Mean parietaria IgE levels in Case fathers was 1.210U/mL, SD 3.508 which was significantly higher than the mean for the Control fathers (0.978U/mL SD 2.737) (p = 0.040).

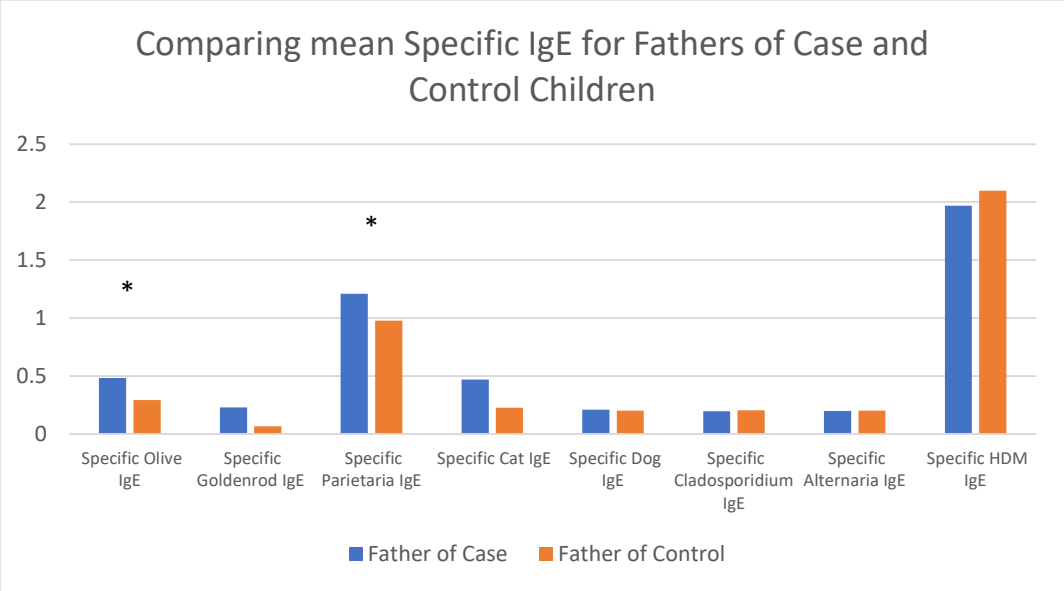


Figure 23: Differences in Mean specific IgE levels for Fathers of Case Children and Control Children

3.4.3 Specific IgE Analysis for the mother cohort

One hundred and sixteen (116) mothers who attended the clinical phase of the study, agreed to have blood letting. Eighty nine (89) of these had were randomly chosen for their blood tested for specific IgE levels (due to funding limitations). The highest mean specific IgE level for the mother who participated in this study was HDM (0.926U/mL SD 2.712), followed closely by parietaria (0.918U/mL SD 2.870). The third highest mean specific IgE level was olive at 0.323U/mL SD 0.471.

Table 35: Mean allergen serum specific IgE for all mothers

	Mean (U/mL)	**SD**
Mother Total IgE	92.267	185.087
Mother Specific Olive IgE	0.323	0.471
Mother Specific Goldenrod IgE	0.220	0.106
Mother Specific Parietaria IgE	0.918	2.870
Mother Specific Cat IgE	0.345	0.950
Mother Specific Dog IgE	0.266	0.569
Mother Specific Cladosporidium IgE	0.189	0.189
Mother Specific Alternaria IgE	0.211	0.211
Mother Specific HDM IgE	0.926	2.712

Figure 24: Mean Specific IgEs for Mothers

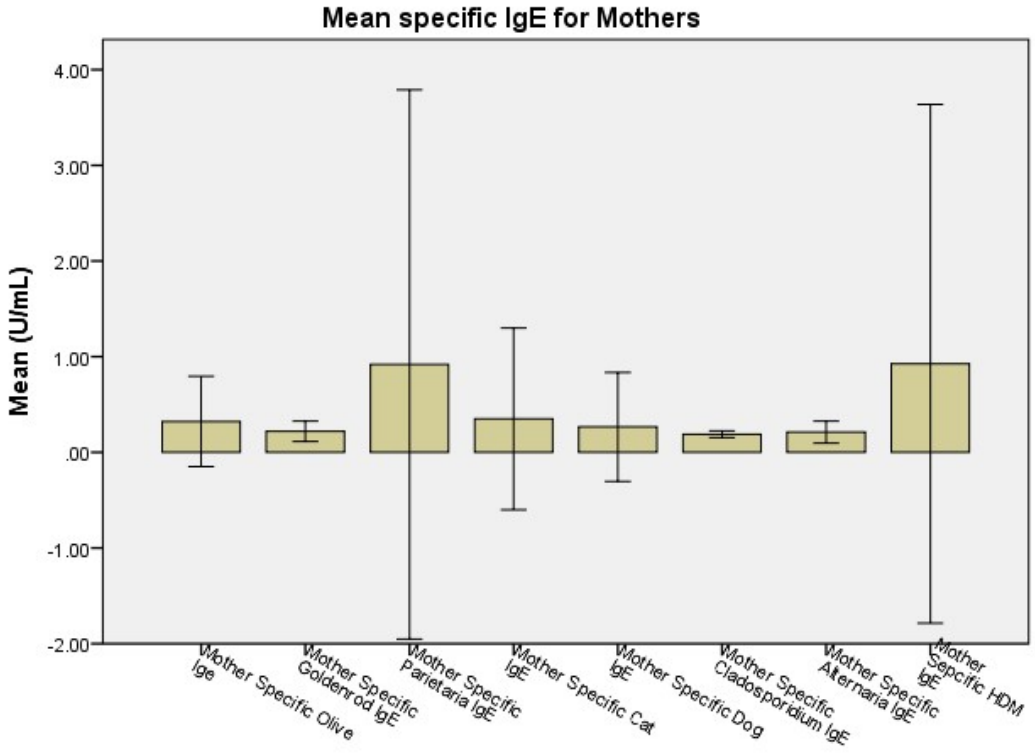


Table 36: Differences in Mean specific IgE levels for Mothers of Case Children and Control Children

		N	Mean (U/mL)	SD	SE	95% CI Lower Bound	95% CI Upper Bound	P Value
Mother's Total IgE	Case	60	95.670	181.986	23.49	48.658	142.682	p = 0.103
	Control	56	88.621	189.934	25.38	37.757	139.486	
Mother Specific Olive IgE	Case	49	0.337	0.464	0.07	0.20	0.47	**p = 0.014**
	Control	40	0.305	0.485	0.077	0.15	0.46	
Mother Specific Goldenrod IgE	Case	49	0.234	0.136	0.02	0.19	0.27	p = 0.088
	Control	40	0.203	0.043	0.07	0.19	0.22	
Mother Specific Parietaria IgE	Case	49	1.112	3.539	0.51	0.10	2.13	p = 0.173
	Control	40	0.681	1.751	0.28	0.12	1.24	
Mother Specific Cat IgE	Case	49	0.461	1.275	0.18	0.10	0.83	p = 0.091
	Control	40	0.214	0.048	0.01	0.20	0.23	
Mother Specific Dog IgE	Case	49	0.322	0.765	0.11	0.10	0.54	**p = 0.031**
	Control	40	0.197	0.041	0.01	0.18	0.21	
Mother Specific Cladosporidium IgE	Case	49	0.195	0.033	0.01	0.19	0.20	**p = 0.050**
	Control	40	0.181	0.035	0.01	0.17	0.19	
Mother Specific Alternaria IgE	Case	49	0.201	0.041	0.01	0.19	0.21	p = 0.171
	Control	40	0.223	0.166	0.03	0.17	0.28	
Mother Specific HDM IgE	Case	49	1.117	2.847	0.41	0.29	1.93	**p = 0.021**
	Control	40	0.698	2.554	0.40	0.12	1.51	

Mann-Whitney test was used.

When comparing mothers of Case and Control children for allergen specific IgE, the most significantly higher mean was Olive IgE (0.3371U/mL vs 0.305U/mL, p = 0.014). HDM specific IgE was much higher in mothers of Cases (1.111U/mL SD 2.847) when compared to those of Controls (0.698U/mL SD 2.554) (p = 0.021).

Mothers of Cases also tended to have higher serum Dog IgE (0.322U/mL SD 0.765) and Cladosporidium IgE (0.195U/mL SD 0.033) when compared to the mothers of their control counterparts (0.196U/mL SD 0.041 and 0.181U/mL SD 0.035 respectively) ($p = 0.031$, $p = 0.05$).

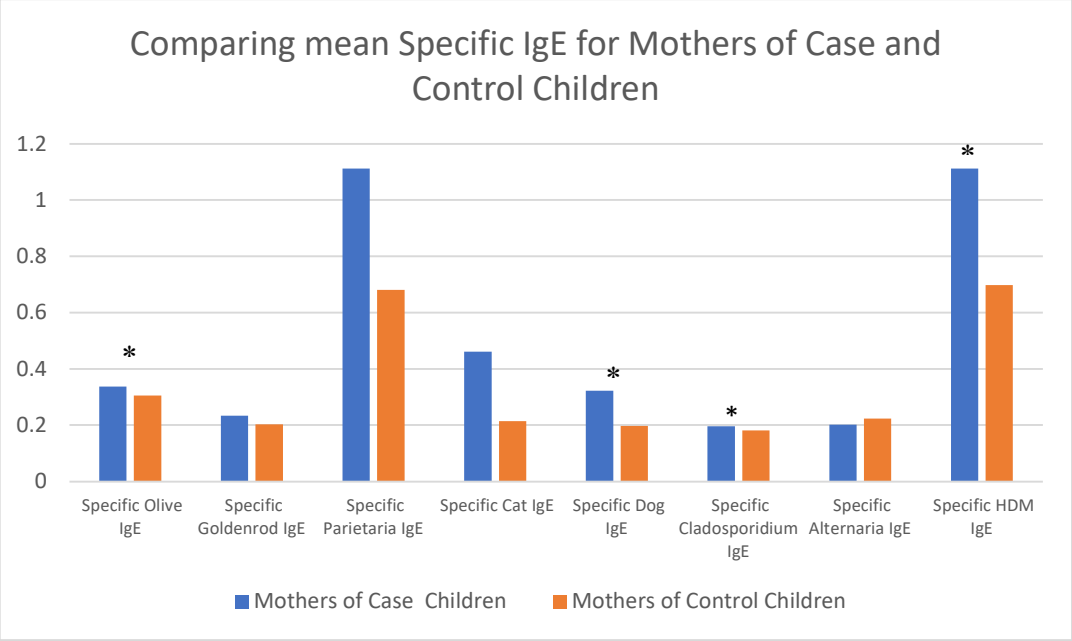


Figure 25: Differences in Mean specific IgE levels for Mothers of Case Children and Control Children

3.4.4 Summary of Differences in Specific IgE findings between groups

In all the groups (children, fathers and mothers), the tested aeroallergens specific IgE which had the highest concentration in the participating subjects serum was specific HDM IgE, followed by parietaria specific IgE. This showed that there was prior exposure by subjects to HDM with a secondary rise in serum specific IgE. Children had significantly higher specific HDM IgE than both fathers and mothers ($p < 0.001$). This result had to be taken in the context of children being selected through a case control process, while the parents were not. In fact, case children had significantly higher serum specific HDM IgE when compared to controls ($p = 0.001$). This would

have increased the mean total IgE levels in this group, and also the mean specific HDM IgE, when compared to the parents [only 16 (12.4%) fathers and 16 (12.4%) mothers had doctor diagnosed asthma in the whole parent cohort].

An interesting observation was the significantly higher specific HDM IgE levels seen in the case of mothers of case children when compared to controls. This was not the case between the specific HDM IgE levels of fathers of case and control children.

The two other aeroallergens to which subjects in this cohort consistently had higher specific IgE concentrations when compared to the other aeorallergens tested for, were Parietaria and Olive tree pollen. These are both anemophilous species, meaning that they are wind pollinators. It was also interesting to note that both fathers and mothers of case children had significantly higher specific Olive IgE levels ($p = 0.018$ and $p = 0.014$ respectively). The levels of these pollens in the environment would depend on the season when these plants flower, and specific IgE levels could vary seasonally. This was explored in the next sections.

3.4.5 Seasonal Changes in IgE levels

The previous section described specific IgE levels for various aeroallergens in both the children and the parent group. It illustrated differences between children case and control groups, and doctor-diagnosed asthmatic parents to non-asthmatic ones.

The study was divided into three different phases, to investigate any impact of seasons on the behaviour of allergic conditions. The first phase was carried out in

spring, the second in autumn and the last in winter. As the Respira Project, was also aimed to study the effect of indoor air quality of school buildings on the respiratory health of children, the summer months were omitted as these were scholastic holidays in Malta, and children would not have been in the schools at that time.

3.4.5.1 Changes in mean serum Total and Specific IgE by season in children cohort

The mean total serum IgE levels were compared for the three seasons into which the study was divided. The results were described in table 37 and Figure 26.

Table 37: Differences in mean Total IgE levels for all children according to season

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Child's Total IgE	Spring	45	86.8511	212.56	31.69	22.9910	150.7112	**0.001**
	Autumn	37	235.5189	329.02	54.09	125.8197	345.2181	
	Winter	32	212.9562	355.68	62.88	84.7191	341.1934	

$p < 0.001$ when comparing spring to autumn (Kruskal-Wallis)

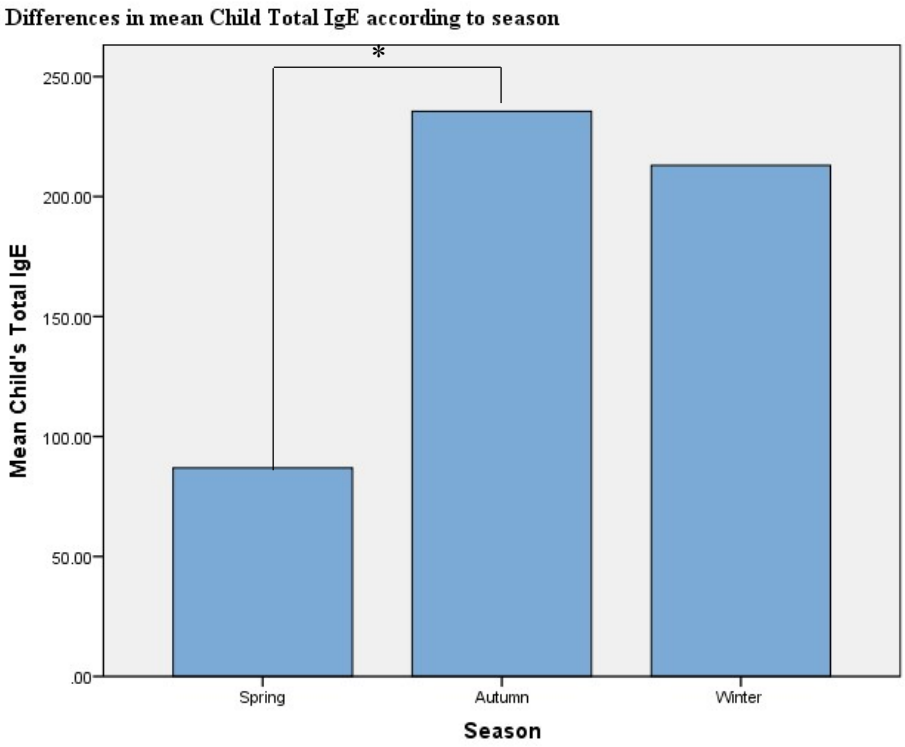


Figure 26: Differences in mean Child Total IgE according to season

Total serum IgE levels in children were lowest in spring and highest in autumn ($p = 0.001$). The children's winter total IgE levels were also high when compared to the levels expressed in spring, though this difference did not reach statistical significance ($p = 0.064$).

The variance in total IgE levels was then analysed separately for the children case and control groups (table 38 and figures 27 and 28).

Table 38: Differences in mean Total IgE levels for case and control children according to season

						95% CI		P
		N	Mean	SD	SE	Lower Bound	Upper Bound	value
Case Mean Total IgE	Spring	23	114.86	261.66	54.56	1.71	228.01	**0.015**
	Autumn	20	321.16	388.28	86.82	139.43	502.88	
	Winter	20	272.38	397.14	88.80	86.51	458.25	
Control Mean Total IgE	Spring	22	57.57	145.54	31.03	-6.96	122.10	**0.002**
	Autumn	17	134.76	211.14	51.21	26.21	243.32	
	Winter	12	113.92	258.80	74.71	-50.51	278.35	

Kruskall-Wallis

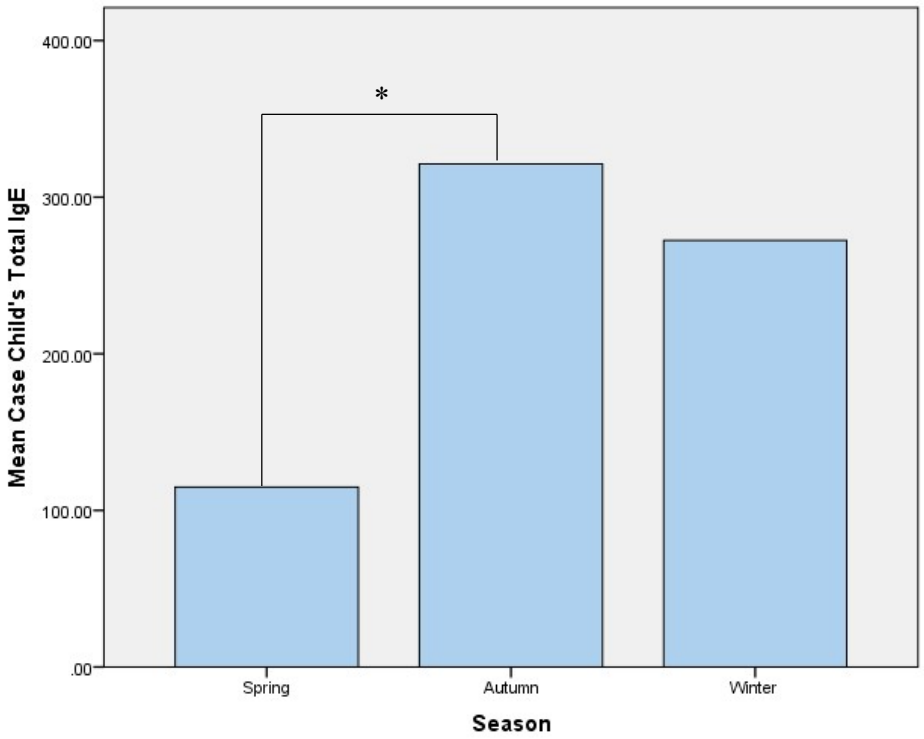


Figure 27: Differences in case children mean Total serum IgE according to season

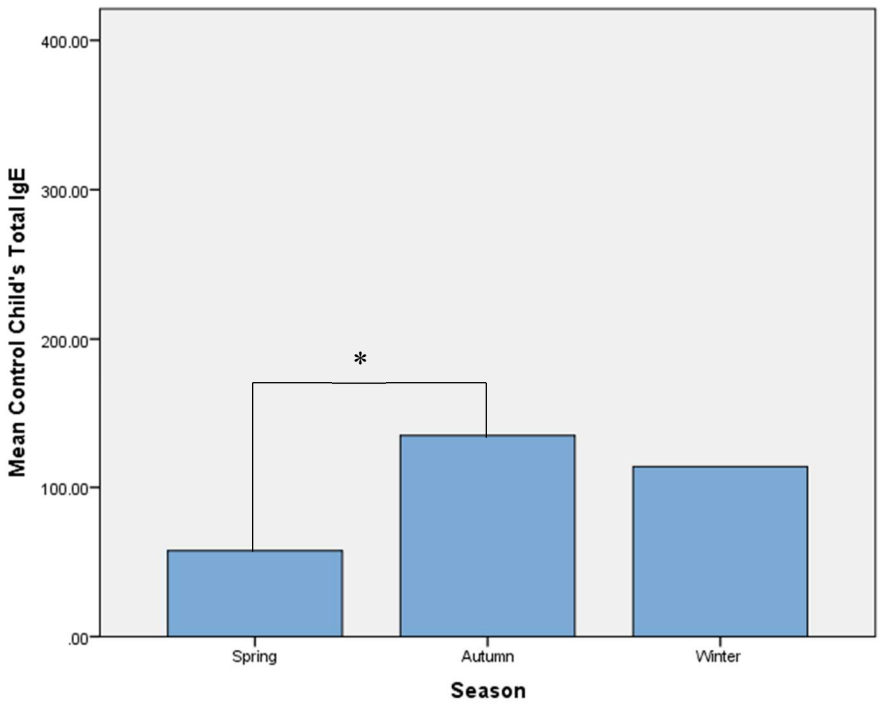


Figure 28: Differences in control children mean Total serum IgE according to season

Levels of mean Total serum IgE in Autumn were significantly higher than the mean levels in spring for both the case children and the control children ($p = 0.015$ and $p = 0.002$ respectively).

Table 39 illustrates the differences in mean specific IgE levels for all the children who attended the clinics according to the season in which the blood sample was obtained.

Table 39: Differences in mean specific IgE levels for all children according to season

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Child Specific Olive IgE	Spring	44	0.24	0.21	0.03	0.18	0.31	
	Autumn	34	0.90	3.83	0.66	-0.43	2.24	**0.005**
	Winter	26	.29	0.21	0.04	0.21	0.38	
Child Specific Goldenrod IgE	Spring	44	0.20	0.06	0.01	0.18	0.22	
	Autumn	34	0.23	0.04	0.01	0.21	0.24	**0.045**
	Winter	25	0.22	0.03	0.01	0.21	0.24	
Child Specific Parietaria IgE	Spring	44	1.17	4.44	0.67	-0.18	2.52	
	Autumn	34	0.76	2.41	0.41	-0.08	1.61	**0.017**
	Winter	25	0.82	2.69	0.54	-0.29	1.93	
Child Specific Cat IgE	Spring	44	0.73	1.35	0.20	0.32	1.14	
	Autumn	34	0.66	1.61	0.28	0.10	1.22	0.092
	Winter	25	0.48	0.85	0.17	0.13	0.83	
Child Specific Dog IgE	Spring	44	0.30	0.26	0.04	0.22	0.38	
	Autumn	34	0.27	0.16	0.03	0.22	0.33	0.406
	Winter	25	0.33	0.48	0.10	0.13	0.53	
Child Specific Cladosporidium IgE	Spring	44	0.19	0.10	0.01	0.16	0.22	
	Autumn	34	0.20	0.03	0.01	0.19	0.21	**0.013**
	Winter	25	0.21	0.03	0.01	0.20	0.22	
Child Specific Alternaria IgE	Spring	44	0.26	0.29	0.04	0.17	0.35	
	Autumn	34	0.21	0.04	0.01	0.19	0.22	**0.020**
	Winter	25	0.22	0.03	0.01	0.21	0.23	
Child Specific HDM IgE	Spring	44	4.89	12.68	1.91	1.04	8.75	
	Autumn	34	4.79	14.06	2.41	-0.11	9.70	**0.024**
	Winter	25	5.14	10.61	2.12	0.76	9.52	

Kruskall-Wallis

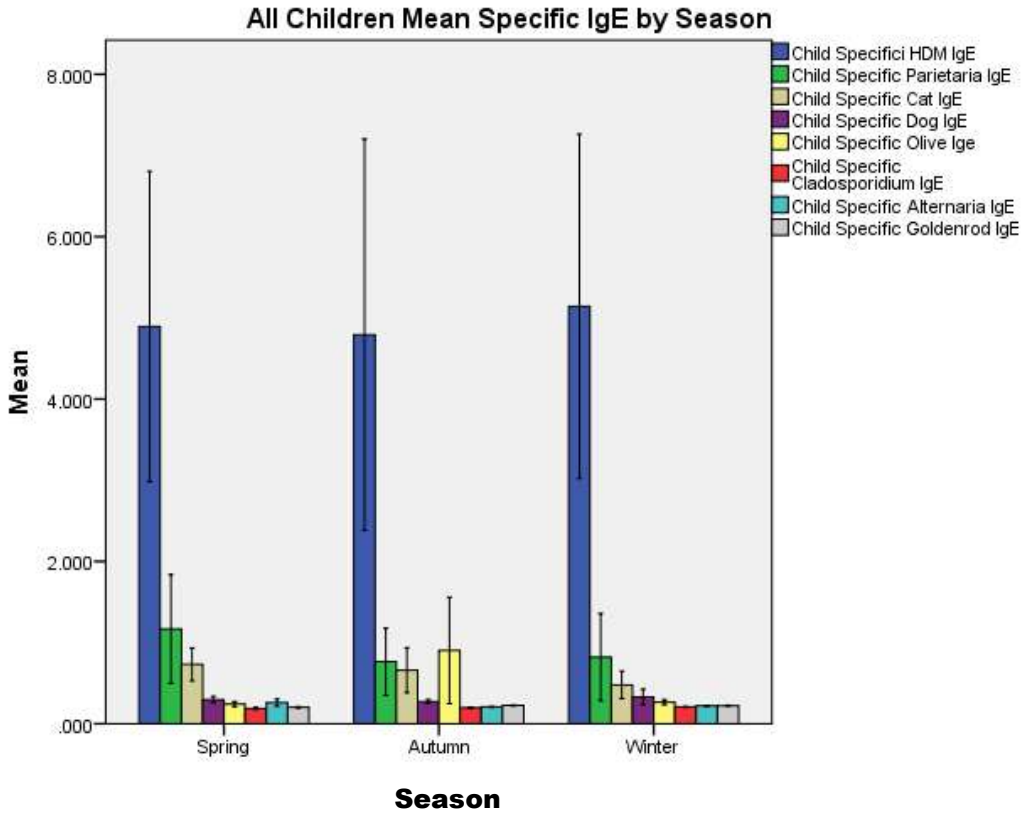


Figure 29: Differences in mean specific IgE levels for all children according to season

Mean specific olive pollen IgE was highest in autumn (0.903U/mL SD 3.825) when compared to spring (0.242 U/mL, SD 0.211) and winter (0.294 U/mL, SD 0.211), p = 0.005.

The second most important difference was seen for mean serum Cladosporidium IgE, which was highest in winter (0.208 U/mL, SD 0.032) when compared to spring (0.189 U/mL, SD 0.095) and autumn (0.0197 U/mL, SD 0.032) (p = 0.013).

Parieteria specific IgE had a higher mean in spring (1.1685U/mL, SD 4.444) when compared to winter (0.820 U/mL, SD 2.688) and autumn (0.765, SD 2.412) (p = 0.017).

The mean specific Alternaria IgE was higher in spring (0.260 U/mL, SD 0.294) as opposed to lower levels in autumn (0.208 U/mL, SD 0.037) and winter (0.220 U/mL, SD 0.033) ($p = 0.020$).

House Dust Mite specific IgE had higher means in winter (2.141 U/mL, SD 10.607), with lower levels in spring (4.892 U/mL, SD 12.681) and autumn (4.792 U/mL, SD 14.056) ($p = 0.024$).

Mean specific IgE for goldenrod was higher in the autumn season (0.226 U/mL, SD 0.042) when compared to spring (0.202 U/mL, SD 0.060) and winter (0.222 U/mL, SD 0.033) ($p = 0.045$).

3.4.5.2 Changes in mean serum IgE by season in case children

The case children (who had current respiratory symptoms, and were likely to be atopic) were then selected separately, in order to investigate how the different mean specific IgE levels vary by season in a population which was predicted to be atopic, due to the participants history of asthma related symptoms. These results are represented in table 40.

Table 40: Differences in mean specific IgE levels for case children according to season

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Child Specific Olive IgE	Spring	23	114.86	261.66	54.56	1.71	228.01	
	Autumn	20	321.16	388.28	86.82	139.44	502.88	**0.013**
	Winter	20	272.38	397.14	88.80	86.51	458.25	
Child Specific Goldenrod IgE	Spring	23	0.26	0.28	0.06	0.14	0.39	
	Autumn	18	0.24	0.05	0.01	0.22	0.27	0.056
	Winter	15	0.29	0.20	0.05	0.18	0.41	
Child Specific Parietaria IgE	Spring	23	0.20	0.06	0.01	0.18	0.23	
	Autumn	18	0.22	0.03	0.01	0.21	0.24	0.113
	Winter	15	0.23	0.02	0.01	0.22	0.25	
Child Specific Cat IgE	Spring	23	2.04	6.08	1.27	-0.59	4.67	
	Autumn	18	1.11	3.28	0.77	-0.52	2.75	0.690
	Winter	15	1.21	3.46	0.89	-0.71	3.13	
Child Specific Dog IgE	Spring	23	0.86	1.73	0.36	0.11	1.61	
	Autumn	18	0.94	2.20	0.52	-0.15	2.03	0.544
	Winter	15	0.66	1.07	0.28	0.06	1.25	
Child Specific Cladosporidium IgE	Spring	23	0.24	0.14	0.03	0.18	0.30	
	Autumn	18	0.28	0.20	0.05	0.18	0.38	**0.022**
	Winter	15	0.41	0.61	0.16	0.07	0.75	
Child Specific Alternaria IgE	Spring	23	0.17	0.04	0.01	0.16	0.19	
	Autumn	18	0.20	0.03	0.01	0.19	0.22	0.180
	Winter	15	0.21	0.03	0.01	0.19	0.22	
Child Specific HDM IgE	Spring	23	0.33	0.39	0.08	0.16	0.50	
	Autumn	18	0.22	0.04	0.01	0.19	0.24	**0.026**
	Winter	15	0.22	0.04	0.01	0.20	0.24	

Kruskall-Wallis

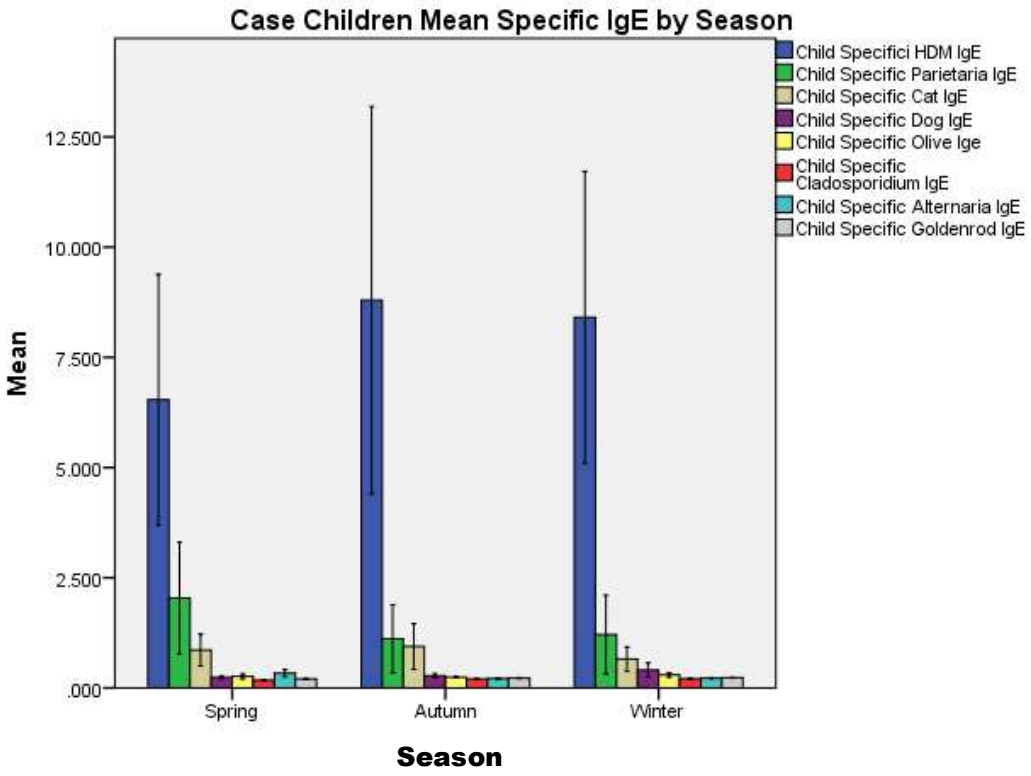


Figure 30: Differences in mean specific IgE levels for all children according to season

While there were where five aeroallergens which showed statistically significant seasonal variation for mean serum IgE in the whole child cohort, this was limited to only three aeroallergens for the case group: Olive, Cladosporidium and HDM.

The most significant difference in mean specific IgE levels for Case children was seen in for olive pollen. This was highest in winter (0.295 U/mL), and lowest in autumn (0.244U/mL; $p = 0.013$).

Mean child specific serum Cladosporidium IgE was significantly higher in autumn and winter (0.204U/mL and 0.207U/mL respectively) when compared to spring (0.175U/mL, $p = 0.022$).

The mean levels for child specific HDM IgE for Case children were significantly higher in autumn and winter (0.877U/mL and 8.409U/mL respectively) when

compared to spring in which the mean level was lower (6. 539U/mL; p = 0.036 and p = 0.017).

3.4.5.3 Changes in mean serum IgE by season in control children

The same analysis was done for the Control children. Table 41 depict these results.

Table 41: Differences in mean specific IgE levels for control children according to season

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Child Specific Olive IgE	Spring	22	57.57	145.54	31.03	-6.96	122.10	
	Autumn	17	134.76	211.14	51.21	26.21	243.32	0.156
	Winter	12	113.92	258.80	74.71	-50.51	278.35	
Child Specific Goldenrod IgE	Spring	21	.22	.08	.02	.18	.26	
	Autumn	16	1.64	5.58	1.39	-1.33	4.62	0.262
	Winter	11	.29	.24	.07	.14	.45	
Child Specific Parietaria IgE	Spring	21	.20	.06	.01	.17	.23	
	Autumn	16	.23	.05	.01	.20	.25	0.154
	Winter	10	.21	.04	.01	.18	.24	
Child Specific Cat IgE	Spring	21	.21	.07	.02	.18	.24	
	Autumn	16	.37	.53	.13	.09	.66	**0.022**
	Winter	10	.24	.04	.01	.21	.27	
Child Specific Dog IgE	Spring	47	.27	.32	.05	.18	.36	
	Autumn	21	.59	.76	.17	.24	.94	0.175
	Winter	16	.35	.24	.06	.22	.47	
Child Specific Cladosporidium IgE	Spring	10	.21	.03	.01	.19	.24	
	Autumn	47	.43	.54	.08	.27	.59	0.185
	Winter	21	.36	.34	.07	.20	.51	
Child Specific Alternaria IgE	Spring	16	.27	.09	.02	.22	.32	
	Autumn	10	.21	.02	.01	.19	.23	0.187
	Winter	47	.30	.24	.04	.23	.37	
Child Specific HDM IgE	Spring	21	.20	.13	.03	.14	.26	
	Autumn	16	.19	.04	.01	.17	.21	0.605
	Winter	10	.21	.03	.01	.19	.23	

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Figure 31: Control Children mean specific IgE by season

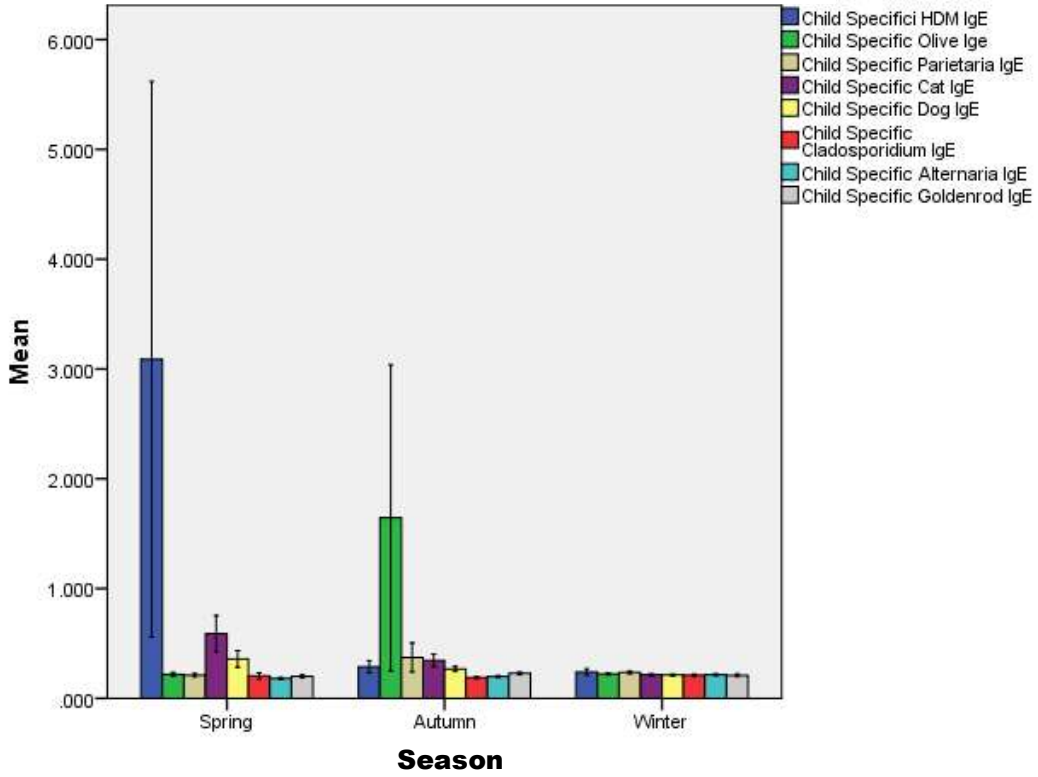


Figure 31: Differences in mean specific IgE levels for control children according to season

The results for the control children were in stark contrast to those obtained from the whole children cohort and the case group. Only one aerollergen has a mean specific IgE which showed significant seasonal variance. This was cat, in which control children had higher mean specific IgE levels in spring, 0.590 U/mL, SD 0.759 as compared to a mean of 0.345U/mL, SD 0.345 in autumn and 0.214 U/mL, SD 0.033 in winter ($p = 0.022$).

Interestingly, mean specific HDM was higher in spring for the control children, with a mean of 3.089 U/mL, SD 11.594 when compared to autumn (0.288, SD 0.222) and winter (0.240 U/mL, SD 0.093), yet due to a large variability, this was not statistically

significant (p = 0.605). This is in stark contrast to the results in the case group, were levels were significantly higher in autumn and winter.

As previously mentioned, the case group had significant seasonal variation in three specific IgEs, Olive, Cladosporidium and HDM, as opposed to the control group in which only happened for mean specific Cat IgE.

The lower mean specific HDM IgE in spring in the case group contrasts with the results obtained from the control group, where the higher mean specific HDM IgE were noted in the warmer spring season. The levels of HDM allergen collected in the houses will be presented in a later section in this thesis.

Of note was the fact that the pattern for specific HDM IgE which was observed in the case children, followed the same pattern for mean specific Cladosporidium IgE (both means were higher in autumn and winter), with similar p values. Given the biology of HDM, and its relationship with mould[41,42], one could postulate that these differences are related and could possibly potentiate each other.

Cladosporidum species is a genus of moulds which comprises species which can thrive in outdoor environments but also others which colonise damp indoor environments[115]. In Malta, the damper months are those of autumn and winter, so one could expect a better environment for these moulds to thrive in this period, especially indoors when windows would be closed promoting condensation, and there could be indoor rainwater ingress due to defects in building construction. Such a situation would explain the higher serum IgE levels in these months when compared to the drier spring season.

Mean specific Olive IgE also behaved inconsistently with the plant's biology. Olive flowers develop between March and May, and the tree is usually dormant in the winter months. For the whole children cohort, the mean levels were higher in the autumn months, and for the case group these were higher in the winter months and lowest in the autumn months. In the control group, specific IgE levels to this aeroallergen had a higher trend in autumn, though this was not statistically significant. One could postulate that these results would be due to persistent pollen in the environment, or perhaps a delayed antibody response by the atopic child. On the other hand, one must note the generally low levels for this aeroallergen, and there is a risk of a potential false positive result.

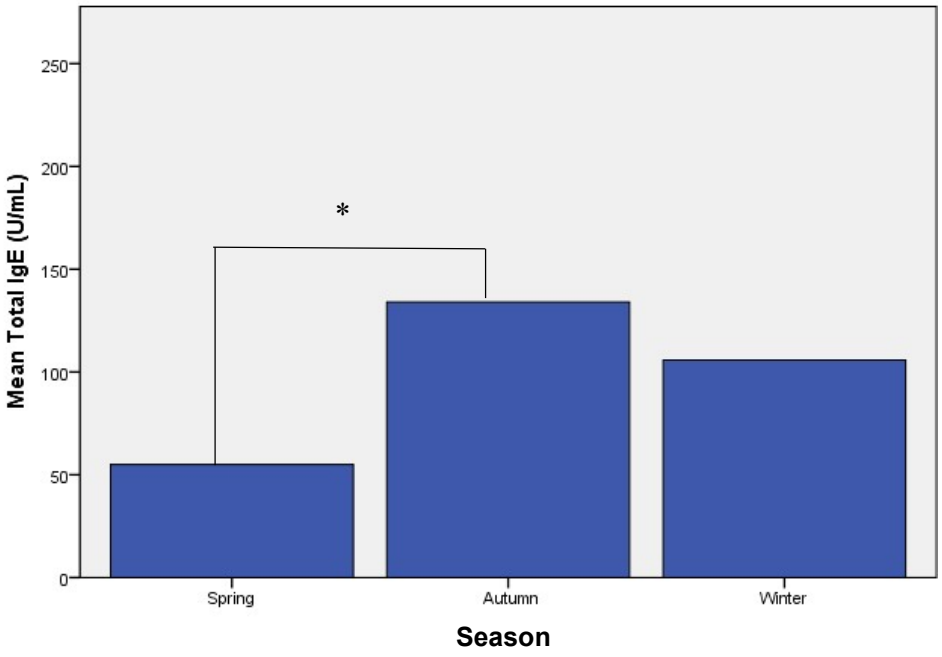
3.4.6.1 Changes in mean serum IgE by season in Fathers

Mean total IgE levels were compared according to season for all the fathers who participated in the clinical phase (N = 91). See table 42.

Table 42: Differences in mean Total IgE levels for all fathers according to season

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Child's Total IgE	Spring	38	55.01	82.57	13.39	27.87	82.14	**0.024**
	Autumn	25	133.99	236.59	47.32	36.33	231.65	
	Winter	28	105.80	169.70	32.07	40.00	171.60	

Figure 32: Differences in mean Total IgE levels for all fathers according to season



There were significant seasonal differences in mean Total IgE levels in the fathers who participated in the study (p = 0.024). Mean total IgE in fathers was significantly higher in the autumn season (133.992U/mL, SD 236.585) when compared to spring (55.005U/mL, SD 82.567; p = 0.006).

The seasonal differences in mean specific IgE are presented in Table 43.

Table 43: Differences in mean specific IgE levels for all fathers according to season

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Father Specific HDM IgE	Spring	28	1.89	4.91	0.93	-0.02	3.79	
	Autumn	21	2.49	4.87	1.06	0.27	4.70	0.136
	Winter	22	1.77	3.95	0.84	0.02	3.52	
Father Specific Parietaria IgE	Spring	28	0.82	3.22	0.61	-0.43	2.07	
	Autumn	21	1.25	3.11	0.68	-0.17	2.66	0.132
	Winter	22	1.33	3.26	0.69	-0.11	2.78	
Father Specific Olive IgE	Spring	28	0.50	1.50	0.28	-0.08	1.08	
	Autumn	21	0.35	0.60	0.13	0.07	0.62	0.161
	Winter	22	0.32	0.22	0.05	0.22	0.42	
Father Specific Cat IgE	Spring	28	0.21	0.07	0.01	0.18	0.24	
	Autumn	21	0.24	0.07	0.02	0.21	0.27	0.097
	Winter	22	0.66	1.14	0.24	0.15	1.16	
Father Specific Goldenrod IgE	Spring	28	0.20	0.05	0.01	0.18	0.22	
	Autumn	21	0.22	0.04	0.01	0.20	0.24	**0.024**
	Winter	22	0.25	0.07	0.01	0.22	0.28	
Father Specific Dog IgE	Spring	28	0.19	0.04	0.01	0.17	0.21	
	Autumn	21	0.20	0.03	0.01	0.19	0.22	0.454
	Winter	22	0.22	0.10	0.02	0.18	0.27	
Father Specific Alternaria IgE	Spring	28	0.19	0.05	0.01	0.17	0.21	
	Autumn	21	0.20	0.03	0.01	0.18	0.21	0.322
	Winter	22	0.22	0.06	0.01	0.19	0.24	
Father Specific Cladosporidium IgE	Spring	28	0.18	0.04	0.01	0.17	0.20	
	Autumn	21	0.19	0.04	0.01	0.17	0.21	0.059
	Winter	22	0.23	0.11	0.02	0.18	0.28	

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Significant seasonal variability throughout all the season for mean specific IgE in fathers was only observed for Goldenrod (p = 0.024). Mean Goldenrod specific IgE was higher in autumn (0.218U/mL, SD 0.044) when compared to spring (0.202, SD 0.048; p = 0.027). Although there was no significant overall seasonal variability for

mean Cladosporidium specific serum IgE in all the fathers, it was significantly higher in winter (0.228U/mL, SD 0.109) when compared to the warmer spring season (0.183U/mL, SD 0.043; p = 0.017).

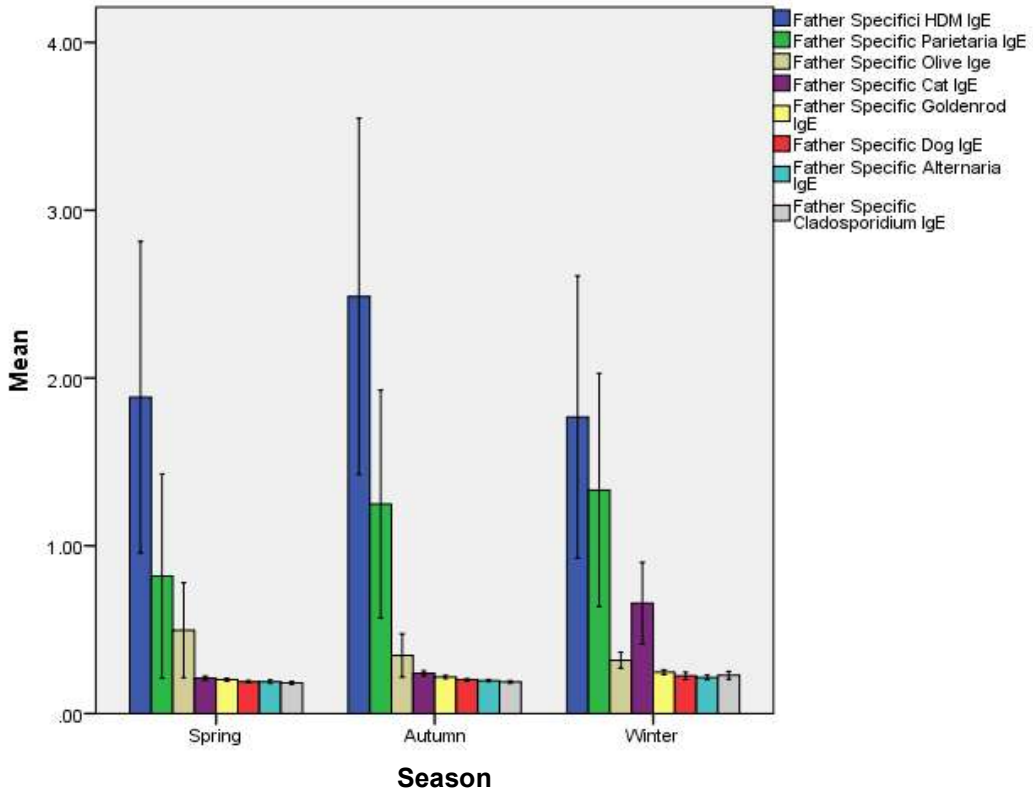


Figure 33: Differences in mean specific IgE levels for all fathers according to season

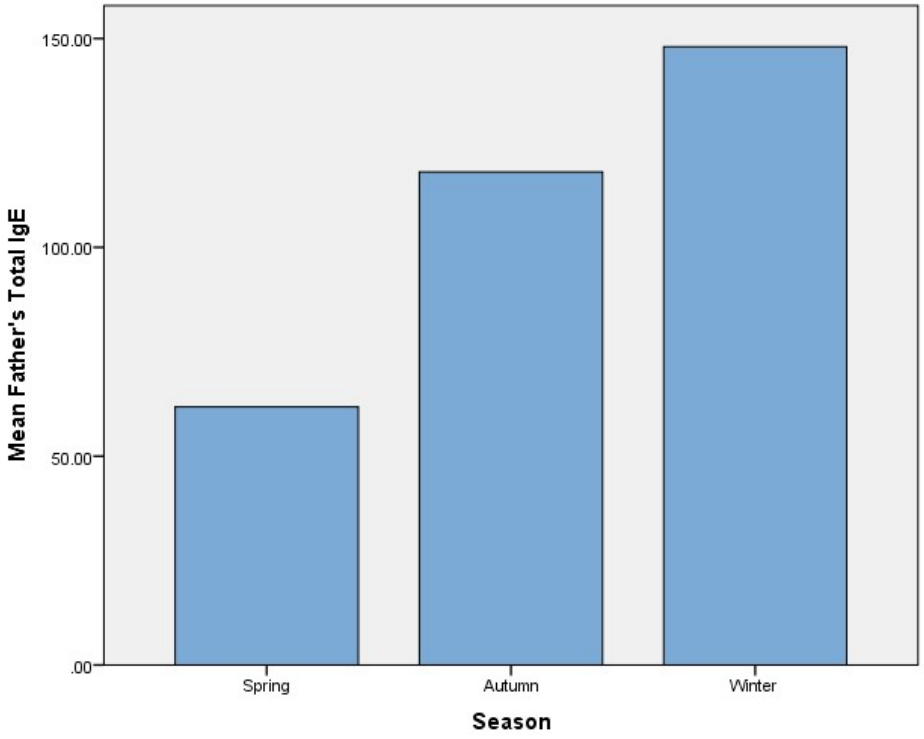
3.4.6.2 Changes in mean serum IgE by season in Fathers with doctor-diagnosed asthma

The above data described a mixed cohort of fathers, who were not selected on a case/control basis, but rather due to the fact they were a father of either a case or control student who participated in the study. Thus one could not have studied them on a specific case or control basis. Instead, some of these fathers had doctor-diagnosed asthma, and the seasonal changes in mean serum IgE in these cases were analysed.

Table 44: Seasonal differences in mean Total IgE levels for fathers who had doctor diagnosed asthma

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Fathers Total IgE	Spring	7	61.80	99.66	37.67	-30.37	153.97	0.054
	Autumn	4	118.08	28.17	14.08	73.26	162.89	
	Winter	5	148.02	129.95	58.12	-13.34	309.38	

Figure 34: Seasonal differences in mean Total IgE levels for fathers who had doctor diagnosed asthma



A total of sixteen (16) fathers in the study had doctor-diagnosed asthma. The mean total IgE on doctor-diagnosed asthmatic fathers was higher in the autumn months (118.075U/mL, SD 28.167) than the spring months (61.800U/mL, SD 99.664; p = 0.048). The overall variation was very close to being statistically significant in this

analysis (p = 0.054). This could have been more significant had the sample been larger (n = 16).

Table 45: Seasonal differences in mean specific IgE levels for fathers who had doctor diagnosed asthma

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Father Specific HDM IgE	Spring	7	3.01	5.33	2.02	-1.92	7.94	
	Autumn	4	4.41	5.42	2.71	-4.22	13.04	0.946
	Winter	4	3.99	6.47	3.24	-6.31	14.30	
Father Specific Parietaria IgE	Spring	7	2.64	6.45	2.44	-3.32	8.60	
	Autumn	4	5.38	6.02	3.01	-4.20	14.96	0.577
	Winter	4	0.23	0.03	0.02	0.18	0.29	
Father Specific Olive IgE	Spring	7	0.26	0.10	0.04	0.16	0.35	
	Autumn	4	0.21	0.07	0.03	0.10	0.31	0.489
	Winter	4	0.41	0.37	0.18	-0.17	1.00	
Father Specific Cat IgE	Spring	7	0.25	0.10	0.04	0.15	0.34	
	Autumn	4	0.21	0.06	0.03	0.11	0.30	0.451
	Winter	4	1.13	1.81	0.90	-1.75	4.01	
Father Specific Goldenrod IgE	Spring	7	0.21	0.04	0.02	0.17	0.25	
	Autumn	4	0.22	0.06	0.03	0.12	0.31	0.940
	Winter	4	0.22	0.04	0.02	0.16	0.27	
Father Specific Dog IgE	Spring	7	0.20	0.05	0.02	0.16	0.25	
	Autumn	4	0.20	0.03	0.02	0.15	0.24	0.981
	Winter	4	0.19	0.01	0.00	0.18	0.20	
Father Specific Alternaria IgE	Spring	7	0.19	0.05	0.02	0.15	0.24	
	Autumn	4	0.19	0.03	0.01	0.14	0.23	0.650
	Winter	4	0.25	0.11	0.06	0.07	0.43	
Father Specific Cladosporidium IgE	Spring	7	0.20	0.06	0.02	0.15	0.25	
	Autumn	4	0.19	0.04	0.02	0.12	0.26	0.944
	Winter	4	0.20	0.03	0.01	0.16	0.24	

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There were no significant seasonal differences in mean serum specific IgE for the various aeroallergens studied in the doctor-diagnosed asthmatic fathers. This could easily be due to a very small sample size in this case (n = 16), but there is a trend towards a higher mean Olive specific IgE level in winter.

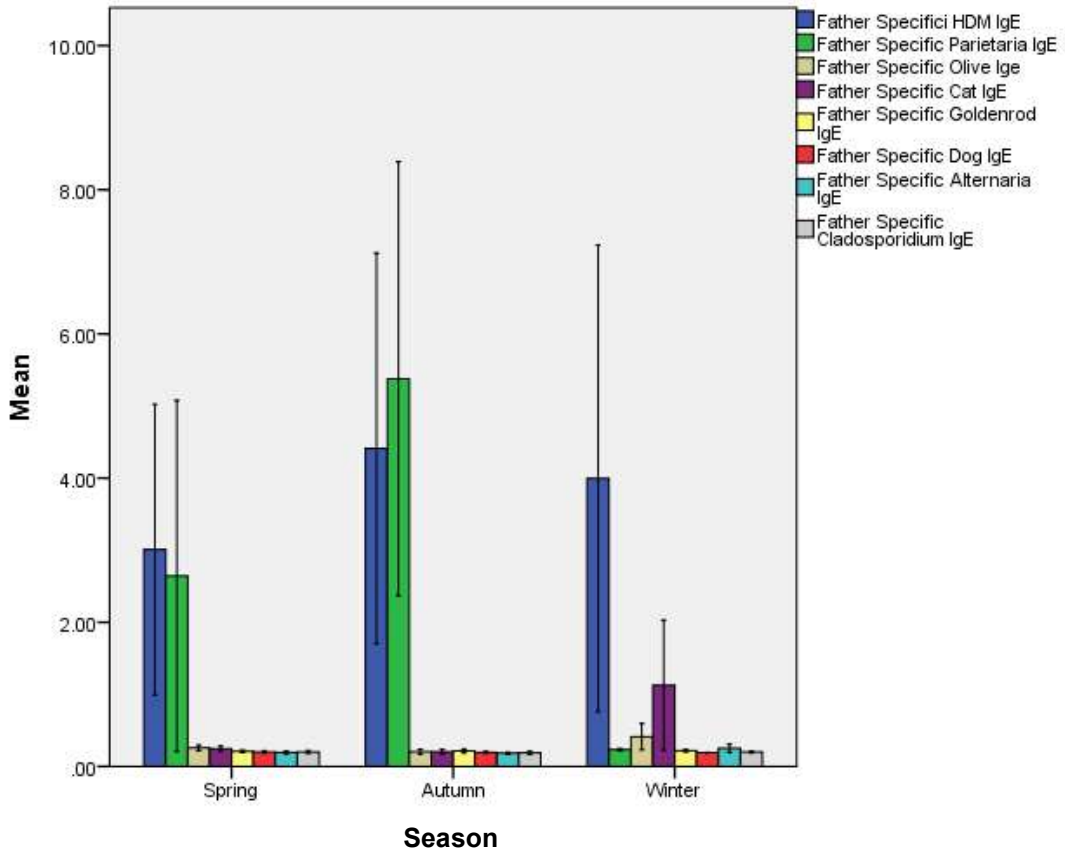

Figure 35: Seasonal differences in mean specific IgE levels for fathers who had doctor diagnosed asthma

3.4.6.3 Changes in mean serum IgE by season in Fathers who did not have doctor diagnosed asthma

Table 46: Seasonal differences in mean Total IgE levels for fathers who did not have doctor diagnosed asthma

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Fathers Total IgE	Spring	31	53.47	80.05	14.38	24.11	82.83	0.143
	Autumn	21	137.02	258.82	56.48	19.21	254.84	
	Winter	23	96.63	178.26	37.17	19.54	173.71	

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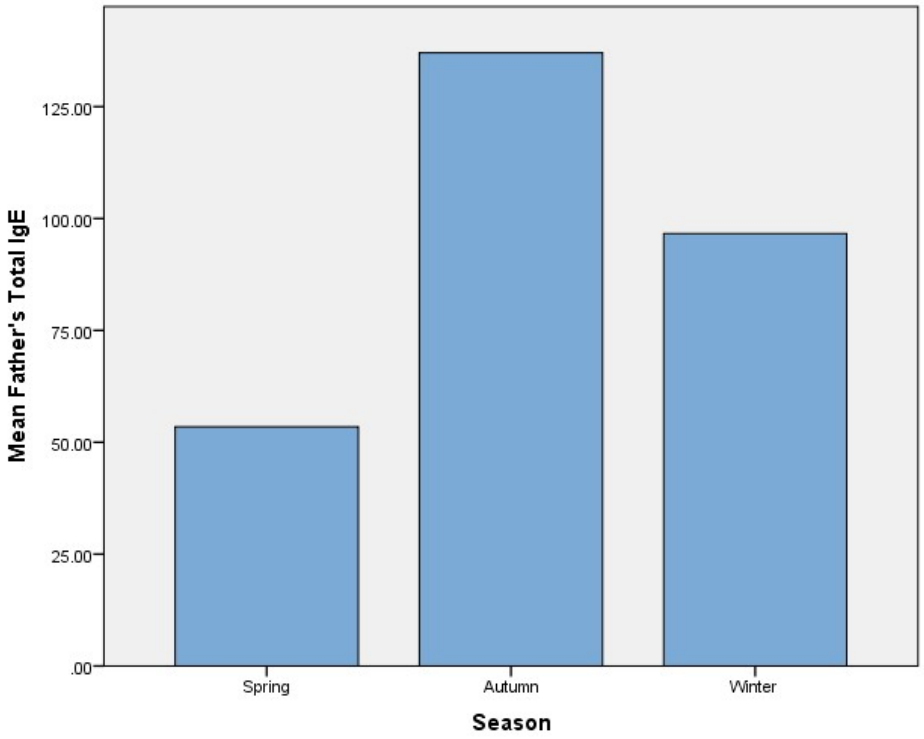
Figure 36: Seasonal differences in mean Total IgE levels for fathers who did not have doctor diagnosed asthma



The seasonal variance in mean serum total IgE for fathers who did not have doctor-diagnosed asthma did not vary significantly.

The seasonal variation in mean specific serum IgE levels for fathers who were not diagnosed with asthma was analysed, and the results were presented in table 47 and figure 37.

Table 47: Seasonal differences in mean specific IgE levels for fathers who did not have doctor diagnosed asthma

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Father Specific HDM IgE	Spring	21	1.51	4.84	1.06	-.69	3.71	
	Autumn	17	2.03	4.79	1.16	-.43	4.50	0.068
	Winter	18	1.27	3.23	.76	-.33	2.88	
Father Specific Parietaria IgE	Spring	21	.21	.04	.01	.19	.23	
	Autumn	17	.28	.16	.04	.19	.36	0.107
	Winter	18	1.58	3.57	.84	-.20	3.35	
Father Specific Olive IgE	Spring	21	.58	1.73	.38	-.21	1.36	
	Autumn	17	.38	.66	.16	.04	.72	0.153
	Winter	18	.30	.19	.04	.20	.39	
Father Specific Cat IgE	Spring	21	.20	.05	.01	.18	.22	
	Autumn	17	.25	.07	.02	.21	.29	**0.050**
	Winter	18	.55	.99	.23	.06	1.04	
Father Specific Goldenrod IgE	Spring	21	.20	.05	.01	.18	.22	
	Autumn	17	.22	.04	.01	.20	.24	**0.017**
	Winter	18	.25	.07	.02	.22	.29	
Father Specific Dog IgE	Spring	21	.19	.04	.01	.17	.20	
	Autumn	17	.20	.03	.01	.19	.22	0.240
	Winter	18	.23	.11	.03	.18	.29	
Father Specific Alternaria IgE	Spring	21	.19	.05	.01	.17	.21	
	Autumn	17	.20	.03	.01	.18	.21	0.338
	Winter	18	.21	.04	.01	.18	.23	
Father Specific Cladosporidium IgE	Spring	21	.18	.04	.01	.16	.19	
	Autumn	17	.19	.04	.01	.17	.21	0.063
	Winter	18	.23	.12	.03	.17	.29	

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There was significant seasonal variability in the mean specific serum IgE in the fathers who did not have doctor-diagnosed asthma in the case of Goldenrod and cat dander. Serum specific Goldenrod IgE was lower (p = 0.017) in spring (0.199U/mL SD 0.050 vs 0.218U/mL SD 0.043 in autumn and 0.253U/mL SD 0.071 in winter). Mean Cat serum specific IgE was highest in winter in this group (0.554U/mL SD 0.986 vs 0.200U/mL SD 0.053 in spring and 0.248U/mL SD 0.073; p = 0.050).

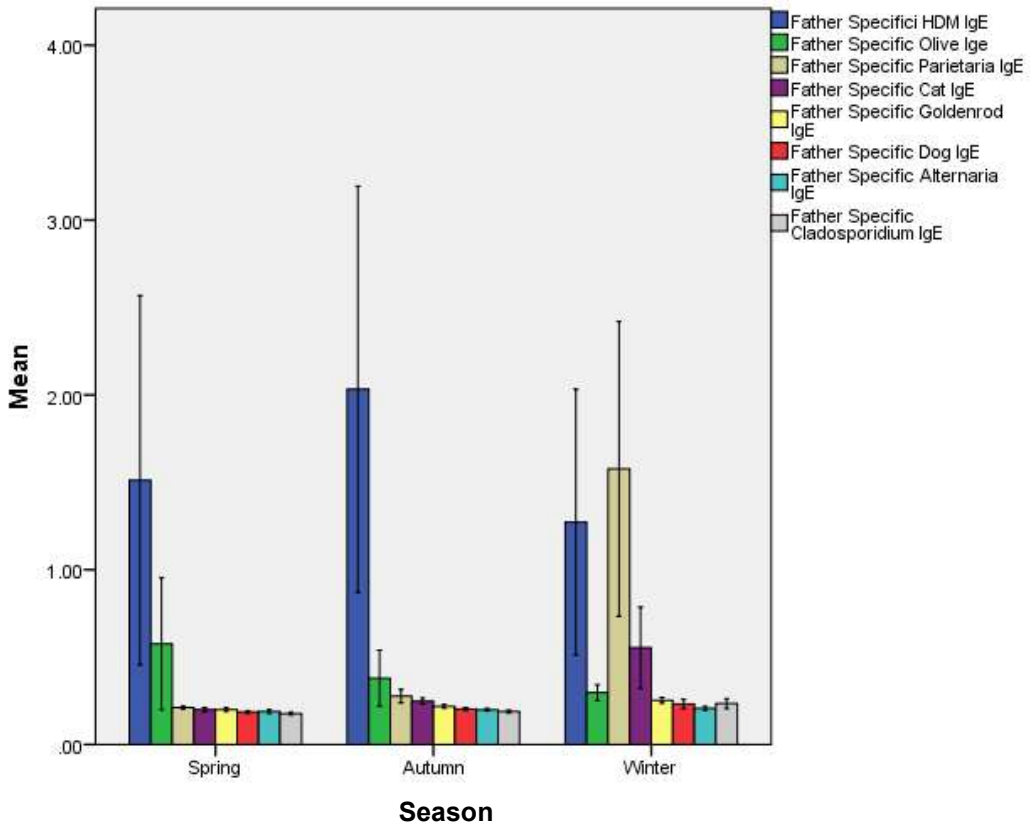

Figure 37: Seasonal differences in mean specific IgE levels for fathers who did not have doctor diagnosed asthma

3.4.6.4 Summary of seasonal serum specific IgE variations in fathers

The seasonal changes noted for goldenrod specific IgE (seen for all the fathers and the group of fathers who did not have doctor-diagnosed asthma) may be due to the fact that this plant blooms in late summer till the end of autumn [123] (hence the lower mean levels noted in spring).

It was interesting to note that none of the mean specific IgE levels had significant seasonal differences in the group of fathers who had doctor-diagnosed asthma. Yet, the total IgE was significantly higher in the damper autumn months for this group. One also notes higher levels of mean serum parietaria IgE in the fathers. In fact, the mean specific Parietaria IgE in fathers was higher (1.105 SD 3.164) when compared to the children's specific IgE for this aeroallergen (0.951 SD 3.456; $p > 0.001$). This could suggest the possibility of asthmatic fathers developing atopy which affects their symptoms to various aeroallergens which they might have been exposed to in their life, while HDM was by far the aeroallergen which stood out in children.

3.4.7.1 Changes in mean serum IgE by season in Mothers

Mean total IgE levels were compared according to season for all the mothers who participated in the clinical phase (N = 116). See table 48.

Table 48: Seasonal differences in mean Total IgE levels for all mothers

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Mother's Total IgE	Spring	47	50.18	125.82	18.35	13.24	87.13	0.054
	Autumn	34	133.73	235.00	40.30	51.73	215.72	
	Winter	35	108.51	190.82	32.25	42.96	174.06	

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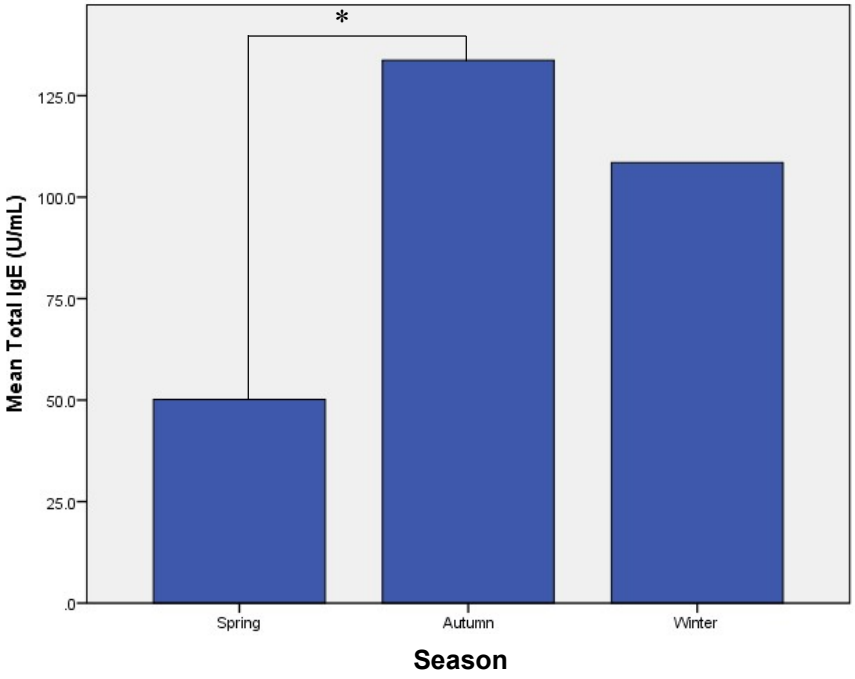
Figure 38: Differences in mean Mothers' Total IgE according to season



The seasonal variability of mean mother total IgE was very close to being statistically significant (p = 0.054). On the other hand, when the mothers' mean Total IgE in autumn was higher than (133.726 U/mL SD 234.997) the same mean in spring (50.183 U/mL SD 125.821; p = 0.009). This was a similar result to the one noted in the fathers.

Table 49: Seasonal differences in mean specific IgE levels for all mothers

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Mother Specific HDM IgE	Spring	37	0.73	2.59	0.43	-0.14	1.60	
	Autumn	26	0.58	1.27	0.25	0.07	1.09	**0.012**
	Winter	26	1.55	3.74	0.73	0.04	3.06	
Mother Specific Parietaria IgE	Spring	37	0.50	1.77	0.29	-0.09	1.09	
	Autumn	26	0.95	2.47	0.48	-0.05	1.95	**0.001**
	Winter	26	1.48	4.22	0.83	-0.22	3.19	
Mother Specific Olive IgE	Spring	37	0.27	0.36	0.06	0.15	0.39	
	Autumn	26	0.48	0.75	0.15	0.18	0.78	**0.005**
	Winter	26	0.24	0.06	0.01	0.21	0.26	
Mother Specific Cat IgE	Spring	37	0.22	0.09	0.01	0.19	0.25	
	Autumn	26	0.31	0.45	0.09	0.13	0.49	**0.038**
	Winter	26	0.58	1.70	0.33	-0.11	1.26	
Mother Specific Alternaria IgE	Spring	37	0.20	0.13	0.02	0.15	0.24	
	Autumn	26	0.20	0.03	0.01	0.19	0.22	**0.006**
	Winter	26	0.24	0.14	0.03	0.18	0.29	
Mother Specific Dog IgE	Spring	37	0.19	0.04	0.01	0.18	0.21	
	Autumn	26	0.21	0.04	0.01	0.19	0.22	**0.006**
	Winter	26	0.43	1.05	0.21	0.01	0.85	
Mother Specific Goldenrod IgE	Spring	37	0.19	0.05	0.01	0.17	0.20	
	Autumn	26	0.26	0.18	0.03	0.19	0.33	**<0.001**
	Winter	26	0.23	0.04	0.01	0.21	0.24	
Mother Specific Cladosporidium IgE	Spring	37	0.17	0.03	0.01	0.16	0.19	
	Autumn	26	0.19	0.03	0.01	0.18	0.21	**0.002**
	Winter	26	0.21	0.03	0.01	0.19	0.22	

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In the mothers, the mean serum specific IgE levels for <u>all</u> the aeroallergens studied showed seasonal variability, which is in stark contrast to the fathers' results, where this was the case for only one aeroallergen when this group was taken as a whole.

Mean serum specific IgE to HDM was highest in winter (1.551 U/mL SD 3.742) when compared to the spring and autumn months (0.730U/mL SD 2.595 and 0.578 U/mL SD 1.267 respectively, p = 0.012).

The same patterns were seen for all the other aeroallergens except for goldenrod and olive. The higher mean specific IgEs for parietaria, cat, alternaria, dog and cladosporidium were also found in the winter months (see table 49, figure 39). On the other hand, goldenrod and olive mean specific IgE levels were higher in autumn.

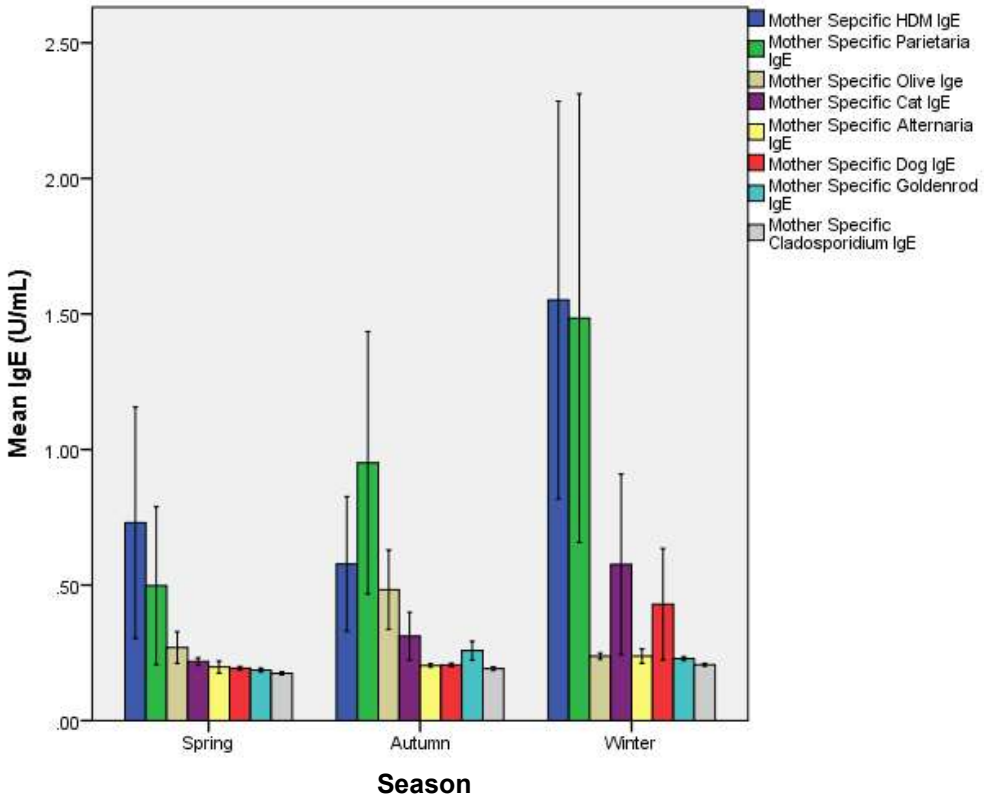

Figure 39: Seasonal differences in mean specific IgE levels for all mothers

3.4.7.2 Changes in mean serum IgE by season in Mothers who had doctor-diagnosed asthma

The mothers who had doctor-diagnosed asthma were then selected, and their mean total IgE and specific IgE levels to aeroallergens were compared according to the three seasons studied.

Thirteen (13) mothers had doctor-diagnosed asthma, and their results were compared in the following tables.

Table 50: Seasonal differences in mean total IgE levels for mothers who had doctor diagnosed asthma

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Mothers Total IgE	Spring	5	201.78	350.54	156.77	-233.48	637.04	0.085
	Autumn	3	12.73	13.01	7.51	-19.58	45.05	
	Winter	5	254.08	239.24	106.99	-42.97	551.13	

The seasonal variance in total serum IgE for doctor-diagnosed asthmatic mothers was not significant (p = 0.085), yet this is likely to be due to the fact that this was a small sample (n = 13). On the other hand, the mean total IgE in autumn (12.733U/mL SD 130.008) was lower than that in winter (254.080U/mL SD 239.239; p = 0.036).

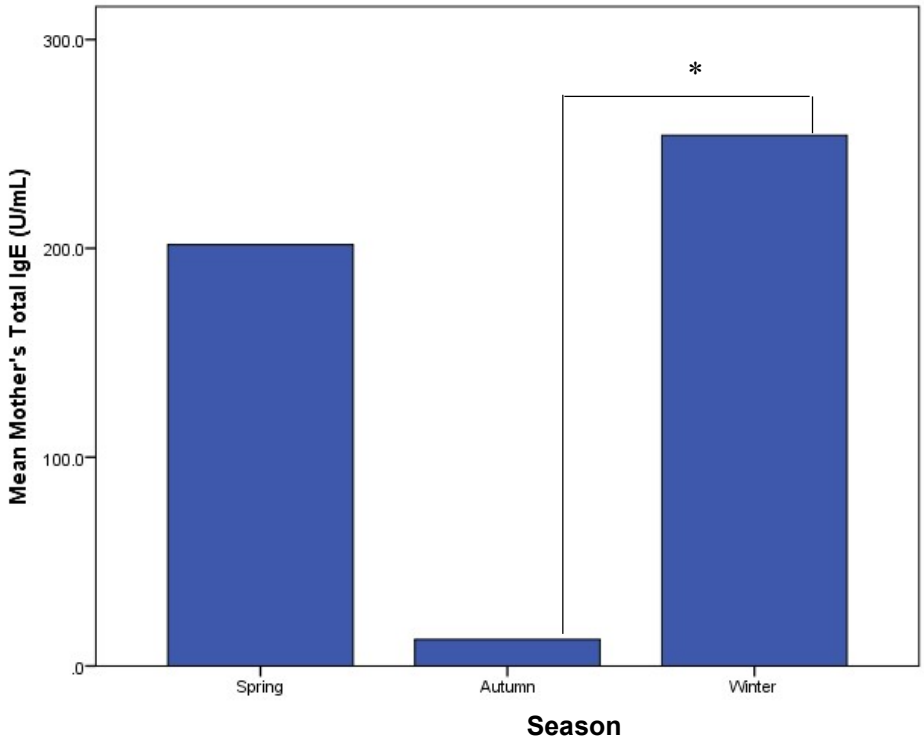

Figure 40: Seasonal differences in mean specific IgE levels for mothers who had doctor-diagnosed asthma.

Table 51: Seasonal differences in mean specific IgE levels for mothers with doctor-diagnosed asthma

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Mother Specific HDM IgE	Spring	4	0.23	0.08	0.04	0.10	0.36	0.059
	Autumn	2	0.16	0.05	0.04	-0.29	0.60	
	Winter	4	6.54	7.06	3.53	-4.69	17.76	
Mother Specific Parietaria IgE	Spring	4	2.89	5.39	2.70	-5.69	11.47	0.245
	Autumn	2	0.19	.04	0.02	-0.13	0.50	
	Winter	4	0.81	1.11	0.55	-0.95	2.57	
Mother Specific Olive IgE	Spring	4	0.73	1.02	0.51	-0.89	2.34	0.191
	Autumn	2	0.17	0.03	0.02	-0.08	0.42	
	Winter	4	0.32	0.11	0.05	0.15	0.48	
Mother Specific Cat IgE	Spring	4	0.20	0.05	0.02	0.13	0.28	0.339
	Autumn	2	0.18	0.02	0.02	-0.02	0.37	
	Winter	4	0.24	0.04	0.02	0.17	0.30	
Mother Specific Alternaria IgE	Spring	4	0.18	0.05	0.02	0.11	0.26	0.141
	Autumn	2	0.17	0.01	0.01	0.10	0.23	
	Winter	4	0.37	0.34	0.17	-0.17	0.91	
Mother Specific Dog IgE	Spring	4	0.20	0.03	0.01	0.15	0.24	0.107
	Autumn	2	0.15	0.01	0.01	0.02	0.28	
	Winter	4	0.21	0.04	0.02	0.15	0.27	
Mother Specific Goldenrod IgE	Spring	4	0.21	0.08	0.04	0.08	0.33	0.226
	Autumn	2	0.17	0.03	0.02	-0.08	0.42	
	Winter	4	0.25	0.01	0.00	0.23	0.26	
Mother Specific Cladosporidium IgE	Spring	4	0.18	0.02	0.01	0.14	0.22	0.179
	Autumn	2	0.16	0.01	0.01	0.03	0.29	
	Winter	4	0.21	0.04	0.02	0.15	0.27	

Kruskall-Wallis

Again, due to the small sample size, none of mean specific IgE levels to aeroallergens had seasonal variability which reached significance at 0.05. The aeroallergen whose mean specific IgE had most variability was HDM ($p = 0.059$). The mean serum HDM specific IgE in doctor-diagnosed asthmatic mothers was highest in the winter season

(6.535U/ml SD 7.057) and lowest in autumn (0.155 U/ml SD 0.049) The highest mean serum IgE for this allergen was recorded in winter (6.535U/mL, SD 7.057).

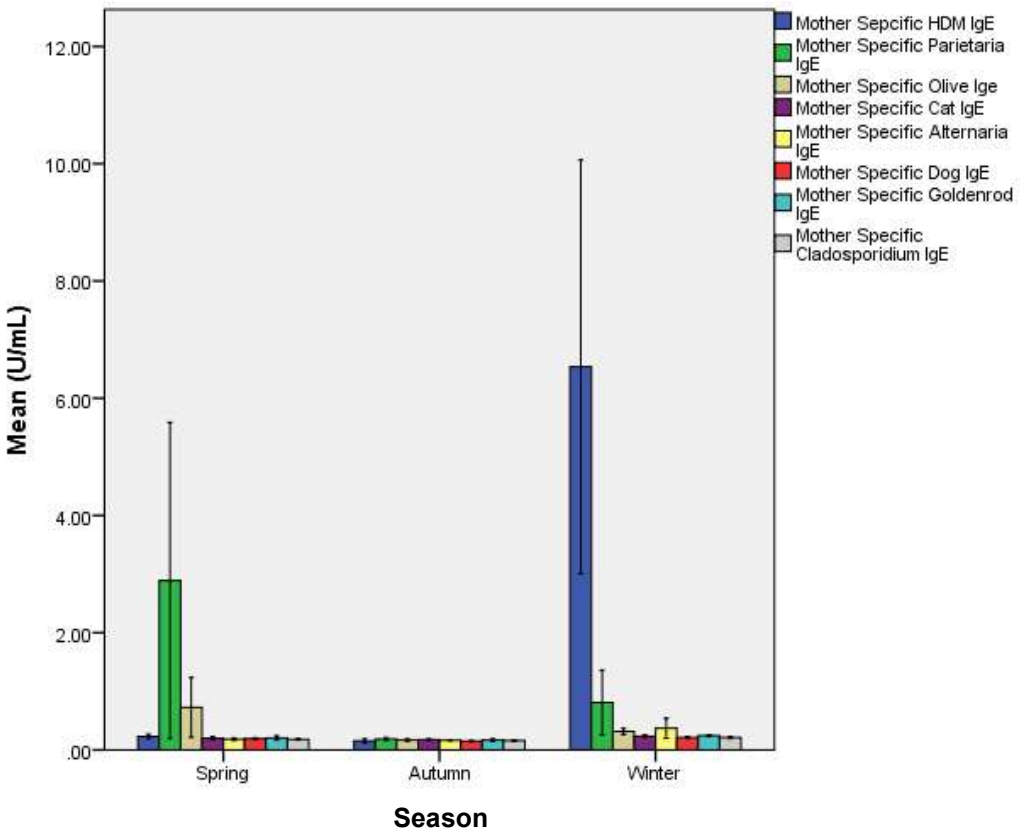

Figure 41: Seasonal differences in mean specific IgE levels for mothers with doctor diagnosed asthma

3.4.7.3 Changes in mean serum IgE by season in Mothers who did not have doctor diagnosed asthma

One hundred and three (103) mothers did not report doctor-diagnosed asthma. All these had total IgE recorded. Seventy nine (79) had specific IgE blood samples collected.

Table 52: Seasonal differences in mean specific IgE levels for mothers who did not have doctor-diagnosed asthma

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	P value
Mother's Total IgE	Spring	42	32.14	51.35	7.92	16.13	48.14	
	Autumn	31	145.44	243.16	43.66	56.24	234.62	**0.009**
	Winter	30	84.24	174.74	31.90	18.99	149.49	

The mothers who did not have doctor-diagnosed asthma had higher mean total IgE in autumn (145.435U/mL SD 243.164) when compared to spring (32.136U/mL SD 51.351) and winter (84.243, SD 174.741).

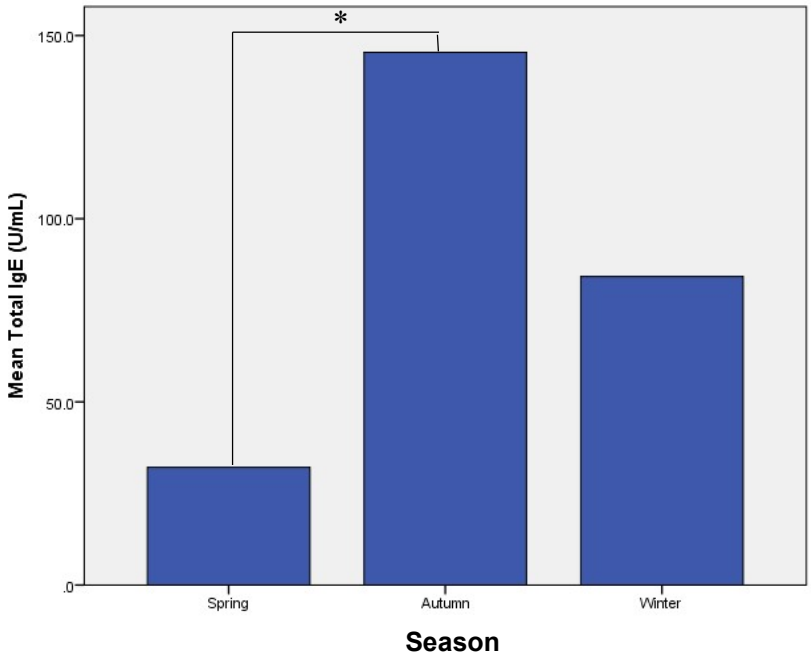

Figure 42: Seasonal differences in mean specific IgE levels for mothers who did not have doctor diagnosed asthma

Table 53: Seasonal differences in mean specific IgE levels for mothers who did not have doctor diagnosed asthma

		N	Mean	SD	SE	95% Confidence Interval for Mean		P value
						Lower Bound	Upper Bound	
Mother Specific HDM IgE	Spring	33	.79	2.75	.48	-.18	1.76	
	Autumn	24	.61	1.31	.27	.06	1.17	**0.024**
	Winter	22	.65	1.99	.42	-.24	1.53	
Mother Specific Parietaria IgE	Spring	33	.21	.05	.01	.19	.23	
	Autumn	24	1.02	2.56	.52	-.07	2.10	**0.001**
	Winter	22	1.61	4.57	.97	-.42	3.63	
Mother Specific Olive IgE	Spring	33	.21	.13	.02	.17	.26	
	Autumn	24	.51	.77	.16	.18	.84	**0.001**
	Winter	22	.22	.03	.01	.21	.24	
Mother Specific Cat IgE	Spring	33	.22	.09	.02	.19	.25	
	Autumn	24	.32	.47	.10	.13	.52	**0.051**
	Winter	22	.64	1.85	.39	-.18	1.46	
Mother Specific Alternaria IgE	Spring	33	.20	.14	.02	.15	.25	
	Autumn	24	.21	.03	.01	.19	.22	**0.006**
	Winter	22	.21	.04	.01	.20	.23	
Mother Specific Dog IgE	Spring	33	.19	.05	.01	.18	.21	
	Autumn	24	.21	.03	.01	.20	.22	**0.006**
	Winter	22	.47	1.14	.24	-.04	.97	
Mother Specific Goldenrod IgE	Spring	33	.18	.04	.01	.17	.20	
	Autumn	24	.27	.18	.04	.19	.34	**<0.001**
	Winter	22	.23	.04	.01	.21	.24	
Mother Specific Cladosporidium IgE	Spring	33	.17	.03	.01	.16	.19	
	Autumn	24	.19	.03	.01	.18	.21	**0.005**
	Winter	22	.21	.03	.01	.19	.22	

Kruskall-Wallis

For mothers who did not have doctor-diagnosed asthma, mean serum HDM specific IgE levels were higher in spring (0.791U/mL SD 2.745) when compared to autumn and winter (0.613 SD 1.314 for autumn, 0.645U/mL SD 13.992 for winter, p = 0.024).

Mean Olive and Goldenrod mean specific IgE were higher in autumn (0.509U/mL and 0.265U/mL respectively).

Mean Parietaria, Alternaria, Dog and Cladosporidum serum specific IgE were all higher in the winter months (1.607U/mL, 0.214U/mL, 0.469U/mL and 0.205U/mL respectively). Again of note, are the higher mean serum specific IgE levels for parietaria (0.843U/mL, SD 2.812) in this group when compared to that for HDM (0.697U/mL SD 2.162, $p < 0.001$).

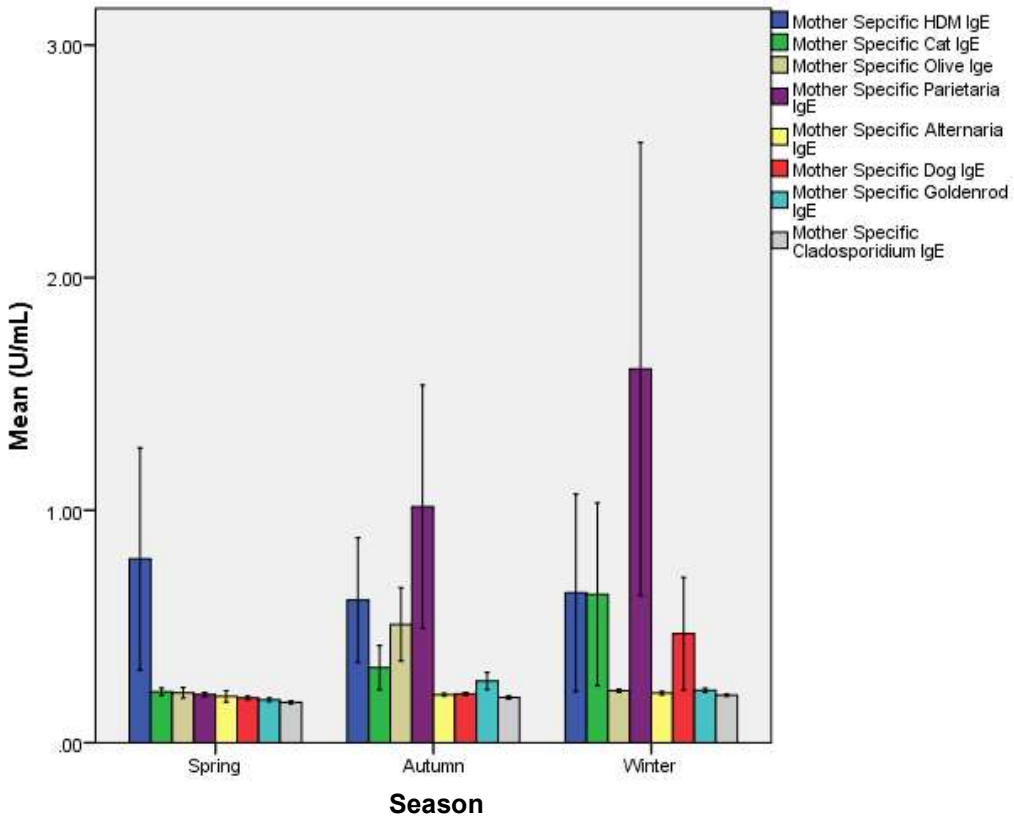

Figure 43: Seasonal differences in mean specific IgE levels for mothers who did not have doctor diagnosed asthma

3.4.7.4 Summary of seasonal serum specific IgE variations in mothers

The results obtained from the analysis of the seasonal variation of mean serum specific IgE levels in the mothers produced results which were in stark contrast with

those obtained from the children and the fathers. First of all, while there was no seasonal variation for specific mean IgE levels for any of the aeroallergens in the father cohort, there were significant seasonal variations for all the specific IgE levels in the mother cohort. One can also note that while the specific IgE levels to HDM were highest in the autumn season in fathers (2.486U/mL SD 4.868) it was lowest in the same season in mothers (0.578U/mL SD 1.267, $p < 0.001$). The other, perhaps surprising finding was that doctor-diagnosed mothers had the lowest mean serum total IgE levels during the autumn season (12.733U/mL, SD 13.008), while case children had the highest levels of serum total IgE in autumn (321.160U/mL, SD 388.282) and doctor-diagnosed asthmatic fathers had the second highest levels after those reached in winter (118.075U/mL, SD 28.167). This was also true for serum specific HDM IgE, where doctor-diagnosed asthmatic mothers had the lowest measured levels in autumn (0.155U/mL, SD 0.049) while these levels were highest in autumn for both case children and doctor-diagnosed asthmatic fathers (8.797U/mL SD 18.638 in the case children group and 4.413U/mL SD 5.422 in the fathers who had doctor-diagnosed asthma).

3.4.7.5 Comparing all children and parents for seasonal serum total and specific IgE variations

The mean total IgE levels for all the children (both cases and controls) and all the parents who participated in the study were compared in figure 44.

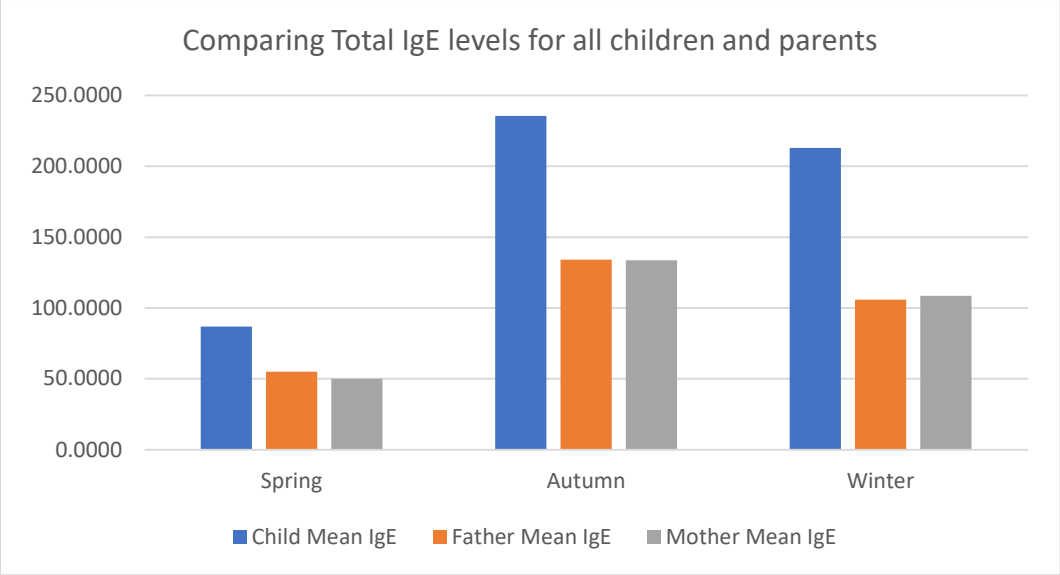

Figure 44: Comparing Total IgE levels for all children and parents

The children and the parents following similar seasonal patterns, although it was evident that the children in this cohort had higher total IgE levels throughout all the seasons. All the groups had their highest mean serum total IgE levels recorded in autumn (235.519U/mL ±329.016U/mL vs 133.992 U/mL ±236.585U/mL in fathers and 133.726U/mL ±234.997UmL in mothers). The lowest levels were recorded in spring (86.851U/mL ±212.560U/mL vs 55.005U/mL ±82.567U/mL in fathers and 50.183U/mL ±125.821U/mL in mothers). One can immediately realise that when all the fathers and the mothers who participated in the study were compared together, the mean in serum total IgE levels throughout the seasons were extremely similar to each other.

In figure 45 the mean serum specific HDM IgE levels for all children and parents were compared according to the different seasons.

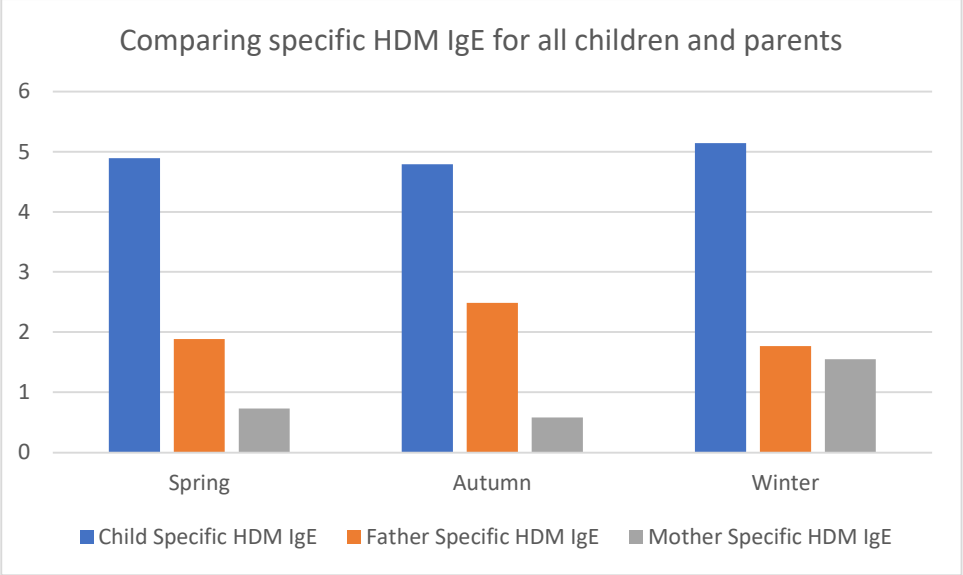

Figure 45: Comparing specific HDM IgE levels for all children and parents

Children had the highest specific HDM IgE levels in winter (5.141U/mL ±10.607mL), while fathers had the highest mean specific IgE levels in autumn (2.486U/mL ±4.868U/mL). The mean specific HDM IgE in mothers had a different seasonal trend, having the highest levels in winter as was the case for children (1.551U/mL ±3.742U/mL) and the lowest mean levels in autumn (0.578U/mL ±1.267U/mL). One can also notice that consistently, the mean seasonal specific HDM levels were the lowest in mothers.

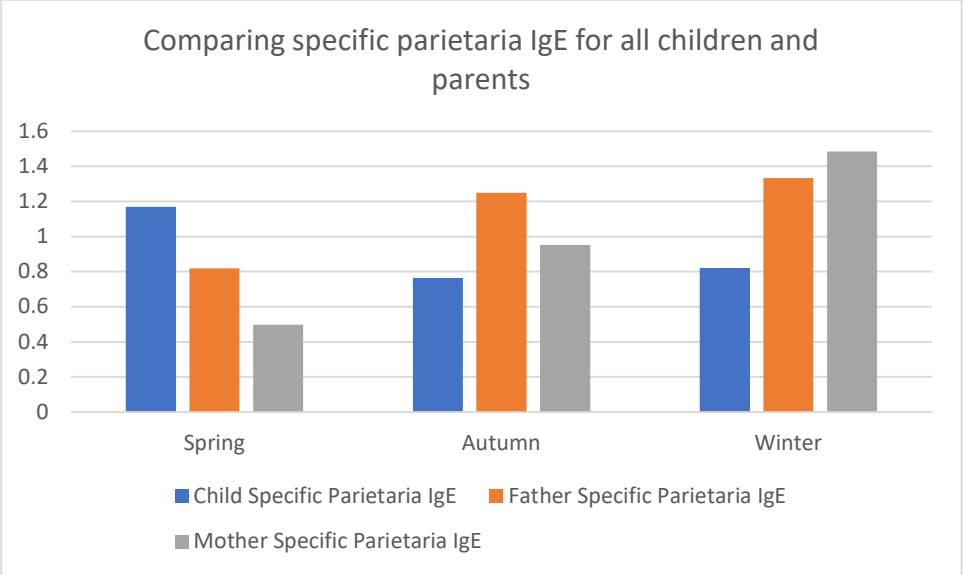

Figure 46: Comparing specific parietaria IgE levels for all children and parents

Children had the highest mean specific *Parietaria* IgE levels in spring (1.168U/mL ±4.444U/mL) while fathers and mothers had the highest specific IgE levels for this aeroallergen in winter (1.332U/mL ±3.260/mL in fathers and 1.485U/mL ±4.218U/mL in mothers).

Specific mean olive IgE levels (another seasonal aeroallergen) was compared by season for the children and parent groups. This is presented in figure 47.

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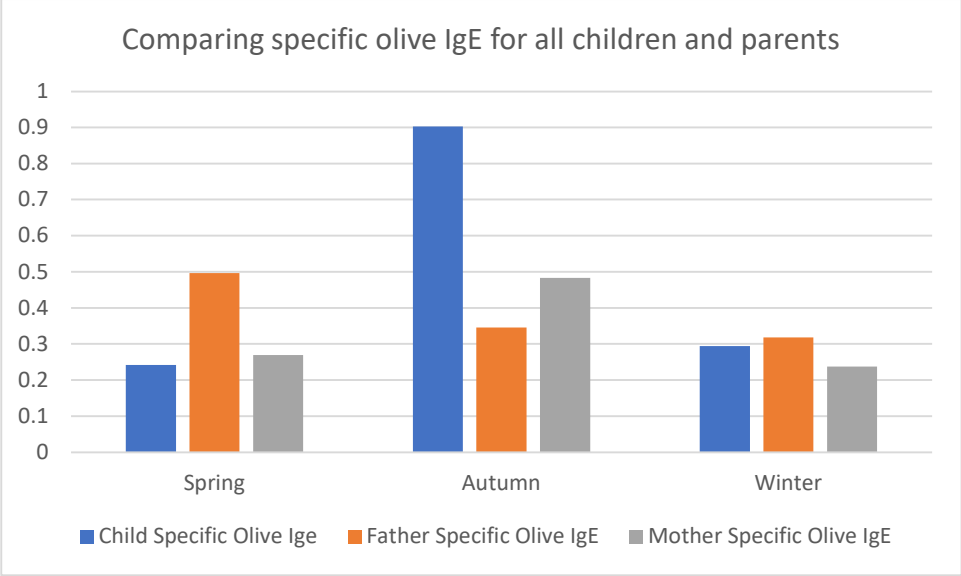


Figure 47: Comparing specific olive IgE levels for all children and parents.

Children and mothers had the highest serum specific IgE levels to olive pollen in autumn (0.903U/mL ±3.825U/mL and 0.483U/mL ±0.746U/mL respectively), while fathers had the highest mean levels in spring (0.496U/mL ±1.496U/mL).

Finally, the mean serum specific cat IgE levels were compared for all children and parents and presented in figure 48.

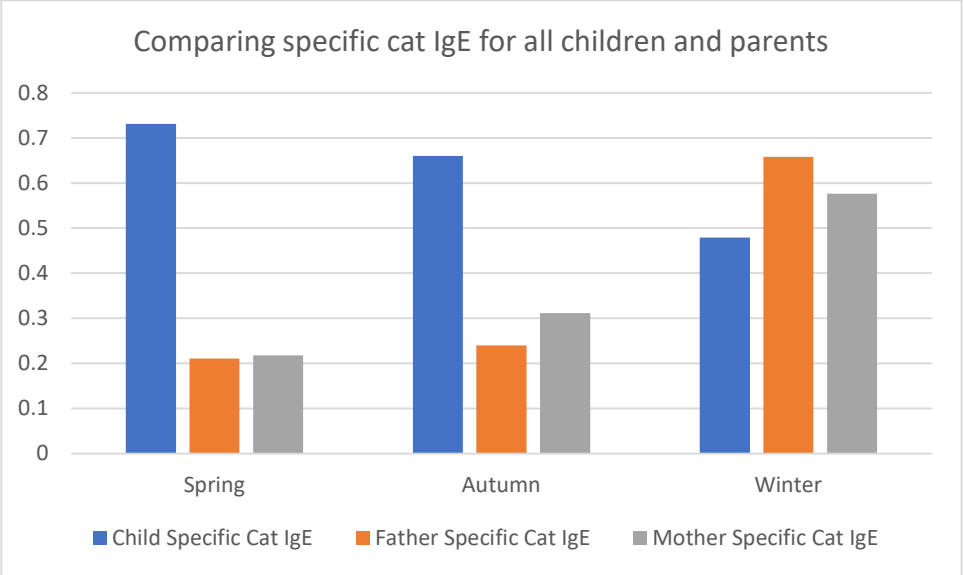


Figure 48: Comparing specific cat IgE levels for all children and parents

Interestingly, while children recorded the highest mean serum specific cat IgE levels in spring (0.731U/mL ±1.348U/mL), parents recorded the highest specific IgE levels for this perennial indoor aeroallergen in winter in fathers (0.658U/mL ±1.143U/mL) and mothers (0.576U/mL ±1.699U/mL).

3.4.7.6 Comparing case children and doctor-diagnosed asthmatic parents for seasonal serum total and specific IgE variations

In this study, case children could have been described as poorly controlled asthmatic children. The serum total IgE levels for this group was compared to doctor-diagnosed asthmatic parents, in figure 49.

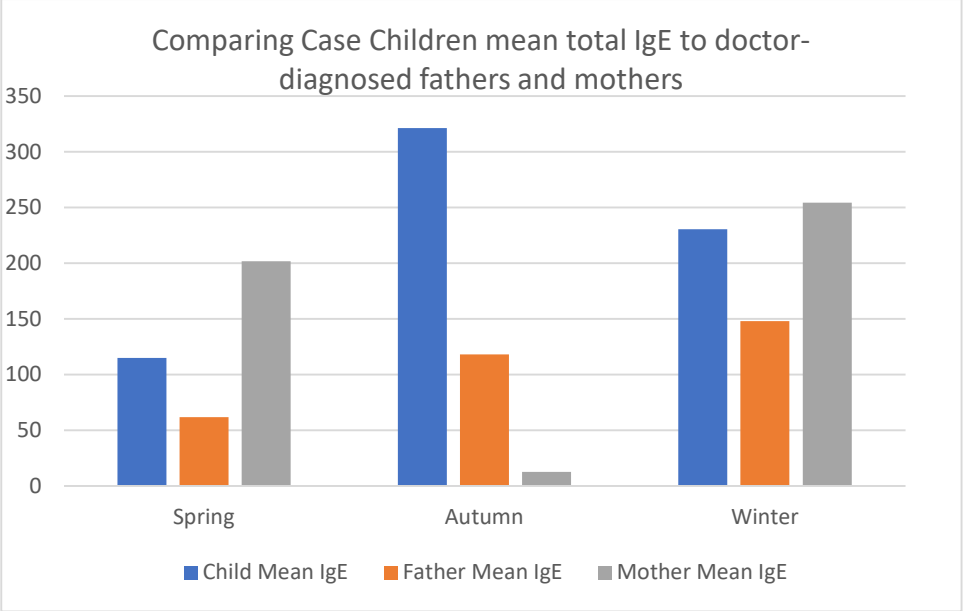


Figure 49: Comparing total serum IgE levels for case children and doctor-diagnosed asthmatic parents

While case children had the highest mean total IgE levels in autumn (321.160U/mL ±388.282U/mL), doctor-diagnosed asthmatic fathers and mothers had the highest total IgE levels recorded in winter (148.020U/mL ±129.953U/mL) in fathers and (254.080U/mL ±239.239U/mL) in mothers. Interestingly, mothers had significantly lower total IgE levels in autumn (13.733U/mL ±13.008U/mL) in contrast to case children and doctor-diagnosed asthmatic fathers who had the lowest levels in spring.

The mean specific HDM levels for case children and doctor-diagnosed asthmatic parents were compared and illustrated in figure 50.

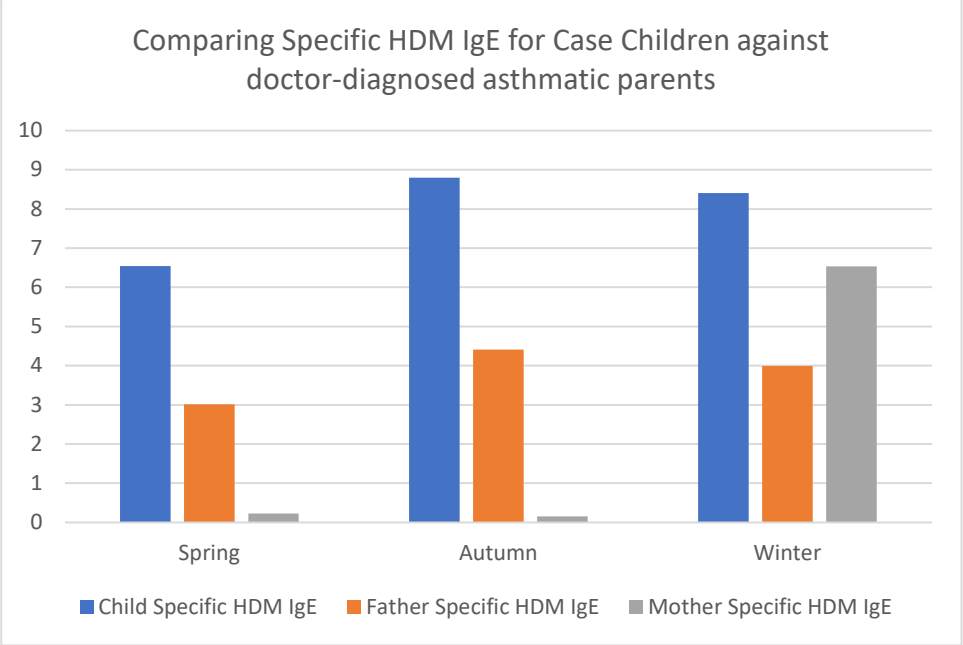


Figure 50: Comparing serum specific HDM IgE levels for case children and doctor-diagnosed asthmatic parents

One could instantly appreciate low HDM specific levels in doctor-diagnosed asthmatic mothers during the spring and autumn seasons (0.230U/mL ±0.080U/mL and 0.155U/mL ±0.049U/mL) while these had the highest levels in winter (6.535U/mL ±7.057U/mL). Case children and doctor-diagnosed asthmatic fathers had similar seasonal patterns to each other, with the highest levels of serum specific HDM IgE levels recorded in autumn (8.797U/mL ± 18.638U/mL in case children and 4.413U/mL ±5.422U/mL in doctor-diagnosed asthmatic fathers) while the lowest mean specific HDM IgE levels in these groups were found during the spring season (6.539U/mL ±13.644U/mL in case children and 3.010U/mL ±5.333U/mL in doctor-diagnosed asthmatic fathers).

The mean serum specific *Parietaria* IgE levels, were compared for case children and doctor-diagnosed asthmatic parents in figure 51.

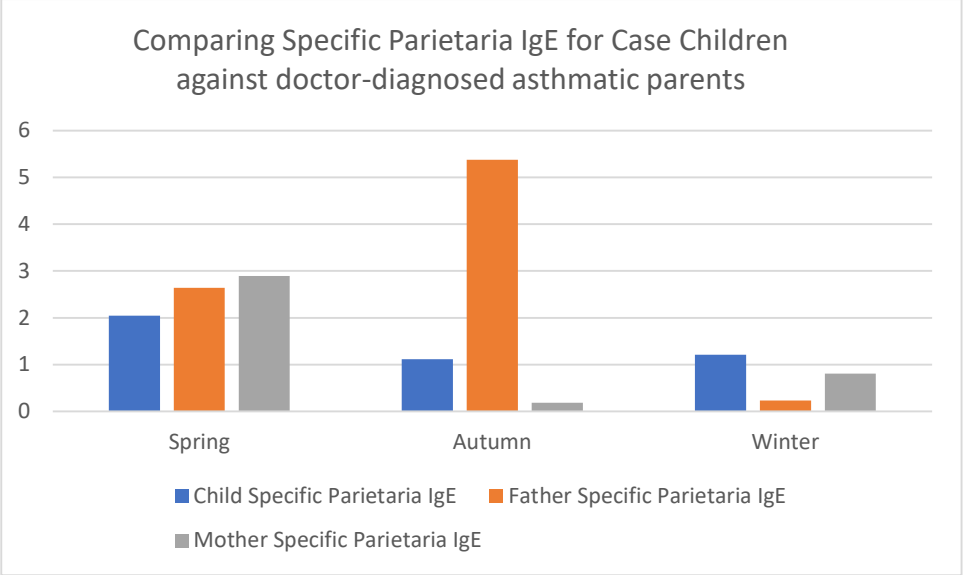


Figure 51: Comparing serum specific *Parietaria* IgE levels for case children and doctor-diagnosed asthmatic parents

Doctor-diagnosed asthmatic fathers had the highest serum specific *Parietaria* IgE levels in autumn (5.377U/mL ±6.021U/mL) while both case children and doctor-diagnosed asthmatic mothers had the highest specific IgE levels to this aeroallergen recorded in spring (2.042U/mL ±6.077U/mL and 2.890U/mL ±5.393U/mL respectively).

Mean serum specific olive IgE levels recorded by case children and doctor-diagnosed parents were compared in figure 52.

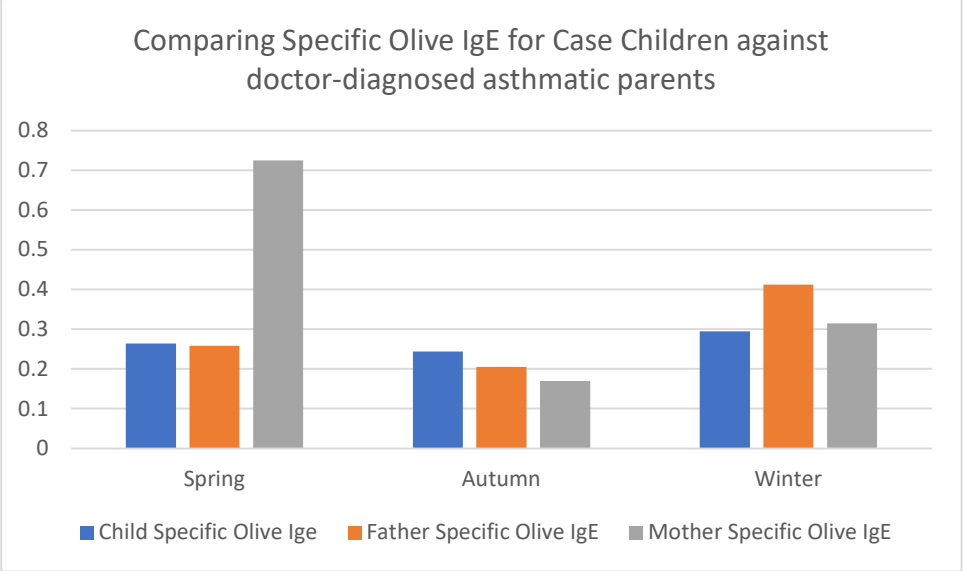


Figure 52: Comparing serum specific olive IgE levels for case children and doctor-diagnosed asthmatic parents

Mothers who had doctor-diagnosed asthma had the highest specific olive IgE levels recorded during the spring season (0.725U/mL ±1.018U/mL) while case children and doctor-diagnosed asthmatic fathers had the highest levels for the aeroallergen recorded during winter (0.295U/mL ±0.200U/mL and 0.413U/mL ±0.366U/mL respectively).

Finally, the mean specific IgE levels for cat were compared between case children and doctor-diagnosed asthmatic parents. This comparison was illustrated in figure 53.

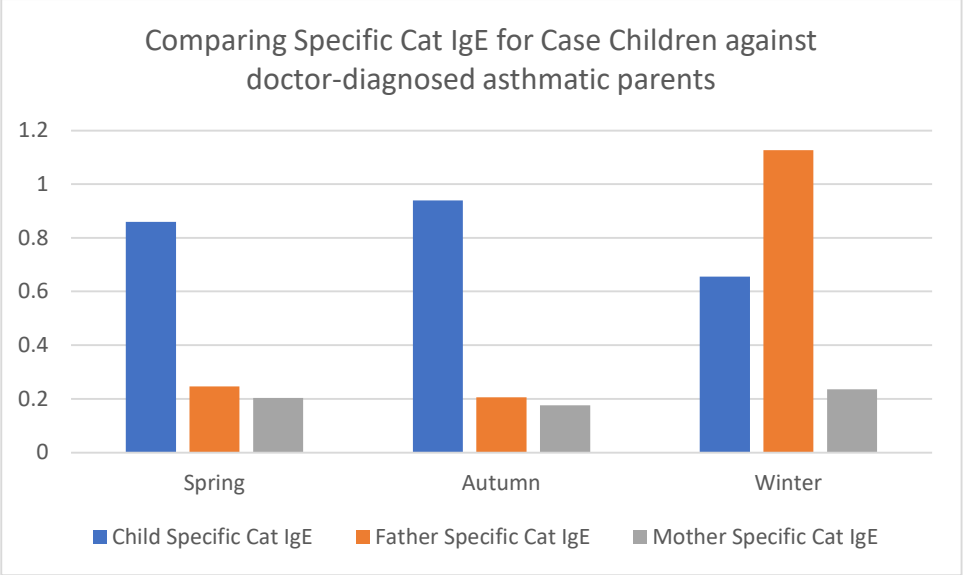


Figure 53: Comparing serum specific cat IgE levels for case children and doctor-diagnosed asthmatic parents

Doctor-diagnosed asthmatic mothers had relatively lower levels for serum specific cat IgE when compared to case children and doctor-diagnosed asthmatic fathers during all seasons. Case children had the highest specific IgE levels recorded for this indoor aeroallergen during autumn (0.940U/mL ±2.196U/mL) closely followed by spring (0.860U/mL ±1.729U/mL). Doctor-diagnosed asthmatic fathers had the highest specific cat IgE levels recorded in winter (1.128U/mL ±1.809U/mL).

3.5 Producing separate groups using cluster analysis

Various authors have postulated the presence of various asthma phenotypes [109,110,111,121]. A landmark study by Haldar et al [109], illustrated the presence of various asthma phenotypes which were present in asthmatic patients at primary and tertiary care levels. Using this knowledge, the "case" children who all had at least two or three symptoms compatible with asthma, were divided into clusters using K-means clustering. K-means clustering is uses the vectors between different variables. K-means clustering aims to group a number of observations into a number clusters in which each observation belongs to the cluster with the nearest mean, which will then act as the defining measure of the cluster. The control group were excluded from this analysis as they would have automatically produced a separate cluster, given the opposite criteria used to select them as subjects with no clinical features of atopic conditions.

3.5.1 Different groups in the children asthma group

The k-means clustering used objective continuous variables, in this case, FEV_1, FEV_1/FVC ratios, exhaled nitric oxide (FeNO) and total IgE levels. This analysis was performed for the Case group only and produced two distinct clusters. These results are presented in tables 54a and 54b.

Table 54: Resulting clusters from case group

	Cluster	
	1	2
FEV_1 ,% predicted	110.4%	102.6%
FEV_1/FVC, %	84.2%	77.0%
Exhaled FeNO, ppb	21	55
Total IgE, U/mL	117.50	1133.20

Table 55: Number of Cases in each Cluster

Cluster	Cluster 1	56
	Cluster 2	7
Valid		63
Missing		3

From the sixty-three (63) cases, sixty (60) were used in this analysis (as there was missing data on three children). K-means clustering produced two clusters from the case group using the abovementioned variables. Case children who were grouped in cluster 2 had lower FEV_1 (mean 102.6% vs 110.4%), lower FEV_1/FVC ratio (mean 84.2 vs 77.0), whilst having higher exhaled nitric oxide (mean 55ppb vs 21 ppb) and total mean IgE level (mean 1133.2U/mL vs 117.5U/mL) when compared to the other case children in cluster 1.

Cluster 1 was the largest group including fifty six (56) subjects. 53.6% (n = 30) were boys, while the rest (n=24, 42.9%) were girls. While cluster 2 consisted of seven (7) children from the case group. Six of these children (85.7%) were boys while 1 (14.3%) was a girl.

Therefore, there were fifty six (56) children who had relatively less airway inflammation (as they had lower FeNO levels), and a lower allergic response (as these had a lower mean total serum IgE) while also having less airway obstruction (as they had higher FEV_1/FVC ratios and $FEV_{1)}$. Seven (7) children had significant allergic response (as these had very high mean serum total IgE levels), more airway inflammation (as these had higher FeNO levels), and more bronchoconstriction (as they had relatively low FEV1/FVC ratio and lower FEV_1).

The clusters were named according to the characteristics described above, and it was decided to call the smaller cluster, the High IgE Cluster, and the larger one, the Low IgE Cluster

Statistical tests were carried out comparing the two groups for various variables and characteristics, and the following results were obtained:

3.5.1.1 Comparison of specific IgE levels for High Total IgE cluster against Low Total IgE Cluster

As the most important variable which separated the two clusters was mean Total IgE, the mean specific IgE levels of the various allergens investigated were also compared. The results are illustrated in table 56.

Table 56: Comparing mean specific IgE levels for various allergens for the two case children clusters

		N	Mean	SD	SE	95% CI Lower Bound	95% CI Upper Bound	p value
Child Specific Olive IgE	High IgE Cluster	6	0.235	0.029	0.012	0.204	0.266	0.747
	Low IgE Cluster	50	0.269	0.220	0.031	0.207	0.332	
Child Specific Goldenrod IgE	High IgE Cluster	6	0.248	0.039	0.016	0.208	0.289	0.069
	Low IgE Cluster	50	0.214	0.046	0.007	0.201	0.227	
Child Specific Parietaria IgE	High IgE Cluster	6	5.695	8.592	3.508	-3.321	14.711	**0.011**
	Low IgE Cluster	50	1.020	3.746	0.530	-0.045	2.084	
Child Specific Cat IgE	High IgE Cluster	6	2.957	4.191	1.711	-1.442	7.355	0.195
	Low IgE Cluster	50	0.576	0.971	0.137	0.300	0.852	
Child Specific Dog IgE	High IgE Cluster	6	0.250	0.038	0.015	0.210	0.290	0.356
	Low IgE Cluster	50	0.304	0.366	0.052	0.200	0.408	
Child Specific Cladosporidium IgE	High IgE Cluster	6	0.200	0.022	0.009	0.177	0.223	0.747
	Low IgE Cluster	50	0.192	0.039	0.006	0.181	0.203	
Child Specific Alternaria IgE	High IgE Cluster	6	0.418	0.521	0.213	-0.129	0.965	0.560
	Low IgE Cluster	50	0.248	0.208	0.029	0.189	0.307	
Child Specific HDM IgE	High IgE Cluster	6	34.962	28.879	11.790	4.655	65.268	**<0.001**
	Low IgE Cluster	50	4.502	8.107	1.146	2.198	6.806	

Mann-Whitney U

The biggest difference in mean serum specific IgE between the clusters was noted for HDM. The mean levels for the High IgE cluster was 35.0U/mL while that for the Low IgE Cluster was 4.50U/mL ($p < 0.001$). The other significant difference in the mean specific IgE levels of was noted in the case of *Parietaria* (5.70U/mL vs 1.02U/mL, $p = 0.011$). There were no significant differences in mean specific IgE levels between the High IgE and Low IgE Case Clusters for the other allergens tested.

Given these findings, a Chi-squared test was then used to analyse whether there were differences in proportions of children in the two clusters, who might have been positive to a skin prick test to the aeroallergens studied. The results are described in table 57.

Table 57: Comparison of Positive SPTs between Case Clusters

	High IgE Cluster		Low IgE Cluster		p value
Allergen	n SPT positive	% SPT positive	n SPT positive	% STP positive	
HDM	6	100	27	49.1	**0.020**
Cockroach	2	33.3	4	7.3	0.102
Olive	1	20	7	12.7	0.524
Grass mix	0	0	0	0	
Parietaria	2	33.3	5	9.1	0.136
Alternaria	2	33.3	4	7.3	0.102
Cat	2	33.3	8	14.5	0.253
Dog	0	0	2	3.6	0.811
Dog tooth grass	0	0	0	0	

All of the cases (n = 6) who formed part of the High Total IgE cluster had an HDM positive SPT (100%), while 49.1% (n = 27/55) of the Low Total IgE cases had SPT positive to HDM (p = 0.020). There were no significant differences in positive skin prick tests for the other aeroallergens between the cases in the two clusters.

3.5.1.2 Regression analysis for Children's Specific IgEs predicting Case Cluster

As both SPTs and specific IgEs were highlighting differences, particularly related to HDM between the two clusters, a multivariate stepwise regression analysis was performed using specific IgE levels as a predictor for the being a member of a High IgE or a Low IgE Case cluster. The results are presented in tables 58.

Table 58: Regression analysis for child specific IgE for case cluster summary

		N	Marginal Percentage
Cluster	High IgE Cluster	6	10.7%
	Low IgE Cluster	50	89.3%
Valid			56
Missing			10
Total			66
Subpopulation			56

A total of fifty-six cases had a complete data cohort of specific IgE levels, in order to be used in this analysis, six (n=6) in the High Total IgE cluster and fifty (n=50) in the Low Total IgE cluster. The pseudo R-Square resuls was of 0.481, meaning that this model could explain 48.1% of the variability in specific IgE levels between the two case clusters.

Table 59: Regression analysis for Specific IgE levels between the two case clusters.

Cluster Number of Case		B	SE	Wald	df	p value	OR	95% CI
High IgE Cluster	Intercept	-3.629	0.837	18.780	1	0.000		
	HDM IgE	0.107	0.037	8.256	1	**0.004**	1.112	1.034-1.196

The stepwise regression analysis found that only mean specific HDM IgE was a significant predictor as a mean specific IgE on the members of a case in being part of the High Total IgE cluster rather than the Low Total IgE cluster. (p = 0.004). A one unit increase in serum specific HDM IgE increases the likelihood of being a member of the High IgE cluster by 1.112 times.

As there seemed to be a consistent trend between the presence of house dust mite atopy and a higher risk of a Case child to be a member of the High IgE cluster, the mean levels of HDM allergen in the dust collected from the homes, together with the

other aerollergens collected from these samples were compared for the two Case clusters. The results are presented in table 60.

Table 60: Comparing mean allergen house dust levels between the two case clusters

	Cluster Number of Case	N	Mean (µg/g)	SD	p value
HDM levels in house dust	High IgE Cluster	2	22.441	26.815	**0.017**
	Low IgE Cluster	36	1.488	3.135	
Cat Levels in house dust	High IgE Cluster	2	2.398	1.582	0.284
	Low IgE Cluster	36	3.225	8.610	
Alternaria Levels in house dust	High IgE Cluster	2	0.176	0.249	0.396
	Low IgE Cluster	35	0.021	0.073	
Timothy Grass Pollen Levels in house dust	High IgE Cluster	2	0.000	0.000	0.728
	Low IgE Cluster	36	0.112	0.268	
LPS Levels in house	High IgE Cluster	2	2.571	1.627	0.518
	Low IgE Cluster	36	1.685	0.930	

Mann-Whitney U

Unfortunately, only two houses of the seven cases of the High Total IgE group were sampled. This was due to either families refusing house dust sampling, or their houses incidentally not being chosen for houses analysis (there were not enough funds to sample the houses of all the families who participated in the clinical phase). House dust mite allergen had the highest mean house dust levels (22.441µg/g) in the High Total IgE Case group when compared to the Low IgE Case group (1.488(µg/g, p = 0.017).

Given the small number of children in the High IgE cluster who had their house dust sampled, one can reference to the paper published by Ruggieri et al (2017), in which the data of the children who participated in this study in Malta had their data used together with the children from Sicily who participated in the same project (Respira). One should note that all these children had their serum IgE and house dust levels analysed in the same labs and under the same conditions. In this cluster analysis,

Cluster 1 (n = 24) had high Specific HDM IgE while Cluster 2 (n = 43) had low specific HDM IgE. Similarly, to the results presented in this chapter, cluster 1 the analysis by Ruggieri et al had higher FeNO than cluster 2. Similarly, the children who were part of cluster 1 had significantly higher (p = 0.007) mean home HDM dust allergen level [2.1µg/g (0.8-30.1 µg/g)] than the second cluster [0.5 µg/g (0.2-2.3 µg/g]. Hence, the results have been replicated when a larger cohort was analysed using the same methodology.

Since damp environments encourage the presence of HDM and mould, features reported through the questionnaires, present in the houses which suggested the presence of humidity were compared for the two Case clusters, and presented in table 61.

Table 61: Comparison of home humid conditions between case clusters

	High IgE Cluster		Low IgE Cluster		p value
Questions on mould presence	n SPT positive	% SPT positive	n SPT positive	% SPT positive	
Water leakage or water damage indoors in walls, floor or ceiling.	2	28.6	9	17.3	0.388
Visible mould growth indoors on walls, floor or ceiling.	4	57.1	23	44.2	0.403
The smell of mould in one or more rooms	4	57.1	17	32.1	0.186
Is it common with dampness/condensation on the lower part of the windows in winter?	7	100	34	65.4	0.066
Is there dampness or visible mould growth in your child's bedroom?	2	28.6	14	25.9	0.597
During the past 5 years, have any dampness problem/water damage/visible mould growth/smell of mould occurred in the dwelling?	4	57.1	17	30.9	0.168
Signs of Humidity at Home?	7	100	38	67.9	0.82

In this analysis, it was noted that there was a strong trend (p=0.066) for cases in the High Total IgE cluster (n=7/7, 100%) to report dampness/condensation in the lower part of the windows in winter more than cases in the Low Total IgE cluster (n=34/55, 65.4%).

This Chi Squared test was performed on a relatively small sample, and this could have been a potential reason for which statistical significance was not reached. This trend increases the suspicion that a damp indoor environment has a role in having a High Total IgE mediated asthma phenotype being expressed.

3.5.2 Different groups in the Father Cohort

The cohort of fathers who participated in the study were also analysed through a K-means cluster analysis, and the same methodology used for the children was applied. In this case, all the fathers (n=81) who had all the data necessary for this analysis collected (Total IgE, FeNO, FEV_1 and FEV_1/FVC ratio), were included rather than including only the fathers of the case children. This was done as there was no particular selection process through which these fathers were chosen, dividing them into cases or controls, and hence the total sample was not meant to be inhomogeneous.

The cluster analysis again resulted in two groups, and these were similarly characterised by a high Total IgE, high FeNO, and low predicted FEV_1 and FEV_1/FVC ratios which comprised of six (6) fathers, and a Low Total IgE, FeNO with higher predicted FEV_1 and FEV_1/FVC ratios group of eighty one (81) fathers. One should note, as previously mentioned, that not all the fathers presented to clinic

together with their family, resulting in not having data on 34 fathers. The results from the clustering exercise are presented in tables 62 and 63.

Table 62: Father Clusters

	Cluster	
	1	2
Father's Total IgE	56.3U/mL	619.7U/mL
Father's FeNO	16ppb	32ppb
Father's FEV_1/FVC ratio	109.88	96.00
Father's Predicted FEV_1	106.01%	101.17%

Table 63: Number of Cases in each Father Cluster

Cluster	1	81
	2	6
Valid		87
Missing		34

Given these results, the first cluster was named the Father Low Total IgE cluster while the second was termed the Father High Total IgE cluster.

It was important to ascertain whether there were any differences in the proportion of fathers who had a past history of asthma between the two groups. This information was obtained through the parent questionnaire and presented in table 64.

Table 64: Father Cluster Members having a history of asthma

			Cluster Number of Case		
			Father High IgE Cluster	Father Low IgE Cluster	Total
Father has asthma	Yes	Count	1	15	16
		percentage	16.7%	18.5%	18.4%
	No	Count	5	66	71
		percentage	83.3%	81.5%	81.6%
Total		Count	6	81	87
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.013$, $p = 0.696$

Interestingly only one (n = 1, 16.7%) of the fathers who were members of the High IgE cluster reported being asthmatic. The other five (83.3%) did not report having asthma. Fifteen (n = 15, 18.5%) fathers from the Low IgE cluster gave a history of asthma, while the other sixty six (n = 66, 81.5%) did not do so.

A similar comparison was performed for a history of allergic rhinitis, and the results illustrated in table 65.

Table 65: Father Cluster Members having a history of allergic rhinitis

			Cluster Number of Case		
			Father High Total IgE Cluster	Father Low Total IgE Cluster	Total
Father has allergic rhinitis	Yes	Count	5	64	69
		percentage	83.3%	79.0%	79.3%
	No	Count	1	17	18
		percentage	16.7%	21.0%	20.7%
Total		Count	6	81	87
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.064$, $p = 0.638$

In this instance, 83.3% (n = 5/6) of the fathers in the High IgE cluster reported having a history of allergic rhinitis. Similarily, 79% (n = 64/81) of the fathers in the Low IgE group gave a similar response, which also illustrates the high likelihood of adults to have had such symptoms and reporting this condition.

3.5.2.1 Comparing specific IgE levels for Father High IgE cluster against Father Low IgE Cluster

In a similar methodology to the one used to analyse the relationships of the mean specific IgE levels in the Case children, the mean specific IgE levels for the aeroallergens which were tested for were compared for the Father High IgE Cluster and the Father Low IgE Cluster. The results are presented in table 66 .

Table 66: Comparing mean specific IgE levels for allergens for the two Father clusters

		N	Mean	SD	SE	95% CI Lower Bound	95% CI Upper Bound	p value
Father Specific Olive IgE	Father High IgE Cluster	5	1.086	1.090	0.488	-0.268	2.440	**< 0.001**
	Father Low IgE Cluster	63	0.351	0.997	0.126	0.100	0.602	
Father Specific Goldenrod IgE	Father High IgE Cluster	5	0.258	0.056	0.025	0.189	0.327	0.156
	Father Low IgE Cluster	63	0.218	0.056	0.007	0.204	0.233	
Father Specific Parietaria IgE	Father High IgE Cluster	5	0.360	.210	0.094	0.099	0.621	0.171
	Father Low IgE Cluster	63	1.206	3.348	0.422	0.363	2.049	
Father Specific Cat IgE	Father High IgE Cluster	5	1.692	1.961	0.877	-0.743	4.127	**0.003**
	Father Low IgE Cluster	63	0.259	0.300	0.038	0.183	0.335	
Father Specific Dog IgE	Father High IgE Cluster	5	0.202	0.008	0.004	0.192	0.212	0.478
	Father Low IgE Cluster	63	0.205	0.070	0.009	0.187	0.222	
Father Specific Cladosporidium IgE	Father High IgE Cluster	5	0.194	0.034	0.015	0.151	0.237	0.820
	Father Low IgE Cluster	63	0.199	0.075	0.009	0.180	0.218	
Father Specific Alternaria IgE	Father High IgE Cluster	5	0.188	0.013	0.006	0.172	0.204	0.750
	Father Low IgE Cluster	63	0.200	0.051	0.006	0.187	0.213	
Father Specific HDM IgE	Father High IgE Cluster	5	10.792	7.038	3.148	2.053	19.531	**<0.001**
	Father High IgE Cluster	5	1.086	1.090	0.488	-0.268	2.440	

Mann-Whitney U

The three-mean specific IgE levels which were significantly higher in the Father High Total IgE group when compared to the Father Low Total IgE group were House Dust Mite, Cat dander and Olive Pollen. In fact, the mean serum specific IgE for HDM for the Father High Total IgE cluster was 10.8U/mL (SD 1.09U/mL) while the mean for the Father Low Total IgE cluster was only 0.35U/mL (SD 1.00 U/mL, $p < 0.001$). Similarly, the mean specific Cat IgE for the Father High IgE cluster was 1.69U/mL (SD 1.96U/mL), while that for the Father Low Total IgE cluster was

0.26U/mL (SD 0.30U/mL, p = 0.003). Interestingly, specific IgE to Olive tree pollen was also much higher in the Father High Total IgE Cluster (1.09U/mL, SD 1.09U/mL) when compared to the mean specific Olive tree pollen IgE in the Low Total IgE Cluster (0.351U/mL, SD 0.997U/mL) with a strong p value of < 0.001).

Given these findings, a Chi-squared test was then used to analyse whether there were significant differences in proportions of fathers in the two groups who had positive skin prick tests for the aeroallergens tested during the clinical phase of the study. The results are presented in table 67.

Table 67: Comparison of Positive SPTs between Father Clusters

	Father High IgE Cluster		Father Low IgE Cluster		p value
Allergen	N SPT positive	% SPT positive	N SPT positive	% SPT positive	
HDM	4	66.7	26	32.9	0.112
Cockroach	0	0	10	12.7	0.460
Olive	2	33.3	10	12.8	0.203
Grass mix	1	16.7	12	15.2	0.643
Parietaria	2	33.3	14	17.7	0.315
Alternaria	1	16.7	7	8.9	0.458
Cat	1	16.7	9	11.5	0.544
Dog	1	16.7	6	7.6	0.413
Dog tooth grass	0	0	6	7.6	0.636

Interestingly, when the Positive SPTs were compared between the two Father Clusters, there were no statistical significant differences for any of the aeroallergens tested.

This contrasts to the findings discussed in the analysis of the specific IgE results. A possible hypothesis for this would be that previous exposure and development of atopy to an allergen would have developed earlier in the life of these adults yet this would not have been expressed at the time of the study due to either avoidance of the allergen or change in the extent of exposure. On the other hand, when specific IgE levels are considered, one is observing the result of current exposure, as serum specific IgE increases according to exposure to the allergen (dust levels), as has been noted for HDM in children earlier in this thesis.

3.5.2.2 Regression analysis for father's Specific IgEs predicting Father Cluster

A multivariate stepwise regression analysis was performed to identify which specific IgE tests are the strongest predictors for whether a Father would be a member of the High Total IgE or Low Total IgE cluster. Sixty eight (68) fathers were analysed in this model, five (5) being members of the High Total IgE cluster while sixty three (63) were in the Low Total IgE cluster. The Pseudo R-Square for this model was 0.486. The results are presented in table 68.

Table 68: Regression analysis for Specific IgE levels between the two Father Clusters.

Cluster	Number of Case	B	SE	Wald	df	p value	OR	95% CI
High IgE Cluster	Intercept	-4.134	0.909	20.706	1	0.000		
	Cat IgE	1.073	0.660	2.642	1	0.104	2.924	0.802 – 10.666
	HDM IgE	0.189	0.078	5.797	1	**0.016**	1.208	1.036 – 1.408

This model found that the father's specific HDM IgE was the strongest predictor for a Father to be a member of the High Total IgE cluster (p = 0.016) with and Odd's Ratio of 1.208.

3.5.3 Different groups in the Mother Cohort

K-means clustering was finally used to classify the mothers who participated in the study. As was the case for the fathers, the mothers were not chosen on a case-control basis, but as being the mothers of the children who participated in the study. Hence, all the mothers were included in this analysis, in total one hundred and thirteen (16 mothers had missing data). The same methodology was used as for the fathers and the children (Mothers' Total IgE, FeNO, Predicted FEV_1 and FEV_1/FVC ratio were used as discriminating variables). The results of this clustering is presented in tables 69 and 70, and as for the two previous chapters, resulted in two clusters: Cluster 1, with 9 members, who were Mothers with a High IgE and Cluster 2, with 104 mothers, who represented a Mother Low Total IgE cluster.

Table 69: Mother Clusters

	Cluster	
	1	2
Mother's Total IgE	660.1U/mL	43.5
Mother's FeNO	18ppb	12ppb
Mother's predicted FEV_1	117.2%	110.9%
Mother's FEV_1/FVC	80.22	80.06

Table 70: Number of Cases in each Mother Cluster

Cluster	1	9
	2	104
Valid		113
Missing		16

The proportion of mothers who were members of the High Total IgE group and who reported having asthma was compared to that of the Low Total IgE cluster. The results are presented in table 71.

Table 71: Mother Cluster Members having a history of asthma

			Cluster Number of Case		
			Mother High IgE Cluster	Mother Low IgE Cluster	Total
Mother has asthma	Yes	Count	2	11	13
		percentage	22.2%	10.6%	11.5%
	No	Count	7	93	100
		percentage	77.8%	89.4%	88.5%
Total		Count	9	104	113
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.103$, $p = 0.276$

Similar to the results obtained from the fathers in the equivalent analysis 22.2% (n=2) of the mothers in the High Total IgE Cluster reported a history of asthma, while 77.8% (n = 7) were not asthmatic. Only 10.6% (n=11) of the Low Total IgE Mothers admitted to a history of asthma in their questionnaires, with the rest, 89.4% (n = 93) not having such a history.

The same comparison was performed for the question where the mothers were asked to report a history of allergic rhinitis. As was in the case of the fathers, the majority (66.7%, n=6) of the High Total IgE mothers, and even the Low IgE mothers (84.6%, n = 88) reported a history of allergic rhinitis (see table 72). In fact, the vast majority of the mothers in this analysis (83.2%, n=94/113) reported rhinitis.

Table 72: Mother Cluster Members having a history of allergic rhinitis

			Cluster Number of Case		
			Mother High IgE Cluster	Mother Low IgE Cluster	Total
Mother has allergic rhinitis	Yes	Count	6	88	94
		percentage	66.7%	84.6%	83.2%
	No	Count	3	16	19
		percentage	33.3%	15.4%	16.8%
Total		Count	9	104	113
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.908$, $p = 0.174$

3.5.3.1 Comparison of specific IgE levels for Mother Total High IgE cluster vs Mother Low Total IgE cluster.

The results of the mothers' mean specific IgE levels for the aeroallergens analysed in the study were compared between the two clusters obtained from the mother cohort. The results are illustrated in table 73.

Table 73: Comparing mean specific IgE levels for allergens for the two Mother clusters

						95% CI		
		N	Mean	SD	SE	Lower Bound	Upper Bound	p value
Mother Specific Olive IgE	Mother High IgE Cluster	6	1.245	1.200	.490	-.014	2.504	**0.005**
	Mother Low IgE Cluster	80	.257	.295	.033	.192	.323	
Mother Specific Goldenrod IgE	Mother High IgE Cluster	6	.235	.032	.013	.201	.269	0.151
	Mother Low IgE Cluster	80	.218	.111	.012	.194	.243	
Mother Specific Parietaria IgE	Mother High IgE Cluster	6	3.758	8.169	3.335	-4.815	12.331	**0.014**
	Mother Low IgE Cluster	80	.732	2.070	.231	.271	1.193	
Mother Specific Cat IgE	Mother High IgE Cluster	6	.217	.043	.018	.171	.262	0.832
	Mother Low IgE Cluster	80	.363	1.002	.112	.140	.586	
Mother Specific Dog IgE	Mother High IgE Cluster	6	.210	.023	.009	.186	.234	0.825
	Mother Low IgE Cluster	80	.273	.600	.067	.139	.406	
Mother Specific Cladosporidium IgE	Mother High IgE Cluster	6	.190	.037	.015	.151	.229	0.885
	Mother Low IgE Cluster	80	.189	.034	.004	.181	.197	

Mother Specific Alternaria IgE	Mother High IgE Cluster	6	.212	.031	.013	.179	.244	0.182
	Mother Low IgE Cluster	80	.211	.121	.013	.184	.238	
Mother Specific HDM IgE	Mother High IgE Cluster	6	2.533	3.856	1.574	-1.513	6.580	**0.027**
	Mother High IgE Cluster	80	.635	2.037	.228	.182	1.088	

The most significant difference for mean specific IgE between the two groups was recorded for Olive tree pollen, with a mean of 1.25U/mL (SD 1.20U/mL) for the Mother High Total IgE cluster against a mean of 0.26U/mL (SD 0.02U/mL) for the Mother Low Total IgE cluster (p = 0.005). On the other hand, the mean specific IgE to *Parietaria* was the highest mean recorded in the Mother High Total IgE cluster at 3.76U/mL (SD 8.17) against 0.73U/mL (SD 2.07U/mL) for the Mother Low Total IgE cluster (p = 0.014). This was followed by mean specific IgE to HDM who in the Mother High Total IgE cluster was 2.533U/mL (SD 3.856U/mL), while this was only 0.64U/mL (SD 2.04U/mL) for the Mother Low Total IgE group (p= 0.027).

A Chi-squared test was used to analyse any differences in proportions of positive SPTs for the aeroallergens investigated in the study, between the two Mother clusters. The results are presented in table 74.

Table 74: Comparison of Positive SPTs between Mother Clusters

	Mother High IgE Cluster		Mother Low IgE Cluster		p value
Allergen	n SPT positive	% SPT positive	n SPT positive	% SPT positive	
HDM	5	55.6	20	19.4	**0.025**
Cockroach	2	22.2	8	7.8	0.187
Olive	3	33.3	12	11.8	0.102
Grass mix	1	11.1	9	8.7	0.583
Parietaria	2	22.2	15	14.6	0.410
Alternaria	1	11.1	8	8.0	0.554
Cat	1	11.1	10	9.7	0.620
Dog	1	11.1	9	8.8	0.587
Dog tooth grass	0	0	5	4.9	0.653

The only significant skin prick test which was significantly more positive in the Mother High Total IgE cluster was that for HDM, with 55.6% (n = 5/9) of the mothers in this group testing positive for this allergen against 19.4% (n = 20/103) in the Mother Low Total IgE group (p = 0.025).

3.5.3.2 Regression analysis for mother's Specific IgEs predicting Mother Cluster

A multivariate stepwise regression analysis was performed to identify the strongest predictors, in terms of specific IgE levels, for a mother to be a member of the High Total IgE cluster. The data of eighty-six (86) mothers was used in this analysis (43 had missing data). Six (6) were members of the Mother High Total IgE cluster while eighty (80) mothers were in the Low Total IgE cluster. The results can be seen in table 75 .

Table 75: Regression analysis for Specific IgE levels between the two Mother Clusters.

Cluster Number of Case		B	SE	Wald	df	p value	OR
Mother High IgE Cluster	Intercept	-12.288	6.226	3.896	1	0.048	
	Olive IgE	7.548	3.701	4.159	1	**0.041**	$1.897x10^3$
	Parietaria IgE	0.421	0.166	6.466	1	**0.011**	1.523

Interestingly, while in the case of the children and father clusters, specific HDM IgE was the strongest predictor, this was not the case for the mothers. In fact, the strongest predictor for a mother to be a member of the High Total IgE cluster was Parieteria specific IgE, with an Odds ratio of 1.523 and a p value of 0.011. This was followed by Olive IgE (p = 0.041).

3.5.4 Associations between child and parent cluster membership

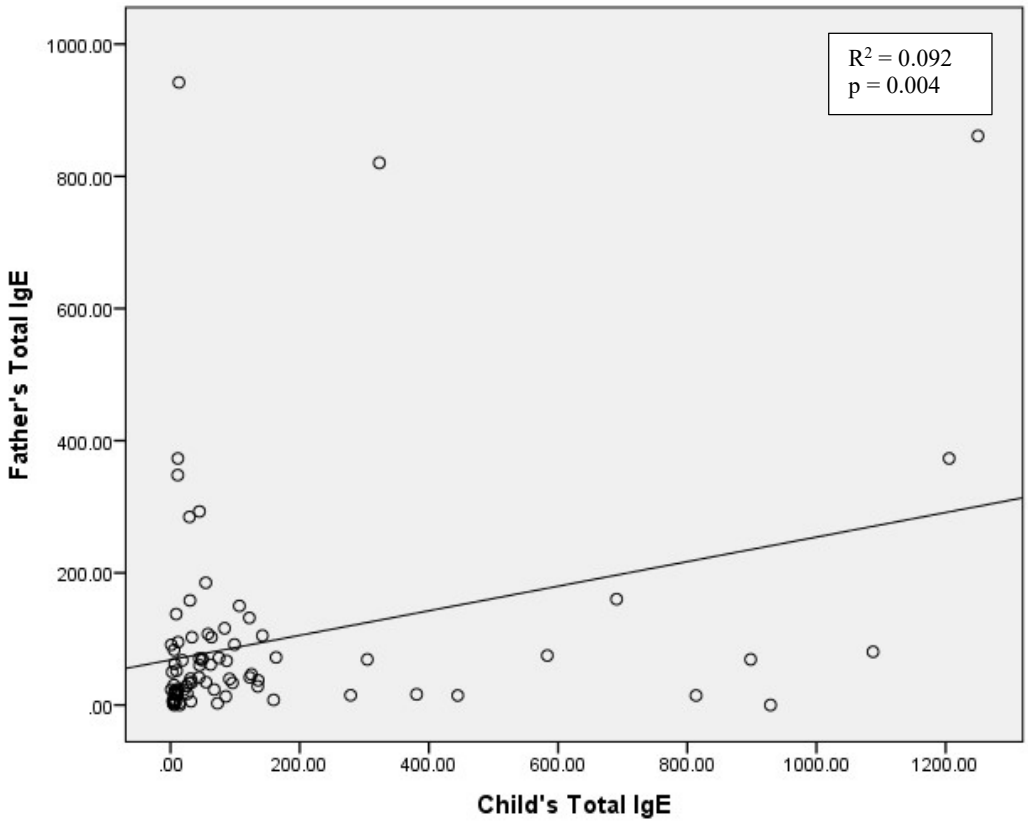
The previous chapters have described how the children and parents who had participated in the study were subdivided using K-means clustering. This consistently resulted in two clusters for each group, a smaller one exhibiting high serum total IgE levels, and a larger one with members who had lower serum total IgE levels. This chapter aims to describe the associations between these groups, most particularly comparing the parents to the children, and revealing any correlations.

As the strongest predictor in the clustering models for the groups was total serum IgE level, a Spearman's correlation method was used to test for a correlation between the children's total serum IgE level and the fathers' total serum IgE level. A weakly positive correlation was found (p = 0.004). (see table 76 and figure 54).

Table 76: Correlation between Fathers and Children's serum total IgE

			Father's Total IgE	Child's Total IgE
Spearman's rho	Father's Total IgE	Correlation Coefficient	1.000	0.314
		Sig. (2-tailed)	.	**0.004**
		N	91	84
	Child's Total IgE	Correlation Coefficient	.314	1.000
		Sig. (2-tailed)	**0.004**	.
		N	84	114

Figure 54: Correlation between Fathers and Children's serum total IgE



The same correlation method was used to test for a correlation between the children's total serum IgE and the mothers' total serum IgE. Again, there was a weakly positive correlation. (p = 0.004). (see table 77 and figure 55).

Table 77: Correlation between Mothers' and Children's serum total IgE

			Mother's Total IgE	Child's Total IgE
Spearman's rho	Mother's Total IgE	Correlation Coefficient	1.000	0.273
		Sig. (2-tailed)	.	**0.004**
		N	116	107
	Child's Total IgE	Correlation Coefficient	0.273	1.000
		Sig. (2-tailed)	**0.004**	.
		N	107	114

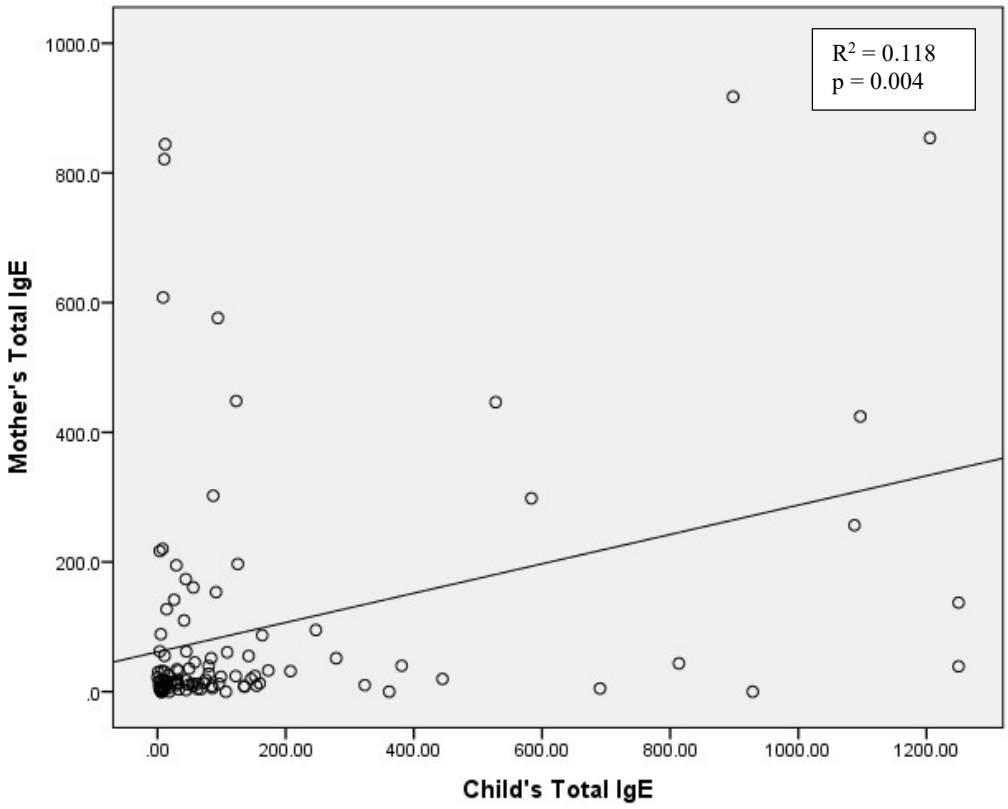


Figure 55: Correlation between Mothers' and Children's serum total IgE

The previous chapters also described the strong relationship between specific serum HDM IgE level and the serum Total IgE levels for each group. It was therefore

important to test for a correlation for the levels of serum specific HDM IgE between the parents' and children.

There was a correlation (p = 0.008) for the fathers' serum specific HDM levels and that for the children who participated in the study (table 78 figure 56).

Table 78**:** Correlation between Fathers' and Children's serum specific HDM IgE

			Father Specific HDM IgE	Child Specific HDM IgE
Spearman's rho	Father Specific HDM IgE	Correlation Coefficient	1.000	0.328
		Sig. (2-tailed)	.	**0.008**
		N	71	64
	Child Specific HDM IgE	Correlation Coefficient	0.328	1.000
		Sig. (2-tailed)	**0.008**	.
		N	64	103

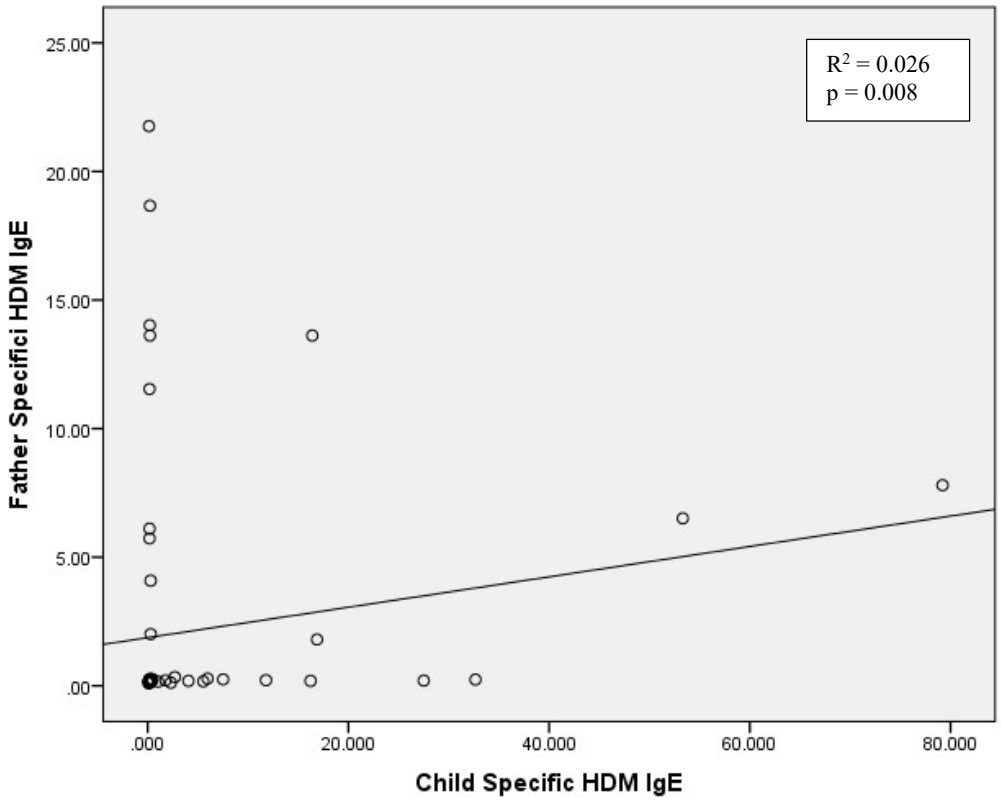


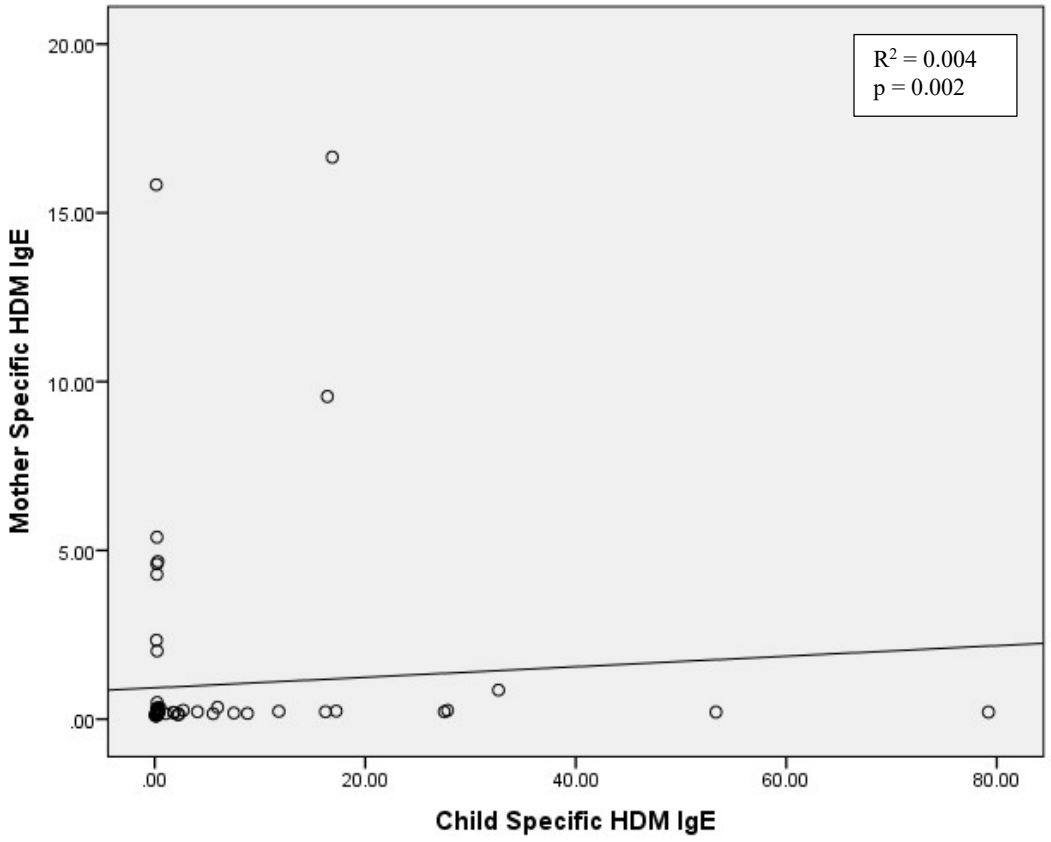
Figure 56**:** Correlation between Fathers' and Children's serum specific HDM IgE

A similar correlation was found between the mothers' serum specific HDM levels and the childrens' specific HDM levels (p = 0.002). The results are presented in table 79 and figure 57.

Table 79**:** Correlation between Mothers' and Children's serum specific HDM IgE

			Mother Specific HDM IgE	Child Specific HDM IgE
Spearman's rho	Mother Specific HDM IgE	Correlation Coefficient	1.000	0.338
		Sig. (2-tailed)	.	**0.002**
		N	89	81
	Child Specific HDM IgE	Correlation Coefficient	0.338	1.000
		Sig. (2-tailed)	**0.002**	.
		N	81	103

Figure 57**:** Correlation between Mothers' and Children's serum specific HDM IgE



These results showed that there were correlations between the serum IgE levels in the children and the parents (both fathers and mothers). This was not only true for

the total IgE levels, but also the specific IgE levels to HDM, which was consistently the most important aeroallergen. The results presented in chapter 3.1.6, which showed that a family history of asthma or another allergic condition, was a strong predictor for a child to be a case rather than a control in this study, one would suspect that this inheritance pattern could be related to IgE. Using such an argument, one could speculate that there could be a relationship between the described High IgE cluster memberships in the separate groups (fathers, mothers and children).

Considering these findings, a Chi-squared analysis was used to assess for a relationship between the High IgE Children group and the High IgE Father and Mother groups.

Table 80: Father Cluster Members having a history of asthma

			Cluster Number of Case		
			Father High IgE Cluster	Father Low IgE Cluster	Total
Child Cluster	High IgE Cluster	Count	2	2	4
		percentage	50.0%	50.0%	100.0%
	Low IgE Cluster	Count	2	34	36
		percentage	5.6%	94.4%	100.0%
Total		Count	6	36	40
		percentage	10.0%	90.0%	100.0%

$X^2(1) = 7.901$, $p = 0.043$

Fifty percent (n=2) of case children in the high IgE cluster had fathers who were also members of the respective High IgE cluster, while 94.4% (n = 34) of cases in the Low IgE cluster had fathers in the respective Low IgE cluster. These differences are significant at $p = 0.043$. While the results are limited by the small numbers in the High IgE clusters, especially since a number of fathers did not attend the clinical

phase of the study, it was evident that is was less likely for a case child in the Low IgE cluster to have a father in the High IgE cluster (5.6%, n=2/36 had this occurrence).

Table 81: Mother Cluster Members having a history of asthma

			Cluster Number of Case		
			Mother High IgE Cluster	Mother Low IgE Cluster	Total
Child Cluster	High IgE Cluster	Count	2	4	6
		percentage	33.3%	66.7%	100.0%
	Low IgE Cluster	Count	3	50	53
		percentage	5.7%	94.3%	100.0%
Total		Count	6	5	54
		percentage	10.0%	8.5%	91.5%

$X^2(1) = 5.321$, $p = 0.076$

In this analysis, there was actually a higher frequency of cases in the High IgE cluster who had mothers in the Low IgE cluster (66.7% n = 4/6, when compared to 33.3%, n = 2/6 in the High IgE group who had mothers in the respective High IgE group). On the other hand, similarly to what had been observed when comparing the Low IgE cases to the Low IgE fathers, 94.3% (n = 50/53) Low IgE group cases had Low IgE group mothers. Only 5.7% (n=3/53) of the Low IgE cases had a mother in the respective High IgE group. These differences failed to reach statistical significance (p = 0.076) but were constrained by the limited number of participants who fell in the High IgE groups.

Finally, a logistic regression model was used to examine whether having a parent in a particular cluster increased the likelihood of a child to be a member of the High IgE case group. The Pseudo R-square test used for the logistic regression being presented

here, predicts that this model will explain a total variation of 24.5% of this population (Nagelkerke = 0.245).

Table 82: Logistic Regression for child and parent cluster relationship

		N	Marginal Percentage
Cluster membership of Child (Case)	High IgE Cluster	4	10.3%
	Low IgE Cluster	35	89.7%
Cluster membership of Mother	Mother High IgE Cluster	3	7.7%
	Mother Low IgE Cluster	36	92.3%
Cluster membership of Father	Father High IgE Cluster	4	10.3%
	Father Low IgE Cluster	35	89.7%

Cluster membership of Case		B	SE	Wald	df	p value	OR	95% CI	
								Lower Bound	Upper Bound
High IgE Cluster	Intercept	-2.803	0.728	14.820	1	0.000			
	[FatherCluster= High IgE]	2.803	1.237	5.135	1	0.023	16.500	1.460	186.409
	[FatherCluster= Low IgE]	0[b]	.	.	0	.	.	.	.

This analysis revealed that cases who had a father who was a member of the High IgE cluster, were **16.5 times** more likely to be a High IgE case (p = 0.023). One must keep in mind that these results were derived from very small numbers, with fathers' High IgE cluster consisting of only four members, and therefore one cannot consider these results as soundly representative of a larger population. On the other hand,

when one goes back to the results presented in section 3.1.6 of this thesis, it was found that having a father who had a history of asthma increased the odds of a child to be a member of the case group (and therefore having symptoms compatible with asthma) rather than the control group by **5.338 times** ($p = 0.022$). These findings could suggest that there could be a tendency for having Th2 mediated asthma which is mediated through IgE which is inherited by an asthmatic child through the father.

Chapter 4

4.1 Homes

4.1.1 Comparing home demographics for case versus control groups

The proportions of each house type were studied, to depict whether these differ between the case and control group. The main houses inhabited by Maltese families included apartments, single family houses which mainly included what locally are known as terraced houses, and semi-detached houses. The participants who chose "others" in the questionnaires, mainly living in dwellings which locally are called maisonettes, which really are apartments which had been designed to have a separate entrance to the street/road rather than a communal shared entrance.

Table 83: Types of houses found in case and control groups

			Group		
			Case	Control	Total
Type of House	Single Family House	Count	26	35	61
		% within case/control	39.4%	56.5%	47.7%
	Semi-detached house	Count	3	6	9
		% within case/control	4.5%	9.7%	7.0%
	Apartment	Count	28	16	44
		% within case/control	42.4%	25.8%	34.4%
	Farm	Count	1	0	1
		% within case/control	1.5%	0.0%	0.8%
	Other	Count	8	5	13
		% within case/control	12.1%	8.1%	10.2%
Total		Count	66	62	128
		% within case/control	100.0%	100.0%	100.0%

$X^2(4) = 7.175$, $p = 0.127$

However, the following differences were observed:

- The proportion of controls living in single family houses (56.5%) was higher than the corresponding proportion in case group (39.4%).
- The proportion of controls living in semi-detached houses (9.7%) was higher than the corresponding proportion in case group (4.5%).
- The proportion of cases living in apartments (42.4%) was higher than the corresponding proportion in the control group (25.8%).
- The proportion of cases living in farms (1.5%) was higher than the corresponding proportion in the control group (0%).
- The proportion of cases living in other type of houses (12.1%) was higher than the corresponding proportion in the control group (8.1%).

These differences in proportions did not reach statistical significance (p-value = 0.127).

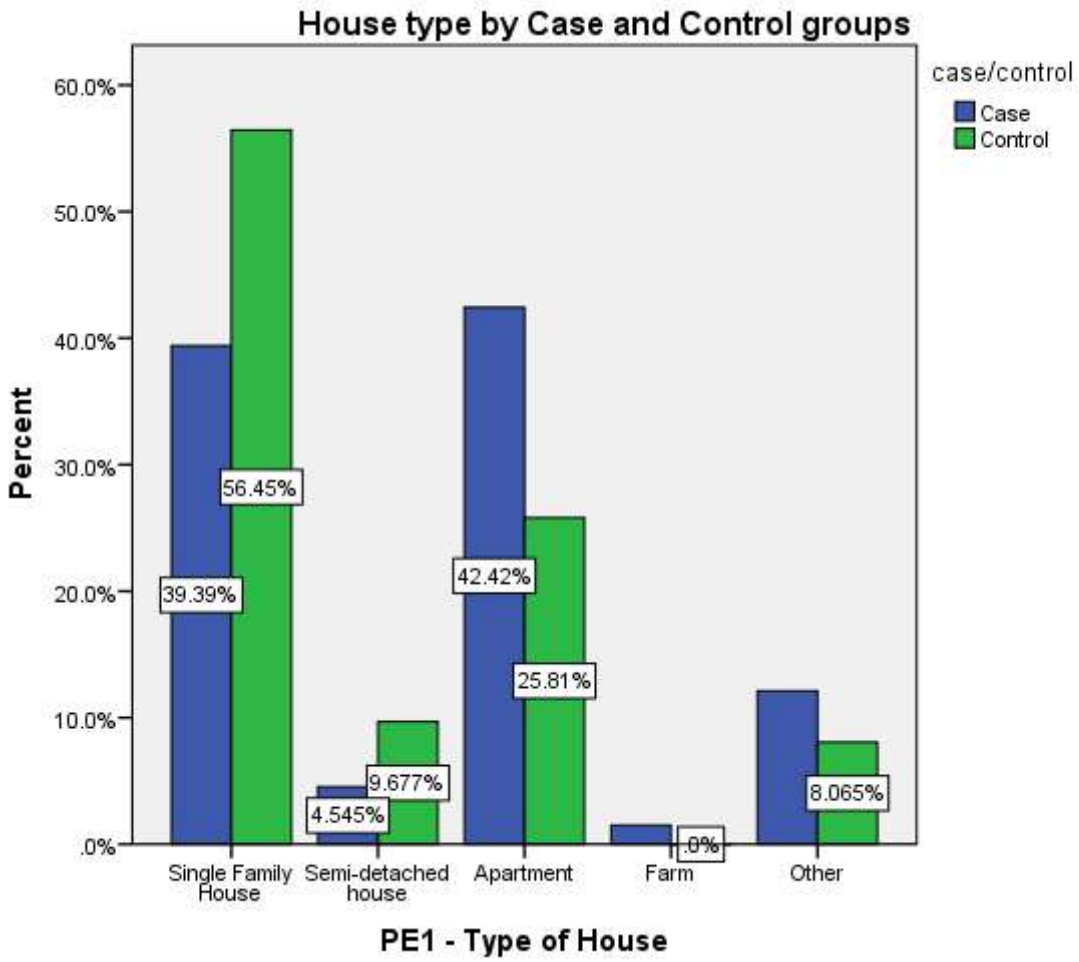


Figure 58: House type by Case and Control groups

When one compared the proportions of type of houses as distributed in the different regions of the study (North, Centre and South of Malta), the following results were obtained, and described in table 84.

Table 84: Proportions of House types according to regions

			Step			
			Central	South	North	Total
Type of House	Single Family House	Count	27	22	12	61
		percentage	56.3%	55.0%	30.0%	47.7%
	Semi-detached house	Count	3	4	2	9
		percentage	6.3%	10.0%	5.0%	7.0%
	Apartment	Count	13	11	20	44
		percentage	27.1%	27.5%	50.0%	34.4%
	Farm	Count	0	0	1	1
		percentage	0.0%	0.0%	2.5%	0.8%
	Other	Count	5	3	5	13
		percentage	10.4%	7.5%	12.5%	10.2%
Total		Count	48	40	40	128
		percentage	100.0%	100.0%	100.0%	100.0%

$X^2(8) = 7.175$, $p = 0.179$

Number of rooms

The mean number of rooms which each household contained was compared for the households inhabited by the case group and those lived in by the control group. Tests for normality was first carried out (Kolmogorov-Smirnov and Shapiro-Wilk), which showed that the mean number of rooms was distributed normally, and thus the independent t-test was used for this comparison.

Table 85: Comparing number of household rooms cases vs controls

	case/control	n	Mean	SD
Number of rooms in the dwelling	Case	59	4.34	1.434
	Control	56	4.91	1.621

$p = 0.195$

The mean number of rooms in the households hosting children in the case group (4.34 rooms) did not differ significantly from those hosting the control group (4.91 rooms) (p-value =0.195).

A similar comparison was performed to compare the mean number of persons living in a child's household, as distributed between the case and control group. Again, normality testing was carried out, and this measure was found to have a normal distribution.

Table 86: Comparing number of rooms in case vs control homes

	case/control	n	Mean	SD
Number of persons living in dwelling	Case	65	4.25	1.238
	Control	62	4.05	1.062

p = 0.219

The mean number of persons living in the houses inhabited by the case group (4.25 persons) did not differ significantly from the mean number of persons inhabiting the control group homes (4.05 persons) (p-value = 0.219).

The mean year in which the houses resided by the case children were built was compared to those resided by the control children. There were no significant differences, as the mean year for the case houses was 1940.66 (SD 266.84) while that for the control houses was 1948.22 (SD 266.84) (p=0.959).

4.1.2 Comparing house location in relation to traffic for case and control groups

The proportion of different house location in relation to traffic was compared for the houses in the case group and those in the control group.

Table 87: Relation of house to traffic

			Case/Control		
			Case	Control	Total
Relation house to traffic	In an area with clean air and far from busy road	Count	16	18	34
		percentage	24.6%	29.5%	27.0%
	In an area with small (reasonably) traffic	Count	30	35	65
		percentage	46.2%	57.4%	51.6%
	Near busy traffic	Count	19	8	27
		percentage	29.2%	13.1%	21.4%
Total		Count	65	61	126
		percentage	100.0%	100.0%	100.0%

$X^2(2) = 4.862,\ p = 0.088$

In this comparison, the one difference which stands out is that the proportion of cases who lived near busy traffic (29.2%) was higher than that of the controls who lived in a similar situation (8%). This was not statistical significanct (p = 0.088).

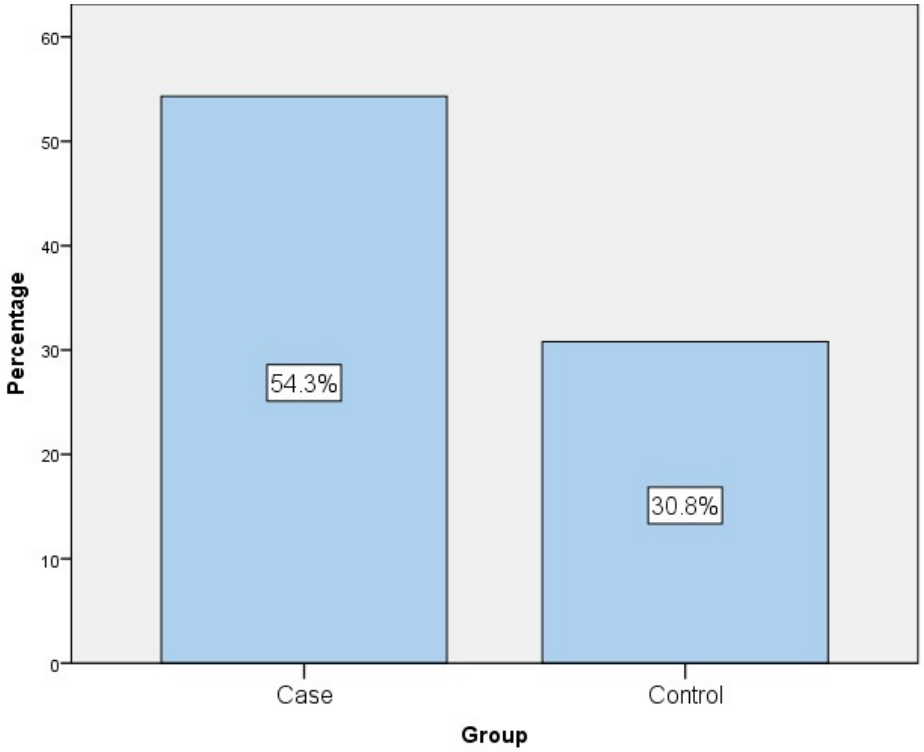
The proportion of children living close to busy traffic was then compared to those living near quiet roads with relatively clean air (thus excluding those living close to moderately busy roads).

Table 88: Relation of house to traffic - analysis comparing busy to clean air roads

			Case/Control		
			Case	Control	Total
Relation house to traffic	In an area with clean air and far from busy road	Count	16	18	34
		percentage	45.7%	69.2%	55.7%
	Near busy traffic	Count	19	8	27
		percentage	54.3%	30.8%	44.3%
Total		Count	35	26	61
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.344$, $p = 0.058$

This showed a higher proportion of cases living close to busy roads (54.3%) when compared to controls, (30.8%) and this was close to being statistically significant ($p = 0.058$). Here one must consider the small sample available for this analysis.



$X^2(1) = 3.344$, $p = 0.058$

Figure 59: Living in an area close to busy traffic

The proportion of children living within 200 metres from a street with heavy traffic was examined.

Table 89: Does your child live within 200 meters from a street with heavy traffic

			Case/Control		
			Case	Control	Total
Does your child live within 200 meters from a street with heavy traffic?	Yes	Count	33	28	61
		percentage	55.0%	46.7%	50.8%
	No	Count	17	27	44
		percentage	28.3%	45.0%	36.7%
	Don't know	Count	10	5	15
		percentage	16.7%	8.3%	12.5%
Total		Count	60	60	120
		percentage	100.0%	100.0%	100.0%

$X^2(2) = 4.349$, $p = 0.114$

Although the was a greater proportion of children in the case group who lived within 200 meters from a road with heavy traffic (55%) when compared to the control group (46.7%) the difference between these two proportions was not statistically significant. (p-value = 0.114).

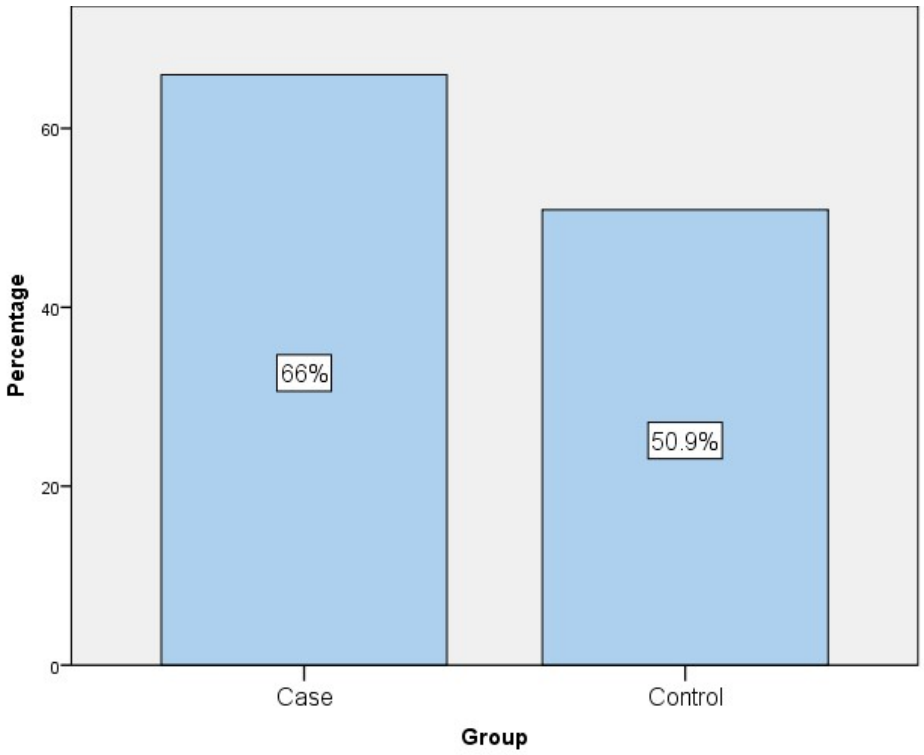
An analysis was then carried out excluding the parents who answered, "do not know" to this answer, and this could have affected the result.

Table 90: Does your child live within 200 meters from a street with heavy traffic excluding "Do not know" answers

			Case/Control		
			Case	Control	Total
Does your child live within 200m from a street with heavy traffic	No	Count	17	27	44
		percentage	34.0%	49.1%	41.9%
	Yes	Count	33	28	61
		percentage	66.0%	50.9%	58.1%
Total		Count	50	55	105
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 2.450$, $p = 0.086$

This analysis showed that the proportion of children who lived within 200m from a street with heavy traffic was higher (66%) in the case group when compared to the control group (50.9%), although not statistically significant (p = 0.086). A large sample could have shown a different result.



$X^2(1) = 2.450$, p = 0.086

Figure 60: Does your child live within 200m from heavy traffic

For those cases and controls who were reported to live within 200 meters from a street with heavy traffic, the parents were asked to describe the type of road which they were commenting on. The following depicts how the homes were distributed.

Table 91: Busy road type cases versus controls

			Case/Control		
			Case	Control	Total
Busy Road type within 200 meters of house	Highway	Count	10	8	18
		percentage	20.0%	21.1%	20.5%
	Street	Count	31	18	49
		percentage	62.0%	47.4%	55.7%
	Boulevard	Count	4	6	10
		percentage	8.0%	15.8%	11.4%
	Other	Count	5	6	11
		percentage	10.0%	15.8	12.5%
Total		Count	50	38	88
		percentage	100.0%	100.0%	100.0%

$X^2(3) = 2.574$, p = 0.462

Although one notes that the proportion of cases who reported to be living near busy streets (62%) was higher than the corresponding proportion in the control group (47.4%), and that there were other small differences related to the proportions or those children leaving close to highways, and to boulevards, these differences were not statistically significant. (p-value = 0.462).

The parents were then asked to report on the estimated distance between the children's home and the said busy road.

Table 92: Distance from busy road cases versus controls

			Case/Control		
			Case	Control	Total
Distance from road	within 20 m	Count	13	8	21
		percentage	31.7%	22.2%	27.3%
	within 50 m	Count	7	2	9
		percentage	17.1%	5.6%	11.7%
	within 100 m	Count	8	16	24
		percentage	19.5%	44.4%	31.2%
	Within 200 m	Count	13	10	11
		percentage	31.7%	27.8	29.9%
Total		Count	41	36	77
		percentage	100.0%	100.0%	100.0%

$X^2(3) = 6.730$, p = 0.081

The proportions of cases who were living within 20 meters (31.7%) and 50 meters (17.1%) from a busy road was larger than the corresponding proportions in the control group (22.2% and 5.6% respectively). On the other hand, the proportions of children in the control group who lived within 100 meters (44.4%) and 200 meters (27.8%) from a busy road was larger than the corresponding proportions in the case group (19.5% and 31.7%) respectively. Yet, these differences were not statistically significant (p-value = 0.081).

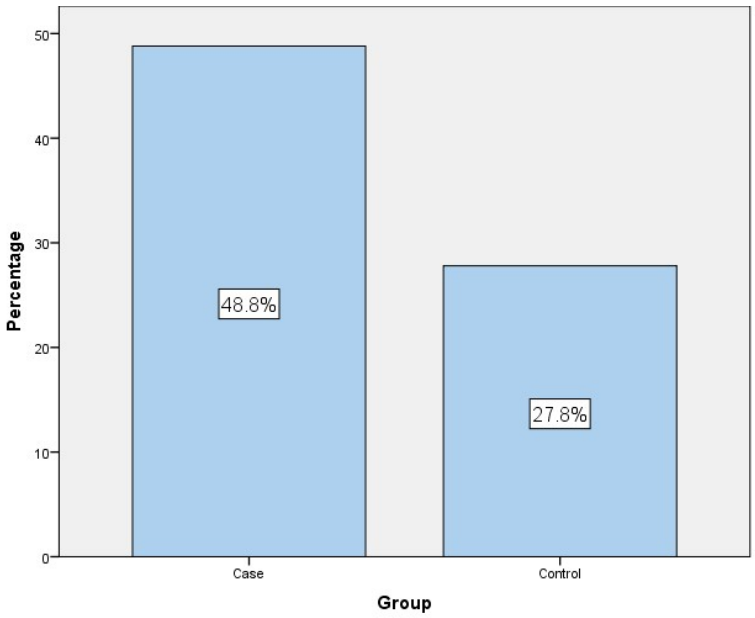
The categories were then combined, in this manner; the respondents who claimed to live within 20m and within 50m were added together into a less than 50m from a busy road category, while those who lived within 100m and within 200m were added together into a great than 50m from a busy road category. The following results were obtained:

Table 93: Distance from busy road comparing less than 50m to more than 50m

			Case/Control		
			Case	Control	Total
Distance from busy road	Less than 50m	Count	20	10	30
		percentage	48.8%	27.8%	39.0%
	More than 50m	Count	21	26	47
		percentage	51.2%	72.2%	61.0%
Total		Count	41	36	77
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.556$, p = 0.049

The proportion of cases who lived within 50m from a busy road (48.8%) was significantly greater than the proportion of controls who lived in such a condition (27.8%) (p = 0.049).



$X^2(1) = 3.556$, p = 0.049

Figure 61: Lives less than 50m from a busy road

The relation of the house to cultivated land as observed between the case and control groups is depicted in the following table. Cultivated land could be interpreted as being a field, orchards or land being used for rural purposes, and hence surrounding houses could be exposed to activities related to this use.

Table 94: Dwelling proximity to cultivated land

			Group		
			Case	Control	Total
Dwelling close to cultivation	True	Count	22	22	44
		Percentage	35.5%	37.3%	36.4%
	False	Count	40	37	77
		Percentage	64.5%	62.7%	63.6%
Total		Count	62	59	121
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.043$, p =0.493

There were no differences between the proportions of children in the case group living in proximity of cultivation when compared to the corresponding proportion in the control group (p = 0.493).

4.1.3 Differences in house indoor characteristics, cases versus control groups

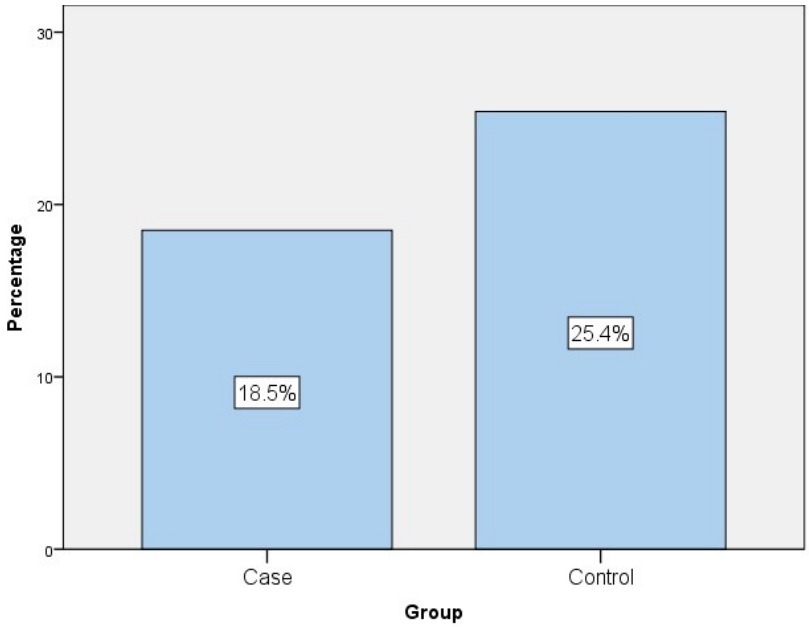
The parents were asked to describe various characteristics pertaining to the heating, interior decoration and possible dampness related problems which could be present in the houses inhabited by the children.

Table 95: Fireplaces

			Group		
			Case	Control	Total
Presence of a fireplace	True	Count	12	32	32
		Percentage	18.5%	25.4%	25.4%
	False	Count	53	94	94
		Percentage	81.5%	74.6%	74.6%
Total		Count	65	61	126
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.408$, p =0.050

The proportion of fireplaces present in the children's homes in the control group (32%) exceeded the corresponding proportion in the case group (18.5%). (p = 0.05).



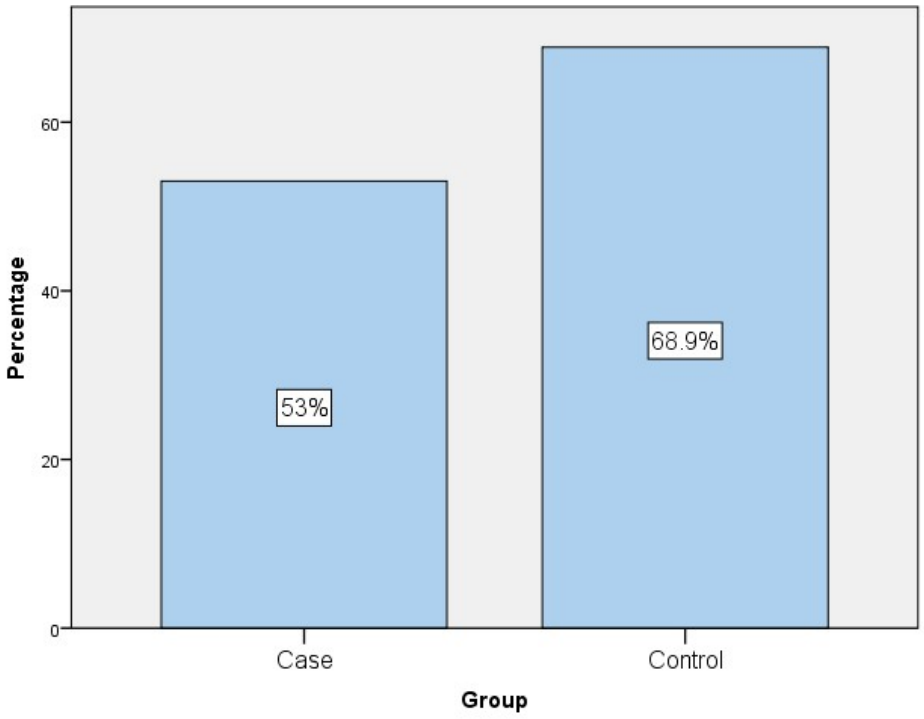
$X^2(1) = 3.408$, p =0.050

Figure 62: Presence of a fireplace in the house

Table 96: Presence of Air conditioner in the house

			Group		
			Case	Control	Total
Presence of an air conditioner	True	Count	35	42	77
		Percentage	53.0%	68.9%	60.6%
	False	Count	31	19	50
		Percentage	47.0%	31.1%	39.4%
Total		Count	66	61	127
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.325$, $p = 0.050$



$X^2(1) = 3.325$, $p = 0.050$

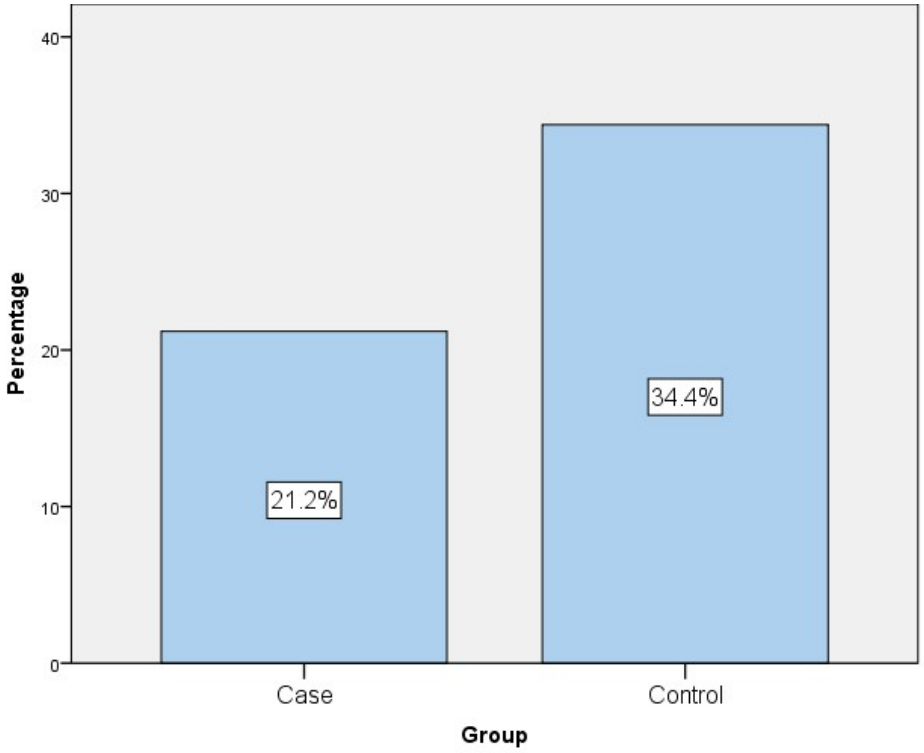
Figure 63: Presence of an air conditioner in the house

The proportion of air conditioners present in the children's homes in the control group (68.9%) exceeded the corresponding proportion in the case group (53.0%). (p-value = 0.050).

Table 97: Presence of an Airconditioner in the child's bedroom

			Group		
			Case	Control	Total
Presence of an air conditioner in child's bedroom	True	Count	14	21	35
		Percentage	21.2%	34.4%	27.6%
	False	Count	52	40	92
		Percentage	78.8%	65.6%	72.4%
Total		Count	66	61	127
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 2.773$, p =0.071



$X^2(1) = 2.773$, p =0.071

Figure 64: Presence of an air conditioner in the child's bedroom

Although the proportion of children who had an air conditioner in their bedroom was higher in the control group (34.4%) when compared to the corresponding proportion in the case group (21.2%), the difference between these two proportions did not reach statistical significance (p-value = 0.071).

Table 98: Type of a cooker used in the child's house

			Group		
			Case	Control	Total
Type of cooker	Only Electric	Count	3	2	5
		Percentage	4.6%	3.2%	3.9%
	Gas Cooker	Count	62	60	122
		Percentage	95.4%	96.8%	96.1%
Total		Count	65	62	127
		Percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.162$, p =0.522

The most popular type of cooker present in the children's houses was a gas cooker, with 95.4% of respondents reporting using this type of cooker in the case group, and 96.8% in the control group. There were no significant differences in these proportions (p-value = 0.522).

Table 99: Type of extractor over cooker

			Group		
			Case	Control	Total
Question - PE15 Extractor to cooker	No	Count	22	19	41
		percentage	33.8%	30.6%	32.3%
	Yes, and connected to outdoor air	Count	21	30	51
		percentage	32.3%	48.4%	40.2%
	Yes, but not connected to outdoor air	Count	22	13	35
		percentage	33.8%	21.0%	27.6%
Total		Count	65	62	127
		percentage	100.0%	100.0%	100.0%

$X^2(2) = 4.053$, p = 0.132

There were no significant differences in the proportions of households who had no extractors, one connected to outdoor air or one not connected to outdoor air between the case and control respondents. (p-value = 0.132).

The children's parents were asked to describe the type of flooring used in the children's house.

Table 100: Type of flooring used in house

			Group		
			Case	Control	Total
What type of floor material is in your child's bedroom	Wood/Parquet	Count	2	1	3
		percentage	3.0%	1.7%	2.4%
	Wall-to-wall carpet	Count	2	2	4
		percentage	3.0%	3.3%	3.2%
	Tiles	Count	60	56	116
		percentage	90.9%	93.3%	92.1%
	Others	Count	2	1	3
		percentage	3.0%	1.7%	2.4%
Total		Count	66	60	126
		percentage	100.0%	100.0%	100.0%

$X^2(3)$ 0.520, p = 0.914

The type of flooring reported to be present in the children's households was almost exclusively tiles. The proportion of cases who had tiles in their household was 90.9%, while the corresponding proportion for the control group was 93.3%. The differences in proportion in the types of flooring used did not differ significantly (p-value = 0.914).

Table 101: Presence of carpets in the child's bedroom

			Group		
			Case	Control	Total
Are there carpets in your child's bedroom	Yes	Count	26	26	52
		percentage	40.0%	45.6%	42.6%
	No	Count	39	31	70
		percentage	60.0%	54.4%	57.4%
Total		Count	65	57	122
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 0.391$, p = 0.532

There were no significant differences between the proportion of children having carpets in their bedrooms in the case group (40.0%) when compared to the corresponding proportion in the control group (45.6%) (p-value = 0.532).

The following questions focused on areas of the house which might have been showing signs of dampness or mouth growth, and the difference in proportion of positive answers to these questions between the case subjects and the control subjects.

Table 102: Water leakage or water damage noted indoors

			Group		
			Case	Control	Total
Water leakage or water damage indoors	Yes	Count	13	6	19
		percentage	21.0%	10.0%	15.6%
	No	Count	49	54	103
		percentage	79.0%	90.0%	84.4%
Total		Count	62	60	122
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 2.790$, p = 0.134

Although the proportion of children who had water leakage or water damage in their dwelling was higher in the case group (21.0%) when compared to the corresponding proportion in the control group (10.0%), the difference between these two proportions did not reach statistical significance (p-value = 0.134).

Table 103: Visible mould in house

			Group		
			Case	Control	Total
Visible mould in house	Yes	Count	29	21	50
		percentage	46.8%	35.0%	41.0%
	No	Count	33	39	72
		percentage	53.2%	65.0%	59.0%
Total		Count	62	60	122
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 1.748$, $p = 0.202$

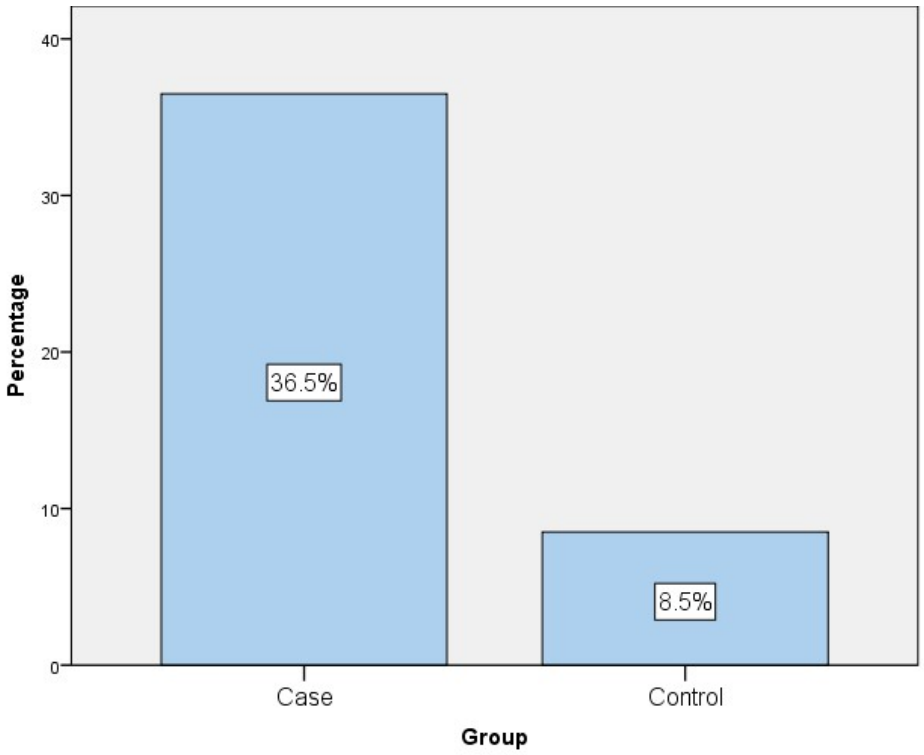
Although the proportion of children who had visible mould reported in their dwelling was higher in the case group (46.8%) when compared to the corresponding proportion in the control group (35.0%), the difference between these two proportions did not reach statistical significance (p-value = 0.202).

Table 104: Smell of mould in the house

			Group		
			Case	Control	Total
Smell of mould in the house	Yes	Count	23	5	28
		percentage	36.5%	8.5%	23.0%
	No	Count	40	54	94
		percentage	63.5%	91.5%	77.0%
Total		Count	23	5	28
		percentage	36.5%	8.5%	23.0%

$X^2(1) = 13.540$, $p < 0.001$

The proportion of children in the case group for whom a smell of mould in the house was reported (36.5%) exceeded the corresponding proportion in the control group (8.5%). (p-value <0.001).



$X^2(1) = 13.540, p < 0.001$

Figure 65: Smell of mould in the house

Table 105: Signs of Dampness or condensation in the house

			Group		
			Case	Control	Total
Signs of Dampness or condensation in the house	Yes	Count	44	34	78
		percentage	71.0%	56.7%	63.9%
	No	Count	18	26	44
		percentage	29.0%	43.3%	36.1%
Total		Count	62	60	122
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 2.705, p = 0.132$

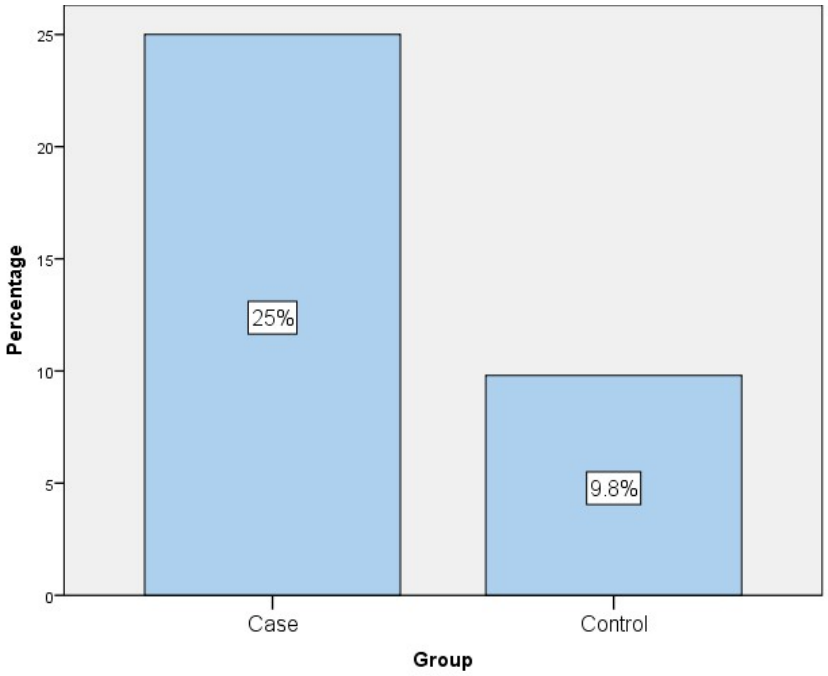
Although the proportion of children who had signs of dampness or condensation reported in their dwelling was higher in the case group (71.0%) when compared to the corresponding proportion in the control group (56.7%), the difference between these two proportions did not reach statistical significance (p-value = 0.132).

Table 106: Visible mould in child's bedroom

			Group		
			Case	Control	Total
Visible mould in child's bedroom	Yes	Count	16	6	22
		percentage	25.0%	9.8%	17.6%
	No	Count	48	55	103
		percentage	75.0%	90.2%	82.4%
Total		Count	64	61	125
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 4.952$, p = 0.034

The proportion of children in the case group for whom visible mould in the child's bedroom was reported (25.0%) exceeded the corresponding proportion in the control group (9.8%). (p-value (0.034).



$X^2(1) = 4.952$, p = 0.034

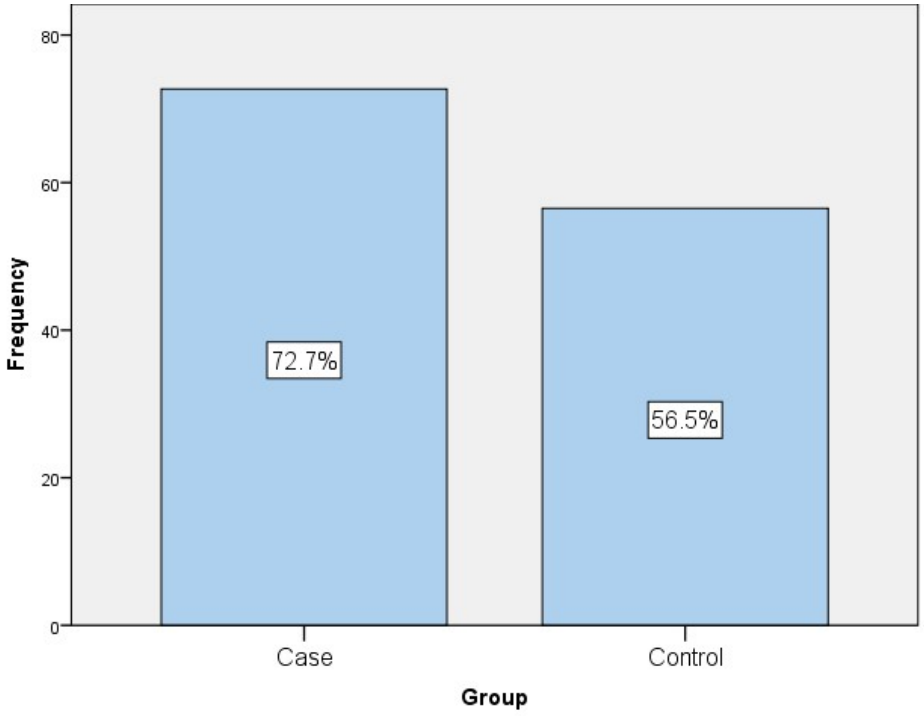
Figure 66: Visible signs of mould in the child's bedroom

Table 107: Signs of humidity in the house

			Group		
			Case	Control	Total
Signs of humidity in the house	Yes	Count	48	35	83
		percentage	72.7%	56.5%	64.8%
	No	Count	18	27	45
		percentage	27.3%	43.5%	35.2%
Total		Count	66	62	128
		percentage	100.0%	100.0%	100.0%

$X^2(1) = 3.715$, p = 0.054

Although the proportion of children who had signs of humidity reported in their dwelling was higher in the case group (72.7%) when compared to the corresponding proportion in the control group (56.5%), the difference between these two proportions was just shy of reaching statistical significance (p-value = 0.054).



$X^2(1) = 3.715$, p = 0.054

Figure 67: Signs of humidity in the house

4.2 House Dust Analysis

Dust samples were collected from a total of seventy-four (74) houses. These were analysed, through an ELISA assay for house dust mite (Der p1 allergen), cat dander (Fel d1 allergen), Alternaria spores (Alt A1 allergen) and timothy grass pollen (Phl p5 allergen). Dust endotoxin levels were measured through a Limulus amebocyte lysate (LAL) test. The allergens were measured in micrograms of allergen per gram of dust (µg/g) while the endotoxin level results were presented in a log of endotoxin level per mg (Eu/mg).

Table 108: Allergen means for all houses

	Mean (µg/g)	SD
HDM levels in house dust	4.198	9.670
Cat Levels in house dust	2.441	7.935
Alternaria Levels in house dust	0.041	0.133
Timothy Grass Pollen Levels in house dust	0.090	0.300

The highest mean dust allergen levels in the houses was recorded for HDM at 4.198µg/g (SD 9.670). This was followed by cat, as the mean Fel d1 levels were 2.441 µg/g (SD 7.935). HDM and Cat were considered to be allergens which had an indoor source. The other two allergens studied, Alternaria spores and Timothy grass pollen a derived from outdoor sources (Alternaria being an outdoor plant pest, and timothy grass being a wild shrub). In fact, indoor levels were much lower than the other two allergens ($p < 0.001$). Mean Timothy grass levels in the indoor dust collected was 0.090µg/g (SD 0.133) and mean Alternaria levels were 0.041 µg/g (0.041).

Table 109: Endotoxin means for all houses

	Mean (Eu/mg)	SD
LPS Levels in house dust	1.875	0.852

Mean endotoxin levels as analysed though LAL in homes was 1.875Eu/mg (SD 0.852).

4.2.1 Comparing mean house dust allergen levels according to house type

The houses were separated into four (4) separate categories. There were thirty eight (38) single family house, commonly known as a terraced house in Malta, six (6) semi-detached houses, twenty five (25) apartments and seven other houses which were classified as others (such as farmhouses and houses of character). The mean allergen levels and endotoxin levels are presented in tables 110 and 111. There were no significant differences in the levels of allergen and endotoxin dust concentrations between the different house types.

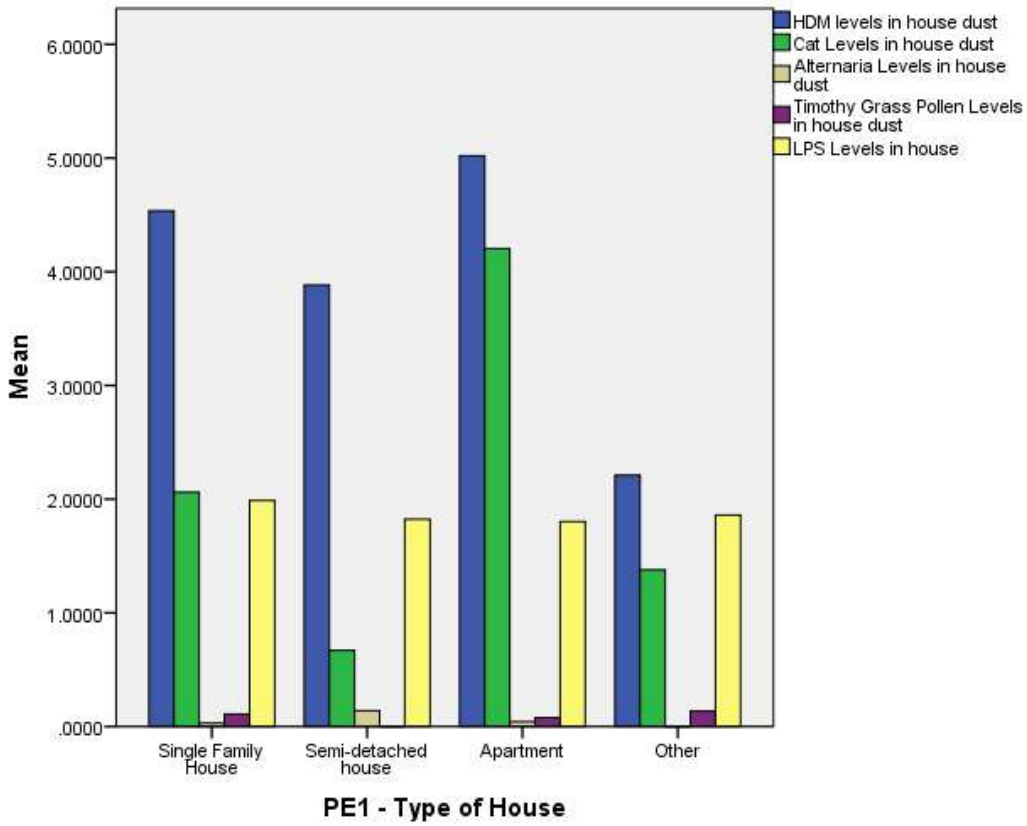
Table 110: Mean allergen levels according to house type

	Single Family House			Semi-detached house			Apartment			Other			
	n	mean µg/g	SD	n	mean µg/g	SD	n	mean µg/g	SD	n	mean µg/g	SD	p value
HDM house dust levels	38	4.341	10.323	6	3.883	5.227	25	4.454	10.604	7	2.211	2.494	0.369
Cat house dust levels	38	2.016	7.355	6	0.670	0.930	25	3.708	10.199	7	1.378	1.862	0.878
Alternaria house dust levels	38	0.030	0.114	6	0.140	0.297	25	0.044	0.112	7	0.000	0.000	0.397
Timothy Grass Pollen house dust levels	38	0.105	0.354	6	0.000	0.000	25	0.088	0.261	7	0.097	0.256	0.831
LPS Levels in house	38	2.028	0.729	6	1.823	0.988	25	1.658	0.908	7	1.861	1.276	0.556

Table 111: Mean allergen levels according to house type

	Single Family House			Semi-detached house			Apartment			Other		
	µg/g	n	SD	µg/g	n	SD	µg/g	n	SD	µg/g	n	SD
LPS Levels in house	2.028	38	0.729	1.823	6	0.988	1.658	25	0.908	1.861	5	1.276

Figure 68: Mean allergen levels according to house type



4.2.2 Comparing mean house dust allergen levels according to season

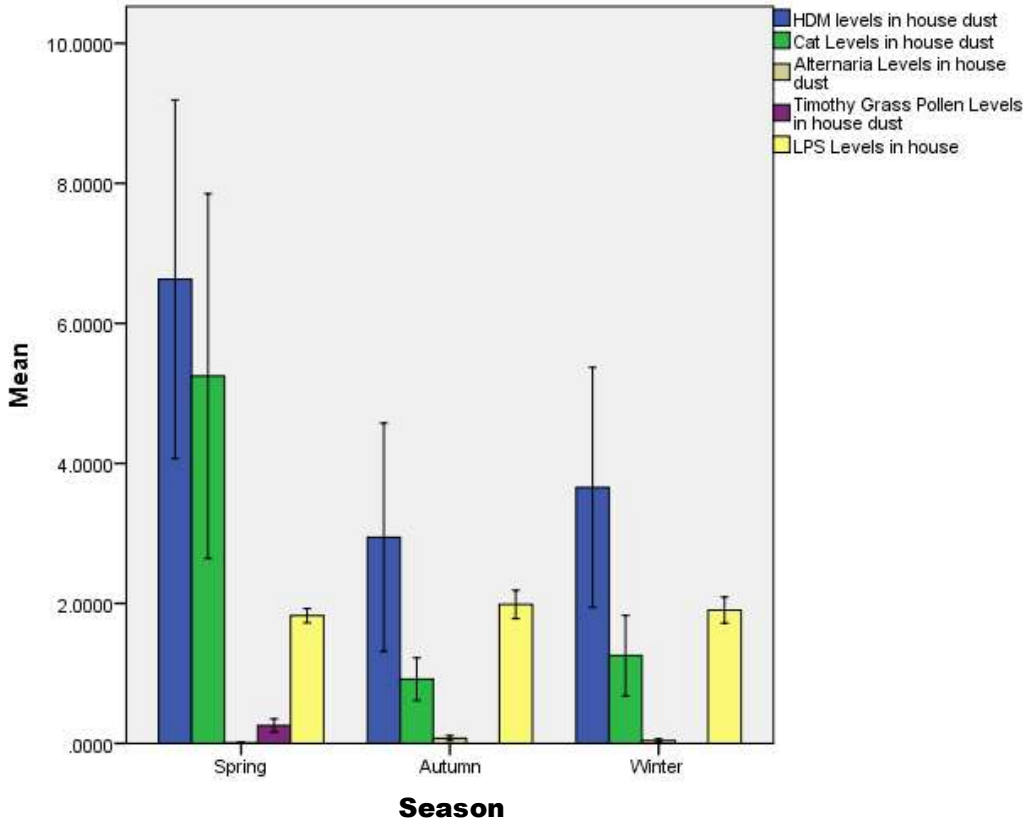
The levels of indoor allergens were compared according to the different seasons in which they were collected (table 112). As expected, Timothy grass pollen was only detectable in spring, when this grass and related grasses would have produced their flowers. Its mean level in indoor dust in spring was 0.261µg/g (SD 0.469; $p < 0.001$). There seemed to be a trend for higher HDM levels in spring, which is a warmer

season in which HDM populations tend to increase. In spring mean HDM dust levels were 6.375µg/g (SD 12.611) while in autumn this was 2.793 µg/g (SD 8.025) and 3.335 µg/g (SD 6.949) in winter. Due to large standard deviations and a relatively small sample size, confounding factors such as different cleaning regimes, these differences were not significant (p = 0.879). Mean cat dust levels also had a higher trend in spring (5.054 µg/g, SD 12.795), when cats moult, than in autumn (0.897, SD 1.525) and winter (1.129 µg/g, SD 2.337) yet again, and probably for similar reasons to HDM, this was not significant (p = 0.519).

Table 112: Mean allergen levels according to season

	Step									
	Spring			Autumn			Winter			
	n	mean	SD	n	mean	SD	n	mean	SD	p value
HDM levels in house dust	26	6.375	12.611	28	2.793	8.025	21	3.335	6.949	0.879
Cat Levels in house dust	26	5.054	12.795	28	0.897	1.525	21	1.129	2.337	0.519
Alternaria Levels in house dust	26	0.009	0.026	28	0.072	0.194	21	0.039	0.103	0.694
Timothy Grass Pollen Levels in house dust	26	0.261	0.469	28	0.000	0.000	21	0.000	0.000	**< 0.001**
LPS Levels in house dust	26	1.824	0.495	28	2.038	1.022	21	1.713	0.954	0.148

Figure 69: Mean allergen levels according to season



4.2.3 Comparing house dust allergen levels according to perceived presence of humidity in the home

Other factors, which were related to the condition of the houses were studied. In the questionnaires, the parents were asked whether there were signs of humidity at home. The following results focus on the relationships the answers reported to the levels of allergens in the vacuumed house dust.

The parents were asked to report whether the were signs of mould growth inside their house. Mean Endotoxin levels were higher in houses in which visible mould growth was reported (2.074Eu/mg, SD 0.832) compared to those in which this was not reported (1.796 Eu/mg, SD 0.765; p = 0.024). There were no other significant differences noted for the other allergens.

Table 113: Relationships of allergen concentration in houses to reported mould growth indoors

	Visible mould growth indoors on walls, floor or ceiling	N	Mean	SD	p value
HDM levels in house dust	Yes	30	3.799	9.302	
	No	41	4.733	10.325	0.351
Cat Levels in house dust	Yes	30	2.014	7.675	
	No	40	2.750	8.445	0.457
Alternaria Levels in house dust	Yes	28	0.038	0.143	
	No	41	0.045	0.131	0.135
Timothy Grass Pollen Levels in house dust	Yes	30	0.091	0.373	
	No	42	0.096	0.251	0.368
LPS Levels in house	Yes	30	2.074	0.832	
	No	41	1.796	0.765	**0.024**

A similar result was obtained for houses in which the parents reported signs of dampness or visible mould growth in the child's bedroom (see table 114). Those houses in which this issue was reported had Endotoxin levels of 2.437Eu/mg (SD 0.430) when compared to 1.780Eu/mg (SD 0.877; p = 0.011).

Table 114: Relationships of allergen concentration in houses to reported dampness or visible mould growth in the child's bedroom.

	Is there dampness or visible mould growth in your child's bedroom?	N	Mean	SD	p value
HDM levels in house dust	Yes	11.000	1.254	2.296	
	No	62.000	4.733	10.448	0.192
Cat Levels in house dust	Yes	11.000	4.266	12.515	
	No	61.000	2.148	6.977	0.942
Alternaria Levels in house dust	Yes	11.000	0.003	0.010	
	No	59.000	0.049	0.145	0.437
Timothy Grass Pollen Levels in house dust	Yes	11.000	0.228	0.580	
	No	63.000	0.068	0.221	0.367
LPS Levels in house	Yes	11.000	2.437	0.430	
	No	62.000	1.780	0.877	**0.011**

There were no significant differences in mean dust allergen levels, between houses in which parents had reported a smell of mould, dampness/condensation on the lower part of the windows in winter, dampness or water related problems in the past 5 years when compared to the ones which did not report these problems.

4.2.4 Comparing allergen dust levels in "Case" children houses to "Control" children houses

The allergen levels for houses which were inhabited by "case" children were compared to those of "control" children, and are presented in table 115. Forty (40) "case" houses were compared to thirty four (34) "control houses".

Table 115: Comparing Mean allergen levels in dust found in "case" houses vs "control" houses

	Case or Control House	Mean	SD	p value
HDM levels in house dust	Case	2.634	7.005	
	Control	6.038	11.932	0.328
Cat Levels in house dust	Case	3.034	8.188	
	Control	1.722	7.680	0.176
Alternaria Levels in house dust	Case	0.045	0.133	
	Control	0.036	0.136	0.545
Timothy Grass Pollen Levels in house dust	Case	0.098	0.254	
	Control	0.081	0.351	0.266
LPS Levels in house dust	Case	1.763	0.959	
	Control	2.007	0.696	0.175

There were no significant differences for any allergen in house dust levels between the houses inhabited by "case" children and those by "control" children. It was

interesting to note a trend for lower mean HDM for "case" houses when compared to those of "control" houses. Perhaps this was due to better cleanliness being kept in these houses as the parents might be aware of the fact their children had asthma symptoms.

4.2.5 Comparing mean dust allergen levels in houses who had doctor-diagnosed fathers to those who were not

In this analysis, the houses were divided between the ones which housed fathers who reported doctor-diagnosed asthma to the ones which homes fathers who did not report this condition. The houses of ten (10) doctor-diagnosed asthmatic fathers, and sixty four (64) fathers who were not diagnosed as having asthma were compared (table 116).

Table 116: Comparing Mean allergen levels in dust found in houses of doctor diagnosed fathers vs houses of fathers who did not have doctor diagnosed asthma

	Father has doctor diagnosed asthma	Mean	SD	p value
HDM levels in house dust	Yes	0.891	1.247	
	No	4.715	10.301	0.287
Cat Levels in house dust	Yes	0.460	0.680	
	No	2.755	8.504	0.576
Alternaria Levels in house dust	Yes	0.013	0.029	
	No	0.045	0.142	0.732
Timothy Grass Pollen Levels in house dust	Yes	0.143	0.284	
	No	0.083	0.303	0.260
LPS Levels in house dust	Yes	1.604	1.009	
	No	1.917	0.825	0.278

There were no significant differences between the mean dust levels of any of the allergens studied in the vacuumed dust when comparing houses of doctor-diagnosed asthmatic fathers to the rest of the sampled houses.

4.2.6 Comparing mean dust allergen levels in houses who had doctor-diagnosed mothers to those who were not

The same analysis was performed, comparing mean dust allergens levels in houses who had mothers with doctor-diagnosed asthma to the houses of mothers who did not report doctor diagnosed asthma. Results were obtained for eight (8) houses which were inhabited by mothers with doctor diagnosed asthma, while sixty six (66) dust samples were collected from houses in which mothers did not have doctor- diagnosed asthma (table 117).

Table 117: Comparing Mean allergen levels in dust found in houses of doctor-diagnosed mothers vs houses of mothers who did not have doctor diagnosed asthma

	Father has doctor diagnosed asthma	Mean	SD	p value
HDM levels in house dust	Yes	0.992	1.459	
	No	4.586	10.167	0.383
Cat Levels in house dust	Yes	1.007	2.580	
	No	2.617	8.356	0.269
Alternaria Levels in house dust	Yes	0.086	0.231	
	No	0.035	0.117	0.453
Timothy Grass Pollen Levels in house dust	Yes	0.079	0.223	
	No	0.092	0.309	0.898
LPS Levels in house dust	Yes	1.797	1.195	
	No	1.884	0.812	0.760

There were no significant differences between the mean dust levels of any of the allergens studied in the vacuumed dust when comparing houses of doctor-diagnosed asthmatic mothers to the rest of the sampled houses.

4.2.7 Differences between allergens in house dust for allergen SPT positive case children and control children

Children who participated in the study and were positive to HDM SPT were selected. The mean levels of HDM allergen in the dust collected in their houses were compared according to whether these were "Case" or "Control" children.

The same process was done for children who were SPT cat positive for cat allergen in the house dust, and Alternaria SPT positive. As a skin prick test solely for Timothy grass does not exist, the children who were positive Grass Mix SPT, in which *Phleum pratense* pollen is included were selected. The results are presented in table 118.

Table 118: Differences between house dust for allergen SPT positive case children and control children

			n	Mean	SD	p value
Child SPT Positive to HDM	HDM levels in house dust	Case	17	3.813	9.939	
		Control	7	0.984	1.410	0.382
Child SPT Positive to Cat	Cat Levels in house dust	Case	6	2.505	3.097	
		Control	3	15.569	24.953	0.431
Child SPT Positive to Alternaria	Alternaria Levels in house dust	Case	3	0.219	0.379	
		Control	1	0.000	0.000	0.564
Child SPT Positive to Grass Mix	Timothy Grass Pollen Levels in house dust	Case	0			
		Control	1	0.000	0.000	N/A

The remaining number of children who were used for this analysis was very small, given the strict selection criteria used in this case. Twenty four (24) children who had their house dust sampled were positive to HDM SPT, nine (9) were positive to Cat SPT, four (4) to Alternaria SPT and only one to Grass Mix SPT. None of the comparisons were significant. The was a high risk of a type 2 statistical error, due to the small numbers, and hence these results could have been false negatives. Of note, there was a trend for mean HDM dust levels to be higher (3.813µg/g SD 9.939) in houses of cases who were SPT positive to HDM when compared to the equivalent control houses (0.9843.813µg/g SD 1.410; p = 0.382). On the other hand, the opposite was true for children who were SPT positive to Cat, where the mean dust levels were higher in the control houses (15.5693.813µg/g SD 24.953 in houses of control children vs 2.5053.813µg/g SD 3.097; p = 0.431). Perhaps, parents who knew that their children had asthma avoided having cats in their houses, leading to lower levels in the dust, though this is only speculative given the high p value and the small sample available.

4.2.8 Differences between allergens in house dust for allergen SPT positive doctor-diagnosed asthmatic fathers and fathers without a diagnosis of asthma

Table 119: Differences between house dust for allergen SPT positive doctor diagnosed fathers and fathers without a diagnosis of asthma

			n	Mean	SD	P value
Father SPT Positive to HDM	HDM levels in house dust	Asthma	6	0.680	0.918	0.231
		Not Asthma	16	6.707	13.524	
Father SPT Positive to Cat	Cat Levels in house dust	Asthma	2	0.000	0.000	0.1430
		Not Asthma	6	3.476	2.838	
Father SPT Positive to Alternaria	Alternaria Levels in house dust	Asthma	0	N/A	N/A	N/A
		Not Asthma	0			
Father SPT Positive to Grass Mix	Timothy Grass Pollen Levels in house dust	Asthma	0			N/A
		Not Asthma	8	0.0758	0.214	

The numbers obtained from similar analysis for fathers who had positive SPTs to collected house dust allergens, were very small. There were no significant differences in any of the house dust allergen levels when comparing the doctor-diagnosed asthmatic fathers to the ones who were not diagnosed with asthma. It was interesting to note that the fathers who had doctor-diagnosed asthma and were SPT positive to HDM and cat seemed to have lower levels of these allergens in the house dust.

4.2.9 Differences between allergens in house dust for allergen SPT positive doctor-diagnosed asthmatic mothers and mothers without a diagnosis of asthma

Table 120: Differences between house dust for allergen SPT positive doctor diagnosed mothers and fathers without a diagnosis of asthma

			n	Mean	SD	p value
Mother SPT Positive to HDM	HDM levels in house dust	Asthma	2	2.082	2.263	0.410
		Not Asthmatic	11	1.430	2.215	
Mother SPT Positive to Cat	Cat Levels in house dust	Asthma	3	2.487	4.240	0.629
		Not Asthmatic	4	9.806	14.843	
Mother SPT Positive to Alternaria	Alternaria Levels in house dust	Asthma	2	0.000	0.000	N/A
		Not Asthmatic	0			
Mother SPT Positive to Grass Mix	Timothy Grass Pollen Levels in house dust	Asthma	2	0.000	0.000	0.857
		Not Asthmatic	6	0.101	0.247	

The numbers obtained from similar analysis for mothers who had positive SPTs to collected house dust allergens, were very small. There were no significant differences in any of the house dust allergen levels when comparing the doctor-diagnosed asthmatic mothers to the ones who were not diagnosed with asthma. It was interesting to note that the fathers who had doctor-diagnosed asthma and were SPT positive to HDM, cat and grass mix seemed to have lower levels of these allergens in the house dust.

4.3 Description of Houses which were the dwelling of High IgE individuals

The previous chapters described children and parents who were classified into clusters, with the more interesting one being the High IgE cluster. These individuals exhibited a high Total serum IgE, which could have been related to an ongoing high Th2 inflammatory pathway, and in fact these had a high FeNO which is a marker of airway inflammation. Their FEV_1 and FEV_1/FVC were also lower than the Low IgE cluster, denoting airway obstruction.

During the sampling made from the houses which participated in this study, a checklist was filled up to obtain descriptive information from the initial appearances of these houses, their type, furniture, heating and cooling appliances, and the regimes regularly used to maintain their cleanliness. The descriptive data from nine households which homed high IgE individuals are being presented in this chapter.

House No. 1 – *Dust HDM level detected 41.402µg/g LPS 3.721*

Child total IgE 1250 HDM IgE 79.210

Father total IgE 861.1 HDM IgE 7.80

Mother Total IgE 137.2 HDM IgE 0.21

Dwelling type: Terraced House (two floored house)

Location: South, Port Area

Location notes: Located in an area close to fishing industry and a fuel storage area.

Type of cooling and heating devices: Combination of gas and electric heaters. Air conditioners used to cool the house.

Furniture: A polyester carpet present in the main living area. Most of the furniture was made of bamboo cane.

Description of floor types, walls and ceilings: Floor in Traditional Maltese tiles. Painted walls in good condition, last painted two years before the study was carried out.

Type of apertures: Aluminium windows. Internal doors made of plywood.

Household Cleaning products used: Disinfectant used for floor cleaning. Alcohol and ammonium hydroxide based glass cleaner for windows.

House Cleaning schedule: House cleaned on a weekly basis

House No. 2 – *Dust HDM level detected 3.480µg/g Alt A1 0.352 LPS 1.420*

Child total IgE 1250 HDM IgE 1.83

Father total IgE N/A HDM IgE N/A

Mother Total IgE 38.9 HDM IgE 0.19

Dwelling type: Second floor apartment

Location: South, Port Area

Location notes: House built around the 1960/70s. Part of a housing estate built by the government to aid families in need of economical help.

Type of cooling and heating devices: Gas heaters to warm the house. Electric fans used to cool the house.

Furniture: Large carpet found in the living room. Furniture made of particle board.

Description of floor types, walls and ceilings: Floor partly in laminated parquet, with the rest covered in traditional Maltese tiles. Walls painted two years previous to the study, with **parts of it covered in mould**.

Type of apertures: Aluminium windows. Internal doors made of laminated wood.

Household Cleaning products used: Liquid detergent originally meant for clothes washing was used for floor cleaning. Plain water was used for window cleaning.

House Cleaning schedule: House cleaned on a twice weekly basis.

House No. 3 – *Dust HDM level detected 0.000µg/g LPS 2.872*

Child total IgE 122.700 HDM IgE 1.030

Father total IgE 41.9 HDM IgE 0.16

Mother Total IgE 448.3 HDM IgE 0.16

Dwelling type: Terraced House (two floored house)

Location: South, Inland Area

Location notes: Located in a green area. Many garages used for car storage are found at ground level in the street.

Type of cooling and heating devices: Gas heaters used for heating. Air conditioners used to cool the house.

Furniture: No carpets were present. Furniture made of solid wood.

Description of floor types, walls and ceilings: Floor in ceramic tiles. Painted walls in good condition.

Type of apertures: Windows in façade made from wood. Aluminium windows were used for the rear end of the building. Internal doors made of solid wood.

Household Cleaning products used: Plain water was used for both floor and window cleaning.

House Cleaning schedule: House cleaned on three times weekly.

House No. 4 – *Dust HDM level detected 0.527µg/g*

Child total IgE 499.7 HDM IgE 15.32

Father total IgE N/A HDM IgE N/A

Mother Total IgE N/A HDM IgE N/A

Dwelling type: Old two roomed house

Location: South, Port Area

Location notes: Extremely small house in poor condition. The house was **unused for over 41 years** and was recently rein habited. It was in fact **very humid**.

Type of cooling and heating devices: None

Furniture: No carpets. Particleboard furniture.

Description of floor types, walls and ceilings: Floor in ceramic tiles. Walls had been painted two years prior to the study, yet parts of the paint was already flaking apart due to issues related to dampness.

Type of apertures: Only a main entrance was present. There were **no windows** to allow for ventilation.

Household Cleaning products used: Disinfectant used for floor cleaning.

House Cleaning schedule: House cleaned on a daily basis.

House No. 5 – *Dust HDM level detected 0.000µg/g Alt A1 0.000 LPS 2.543*

Child total IgE 11.900 HDM IgE 0.270

Father total IgE N/A HDM IgE N/A

Mother Total IgE 844.3 HDM IgE 0.23

Dwelling type: Terraced House (two floored house)

Location: South, Inland Area

Location notes: This was a post war house (hence around 70 years old), found in the core of an old village. Parts of the house had never been finished, including the staircase which still had exposed concrete. Construction works were being carried out in an adjacent property.

Type of cooling and heating devices: Gas heaters to warm the house. Electric fans used to cool the house.

Furniture: No carpets. Fabric sofa in the living room. Mostly old wooden furniture was found around the house.

Description of floor types, walls and ceilings: Floor covered in a combination of old Traditional Maltese tiles and ceramic tiles. Painted walls in poor **(damp) condition** with the lower areas of the ground floor wall showed signs of humidity with the paint flaking apart. One of the ceiling was actually a glass ceiling with an aluminium frame, and signs of moisture could bee seen around the frame.

Type of apertures: Windows in façade made out of wood. Aluminium windows were used for the rear end of the building. Internal doors made of solid wood.

Household Cleaning products used: A mixture of orange peel, vinegar and lemon juice was used as a detergent for floor cleaning. Ethanol and ammonium hydroxide based glass cleaner was used to clean the windows.

House Cleaning schedule: House cleaned on a three times weekly basis.

House No. 6 – *Dust HDM level detected 0.000µg/g Fel d1 0.092 Alt A1 0.128 LPS 2.594*

Child total IgE 11.4 HDM IgE 0.22

Father total IgE 373.3 HDM IgE 13.62

Mother Total IgE 4.7 HDM IgE N/A

Dwelling type: Third floor apartment.

Location: North of island.

Location notes: This dwelling is in a block which was **still under construction and unfinished**. Most of the internal areas were finished.

Type of cooling and heating devices: Gas heaters were used to warm the house. Air conditioners were used to cool the house.

Furniture: Wool carpet in the living room. Furniture was made from fibreboard.

Description of floor types, walls and ceilings: Floor covered in ceramic tiles. The walls were covered in gypsum.

Type of apertures: Aluminium windows were used as external apertures. The internal door was a temporary piece of wood which acted as a door.

Household Cleaning products used: Floor detergent was used for floor cleaning. Ethanol and ammonium hydroxide based glass cleaner was used to clean the windows.

House Cleaning schedule: House cleaned on a twice weekly basis.

House No. 7 – *Dust HDM level detected not available*

Child total IgE 8.7 HDM IgE 0.20 Alt 0.22

Father total IgE 137.7 HDM IgE 0.16 Alt 0.42

Mother Total IgE 607.9 HDM IgE 4.6 Alt 0.21

Dwelling type: Semi-detached two floored house

Location: North

Location notes: This dwelling had a garden in front of the house.

Type of cooling and heating devices: Gas heaters were used to warm the house. Air conditioners were used to cool the house.

Furniture: Carpets were present around the house, made of wool. The furniture was made of solid wood.

Description of floor types, walls and ceilings: Floor covered in traditional Maltese tiles. There were **dampness related issues**, with the paint flaking away in the ground floor walls, and the ceiling damaged by dampness and needing re-plastering.

Type of apertures: Aluminium windows were used as external apertures. Internal doors made of solid wood.

Household Cleaning products used: Floor detergent was used for floor cleaning. Plain water was used to clean the windows.

House Cleaning schedule: House cleaned on a weekly basis.

House No. 8 - – *Dust HDM level detected 0.820µg/g Alt A1 0.000 LPS 2.471*

Child total IgE 323.40 HDM IgE 7.52

Father total IgE 820.4 HDM IgE 0.25

Mother Total IgE 10.1 HDM IgE 0.18

Dwelling type: Maisonette (First floor apartment with a separate entrance rather than a common entrance).

Location: North part of the Island

Location notes: This dwelling was built on top of garages used for car storage. Adjacent to the property there was a large children's playing field.

Type of cooling and heating devices: Gas heaters were used to warm the house. Air conditioners were used to cool the house.

Furniture: No carpets. Fabric sofa in the living room. Mostly furniture made out of particleboard was found around the house.

Description of floor types, walls and ceilings: Floor covered in traditional maltese tiles. The were signs of dampness, with most of the walls having flaking paint. The walls were also very dirty, and there were **signs of moisture**.

Type of apertures: Aluminium windows were used as external apertures. There were no doors present in the house.

Household Cleaning products used: Floor detergent was used for floor cleaning. Plain water was used to clean the windows.

House Cleaning schedule: House cleaned on a weekly basis.

Summary of above descriptions

Only three of the nine houses (2/8) described here could have been described as being in a good condition without any structural or dampness issues. Five houses (5/8) had dampness issues, which would have consisted of flaking paint due to damp walls, or clear signs of moulds. The dampness issues were immediately evident, as it immediately struck the investigators attention once he entered the house. Two dwellings (2/8) had not been finished, one of them not having proper apertures and both did not have finished stairs. One of the houses (house 4), had no sources of ventilation, and consisted of only two rooms. By today's standards, this house would not be considered habitable. Interestingly, eight out of nine of these households used gas heaters for heating purposes. While gas heating is very often used in Maltese household, gas combustion produces water, which increases indoor moisture.

Interestingly, four of the nine houses had house dust mite allergen detected in the dust samples, three did not have detectible levels, and two unfortunately did not have dust levels available due to the dust not being analysed due to budgetary restrictions. House number one, which looked clean, tidy and well maintained, had the highest HDM allergen levels in the dust collected at 41.402µg/g. House number 3, which again looked well maintained, had detectable HDM levels at 0.527µg/g. Houses 2 and 9, which both had signs of dampness, had levels of 3.480µg/g and 0.821µg/g respectively.

Interestingly, two houses which had severe humidity issues with mould growth, houses 4 and 5, did not have detectible HDM allergen levels in the dust collected. This could indicate that either there are other factors in these houses which are triggering an IgE mediated inflammatory pathway in their residents, or HDM present in another room rather than the living room which was sampled as per protocol. House number 7 also missed having detectible HDM levels in the dust collected, yet again parts of this house were still in a construction phase, and hence other factors might have been responsible for a member of the residing family to have a high serum IgE.

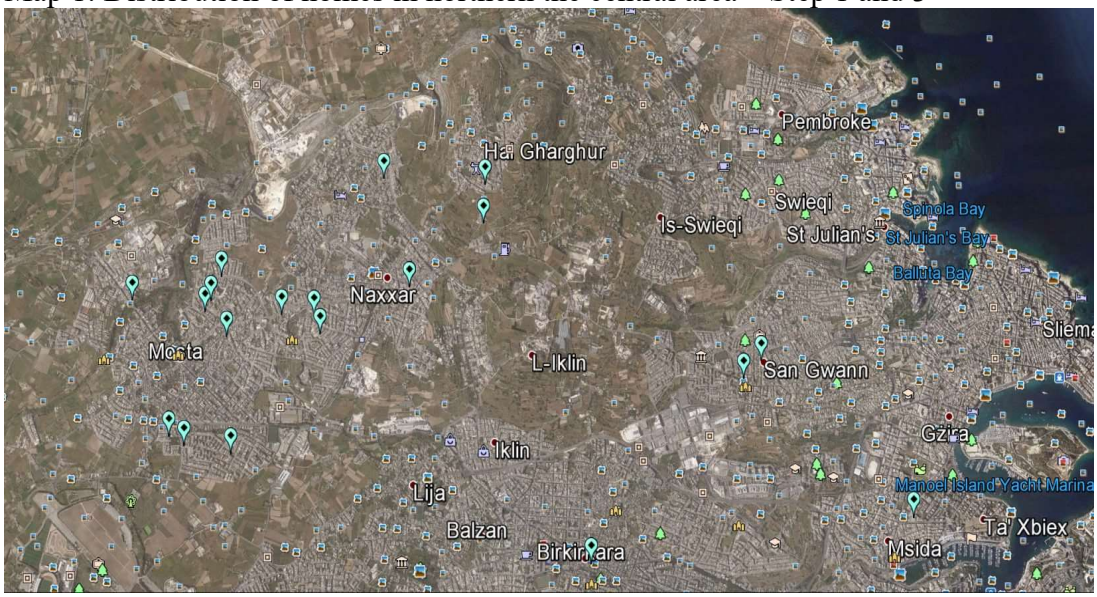
Table Comparing houses which homed individuals in the High IgE clusters

House (Location)	House Type	Child Total IgE Child HDM IgE	Father Total IgE Father HDM IgE	Mother Total IgE Mother HDM IgE	Dust Results Der p1 HDM	 Alt A1 Alternaria	 LPS Endotoxin	Notes
House No. 1 (South, Port area)	Terraced House (two floored house)	**1250U/mL** 79.21U/mL	**861.1U/mL** 7.80U/mL	137.2U/mL 0.21U/mL	41.40*µg/g*	***0.000µg/g***	3.721	Located in an area close to fishing industry and a fuel storage area.
House No. 2 (South, Port area)	Second Floor Apartment	**1250U/mL** 1.83U/mL	N/A N/A	38.9U/mL 0.19U/mL	3.480µg/g	0.352 µg/g	1.420	1970s build. Part of walls covered in mould.
House No.3 (South, inland area)	Terraced House (two floored house)	122.7U/mL 1.030U/mL	41.9U/mL 0.16U/mL	**448.3U/mL** 0.16U/mL	0.000µg/g	*0.000µg/g*	2.872	Many garages used for car storage are found at ground level in street.
House No. 4 (South, Port area)	Old Two roomed house	**499.7U/mL** 15.32U/mL	N/A N/A	N/A N/A	0.527*µg/g*	*0.000µg/g*	1.483	Had been unused for 41 years and was very humid.
House No.5 (South, inland area)	Terraced House (two floored house)	11.90U/mL 0.270U/mL	N/A N/A	**844.3U/mL** 0.23U/mL	*0.000µg/g*	*0.000µg/g*	2.543	70 years old with some incomplete. Damp walls were noted.
House No. 6 (North of Island)	Third floor apartment.	11.4U/mL 0.22U/mL	**373.3U/mL** 13.62U/mL	4.7U/mL N/A	0.000*µg/g*	0.128 *µg/g*	2.594	This dwelling is in a block which was **still under construction.**
House No. 7 (North of Island)	Semi-detached house	8.7U/mL 0.20U/mL	137.7U/mL 0.16U/mL	**607.9U/mL** 4.60U/mL	N/A	N/A	N/A	Signs of dampness in both walls and ceilings.
House No. 8 (North of Island)	Maisonette (First floor apartment with a separate entrance)	323.4U/mL **7.52U/mL**	**820.4U/mL** 0.25U/mL	10.1U/mL 0.18U/mL	0.821U/mL	0.000U/mL	2.471	Dampness and signs of moisture seen in the house.

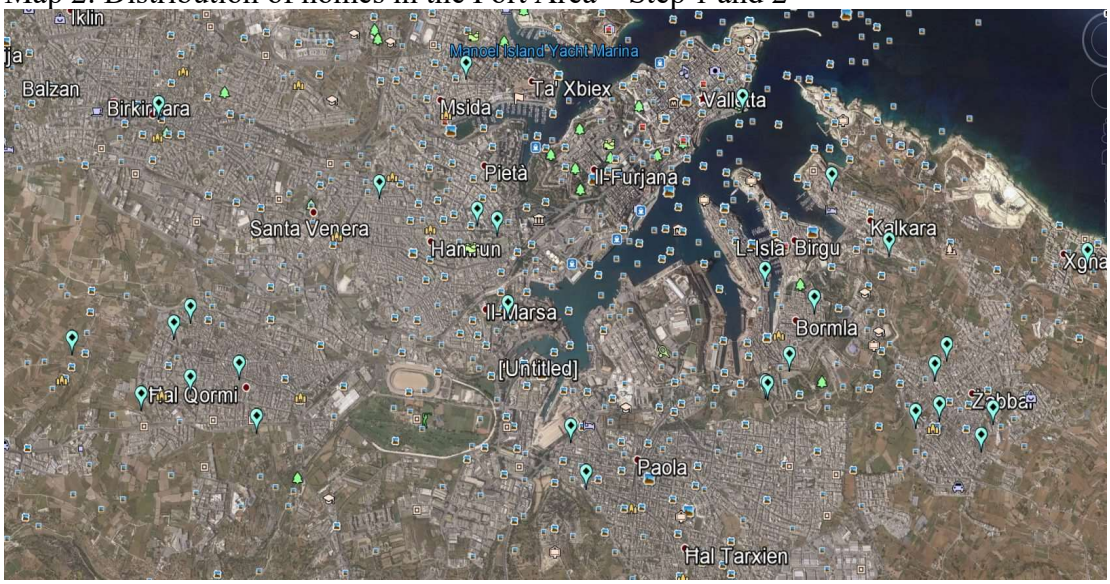
4.4 Home distribution throughout the Malta.

The following maps depict who the homes were distributed locally over the three steps of this study.

Map 1: Distribution of homes in northern the central area – Step 1 and 3



Map 2: Distribution of homes in the Port Area – Step 1 and 2



Map 3: Distribution of home in the Northern area – Step 3

As one can see from these maps, the homes were distributed fairly homogenously throughout the built up areas of the island. One can appreciate the difference in building densities when one compared the northern areas (e.g Mellieha, San Pawl il-Bahar) to the southern and central areas (maps 2 and 3). As the Respira project aimed to compare differences between Malta, and its neighbouring island Sicily, one can appreciate that the areas chosen were focused on the coastal area closest to Sicily.

5.1 Discussion

This study is one of the first carried out in the Maltese islands on respiratory health, which includes well-selected case control cohorts. Other studies such as the ISAAC[73] and the SINFONIE[81] studies are general studies on respiratory health which looked into prevalence and severity of asthma and related conditions in selected age group. In the ISAAC study, further sub-analysis was presented comparing wheezers to non-wheezers. The SINFONIE study went into more detail, adding actual indoor air quality assessment in the schools, including allergens and indoor air quality (e.g. $PM_{2.5}$, NO_2 etc) and selected a cohort of students to undergo clinical testing, including spirometry, FeNO, nasal lavage and urine cotinine.

The study being presented in this thesis selected cases with current respiratory symptoms compatible with uncontrolled asthma, against controls who did not have such symptoms and did not have a history of other allergic conditions. This design permitted using a smaller sample size and analysing their clinical characteristics which were strikingly different between the two groups. It also helped in understanding whether any factors in the domestic environment had contributed to active symptoms and differences, by visiting the households. This would have not been possible with a cohort study, as visiting a large number of households would have needed an extensively larger resource and equipment means in order to cover them within the predetermined time window. On the other hand, one could argue that the control group had an extremely strict exclusion criteria, and that this design excluded children who had asthma or related allergic conditions but who were well controlled due to a number of potential factors (e.g. compliance to asthma medications).

5.1.1 Effects of smoking on children

The first striking results were related to smoking exposure. Although the proportion of children whose mothers smoked during pregnancy was not significantly higher in the case group when compared to the control group, this was not the case for exposure to second hand smoke in the first year of life. 30.3% of cases were exposed to SHS in the first year of life when compared to 6.6% in the control group. A similar trend had already been reported by Montefort et al[83] as an observation noted in the ISAAC study locally. SHS in the first year of life was noted to be associated with wheezing 'ever', exercise-induced wheeze and being diagnosed with asthma. As mentioned earlier, literature has reported a greater incidence of atopic conditions in individuals who were exposed to tobacco smoke[25].

When one explores the current exposure to tobacco smoke, the aforementioned trend was also true, as 40.9% of cases were being exposed to tobacco smoke when compared to 18.3% who reported this in the control group. One could speculate that the parents of cases, who had active respiratory symptoms, would be more aware of such exposure as they would be more concerned about their children's health and thus tend to have a higher tendency of reporting these situations when compared to the parents in the control group. On the other hand, these proportions were similar to those reported for exposure to SHS in the first year of life, and thus it was likely that children who were born in an environment exposed to smokers continued to live in such a situation as they grow older.

A large survey which has been extensively reported and which shed more light on the effect of tobacco smoke on allergy and asthma is the European Community

Respiratory Health Survey (ECRHS)[94]. This was a multicentre survey spanning various European countries. Amongst various findings, it was found that maternal smoking was associated with current respiratory symptoms in adult females, and paternal smoking was associated with symptoms in adult males. This was not replicated in this study, though this could be due to the fact that the "case" group was composed of a relatively small number of individuals, and only focused on the children's current symptoms, while the ECRHS was a much larger epidemiological survey.

The ECRHS also made interesting findings related to allergic conditions and sensitisation. It found that smokers were more likely to have higher total IgE levels, and were also more likely to have detectable serum-specific IgE to house dust mite, yet less likely to be IgE-sensitized to grass and cat. Mlinaric et al reported similar effects with passive smoking in adolescents[95], and found that there was an increased prevalence of allergic disease in both active and passive smokers. Potentially, this could be due to an activation of interleukin-4 (IL-4) which stimulates IgE synthesis[96]. Mlenaric et al interestingly reported that total IgE levels were even higher in passive smokers when compared to active smokers.

These findings somehow correspond to the results presented in this thesis. The case group had a higher exposure to SHS in the first year of life ($p = 0.001$) and current SHS exposure at home ($p = 0.006$). The case group also had significantly higher serum total IgE levels ($p = 0.027$). One has to look into these findings in more detail and understand whether these are directly related to each other, or whether the higher

IgE levels were unrelated to smoking, but rather a consequence of choosing children with active symptoms secondary to allergic-conditions.

5.1.2 Family history of atopy and its effects

Family history of atopic conditions is also a strong risk factor for bronchial asthma[84, 97]. Besides univariate analysis showing higher proportions of family members having a history of asthma, nasal allergy and eczema in direct relatives of cases when compared to control children, a logistic regression model which is more statistically solid, confirmed this finding. The presence of a sibling who had been diagnosed with asthma was the strongest predictor for a child to be part of the "case" group and hence more likely to have current respiratory symptoms, with an odds ratio of 6.1. The second strongest predictor was having a father who had asthma (OR 5.338) followed by a mother having rhinitis (OR 3.038). This model had a Pseudo R-Square value of 0.272, which meant it could predict 27.7% of the total variation in these family history-related responses which differed between the case and the control group. In the ECRHS, the odds ratio for a subject to develop asthma if the father had asthma was 2.9, while that for having an asthmatic mother was 3.2[99]. One cannot compare these results directly to this study, as here the results focused on a case group who had uncontrolled asthma, and excluded controlled cases, while the ECRHS reported a general prevalence of asthma.

Hence, here we are noticing a hereditary aspect and hence the probable importance of genetics in the development of respiratory and atopy-related problems in Maltese children. Skadhauge et al[98] studied 11,688 twin pairs born in Denmark, aged 12-41

years, and found greater concordance in monozygotic rather than dizygotic twins, pointing to a genetic component. The ECRHS again sheds some more information on the genetic importance on the risk of developing asthma[99]. Yet, while they noticed the importance of a genetic aspect in various European countries, Burney et al[99] noted significant variations in the risk of asthma within single countries, and concluded that besides a genetic heterogeneity a variation in exposure to common environmental factors such as air pollution, allergen load, or viral epidemics could also have led to these variations.

Holloway et al [105-106] have described extensively the genetics related to asthma. Genome-wide association studies (GWAS) analysis have identified an association between functional variance in the gene encoding the alpha chain of the high-affinity receptor for IgE (FcεR1α) on chromosome 1q23. Foster et al[113] have in fact demonstrated that subjects who have been diagnosed with allergic asthma had precursor dendritic cells which had higher expression of FcεR1α and this correlated to higher serum IgE levels in these subject when compared to a control group. Other foci have been also identified for genes encoding Th2 cytokines typically associated with upregulated atopmic disease such as IL-4 and IL-13.

Interestingly in the population presented in the thesis, there were strong correlations between children mean total IgE and father mean total IgE ($p = 0.004$) and the same correlation was found between children's and mothers mean total IgE ($p = 0.004$). This could indicate that Maltese children were also inheriting the above described gene from their parents, leading to IgE-driven allergic conditions. This theory is further strengthened by the associations found between the child and parent clusters,

were a logistic regression model showed that having a father who had a history of asthma and had a high serum IgE increased the risk of the child being a case with a high serum IgE by an odds ratio of 16.5 ($p = 0.023$). Perhaps identifying phenotypes such as the ones described in this thesis and then focusing the genetic research in these families could identify genes which are specifically involved in the pathways, and the identification of these genes could also act as markers for predicting treatment response of high cost treatment for asthma such as omalizumab.

5.1.3 Atopy in this local population

House dust mite sensitisation was the predominant aeroallergen in all the groups in this study (38.8% in children, 34.8% in fathers and 22.3% in mothers), as detected through skin prick testing (N.B. A *Dermatophagoides* mix SPT was used, and hence one cannot differentiate between Der p1 and Der f1 in this case). These frequencies were similar to those found in the GA^{2}LEN study where the mean European sensitization rates were of 31.3% to Der p1 and 28.9% to Der f1 were reported[107]. Interestingly, the GA^{2}LEN study also reported that rates were higher in the Mediterranean countries such as Portugal where a sensitisation of 68% to Der p1 and 68% to Der f1 was reported[107]. Such a finding contrasts to what was described in the first phase of the ISAAC study[6], where Malta placed 15th in the prevalence of asthma in 13-14 year olds while Portugal was 36th within the countries which participated in the large study. Therefore, although HDM sensitisation was much higher in this Mediterranean country, other factors in Maltese children must have been resulting in increased symptoms related to asthma. This could be due to environmental factors, factors which could be synergistic together to HDM, or other pathways unrelated to HDM atopy which would be leading to a greater prevalence

of asthma in Malta. Similarly, in a conference paper presented by our group, presenting the Respira Study findings at the European Respiratory congress in Munich[108] in 2014[108], we reported that children in Malta had a higher risk of developing asthma when compared to children who lived in the neighbouring Mediterranean Island of Sicily.

5.1.3.1 Variations in Total and specific IgE levels

Serum IgE levels are highly variable, being undetectable in early life, and depending on the atopic status of the individual elevates according to sensitisation [116]. It can vary by season, and it has been reported that there is a delay in the elevation of these levels after exposure, as these peaked 4-6 weeks after exposure in pollen allergic individuals [117]. Levels also elevated in response to certain viral infection and parasitic infestations [116]. It was therefore not surprising to see varying levels of both serum total and specific IgE levels in the subjects who participated in this study. The following sections describe the results obtained in the various groups and how these varied according to different variables. It has been customary in many publications presenting results pertained to serum IgE, to use a geometric mean through using a Log_{10} transformation of the IgE results. This leads to normalisation of the data, and the ability to use parametric tests such as t-test and one-way Anova. Analysis on Log_{10} IgE data (kU/mL) has been performed, and these led to the same results (see appendix 5 section). As only results for mean serum total IgE and mean specific HDM were normalised through a geometric mean, while the other mean specific IgE levels did not have a normal distribution after a Log_{10} transformation, it was chosen to standardise the analysis by using non-parametric tests for all the IgE data in U/mL.

5.1.3.1.1 Variations in Total and specific IgE levels in children

The mean total IgE levels in case children were more than two times higher than those in control children (230.36U/mL vs 96.56U/mL, p = 0.025). This was an expected result, as atopic individuals have been reported to have higher IgE levels when compared to non-atopic individuals. In fact, most of the case children (60.6%, n=40/66) had at least one positive skin prick test. The control group were selected with the proviso that these had no history of atopic conditions, although some of these still had sensitisation to at least one aeroallergen (as 19/63 children 30.2% in the control group had positive SPTs).

House dust mite was the aeroallergen for which the highest mean serum specific IgE was recorded in all the children (4.919U/mL, SD 12.578). Der p 1 allergen, which is most commonly produced by HDM has been shown to be able to cleave CD23 which leads to increased serum IgE levels [117]. Specific HDM IgE was also the only specific IgE tested for in this study which was found to be significantly higher in the case group when compared to the control group (p = 0.001). It has been postulated that mutations in gene encoding CD23 can result in more exacerbations in asthmatic children [118], and this could be a genetic explanation for the differences in specific IgE levels found in this study. There were no significant differences in the levels of Der p 1 levels found in the dust in the living rooms in the houses of case and control children and hence, one cannot attribute different exposures to HDM as an explanation for the higher specific IgE levels in case children when compared to control children.

Seasonal changes in serum IgE levels were clearly observed in children who participated in this study. The lowest total serum IgE levels were recorded in the spring season, while the highest levels were recorded in the autumn season ($p = 0.001$). The mean IgE levels in winter were also higher than the spring season, though this difference was just shy of statistical significance ($p = 0.064$).

The children who had their blood sampled in Spring, had this done between March and April 2012, and lived in the central part of the island. Those who had blood letting during Autumn, had this done in October 2012, and resided in the southern part of the island, while the children who had sampling in Winter lived in the northern part of the island, and had blood letting in January 2013. The same timescales were used to obtain samples from the adults who participated in this study. There could be an argument for these differences to be a result of these children living in geographically different part of the island. The maps presented in section 4.3, showed that given the small size of Malta, the children did not live far from each other, and hence it is unlikely that geographical distribution could explain these differences. The central part of the island had a higher vehicular density and was generally more polluted, as air station data from the Environment and Resources Authority showed higher pollutant levels in the Msida (central) air quality station rather than the Zejtun (southern) air quality station [119]. Environmental air pollution has been associated with higher serum IgE levels in asthmatic individuals [120]. This was not the case in this study, where children who lived in the more polluted central area of the island and who were sampled during the spring season had the lowest mean serum IgE levels. These observations would lead to the conclusion that these results were truly

the consequence of different seasonal circumstances, resulting in the lower serum IgE levels in spring, and higher in autumn and winter.

Case children and control children followed similar patterns, with both groups having lower mean IgE levels in spring and higher levels in autumn, albeit, as expected the mean levels were higher for the case children. Potentially, this could be due to environmental conditions in the colder, damper autumn and winter seasons which induce higher serum IgE levels in children.

Interestingly, in case children, serum specific HDM IgE followed the same seasonal variation as was the case for total IgE. The levels of specific HDM IgE were significantly lower in spring when compared to autumn ($p = 0.036$) and winter ($p = 0.017$). It could be argued that since the total serum IgE levels were markedly raised during these seasons, then specific HDM IgE was following the same pattern in a proportional manner. On the other hand, another specific IgE level to a seasonal aeroallergen such as olive pollen was higher in spring, when olive tree are flowering. Control children, who had significantly lower serum IgE levels in spring, did not show the same seasonal variation for specific HDM IgE as children did, with the mean specific HDM IgE levels in this group which actually tended to be higher during spring (not statistically significant, $p = 0.605$).

A study by Gouder et al [118], described attendances at the Accident and Emergency Department in the main hospital in Malta. In this publication, the authors present the months in which these attendances were distributed, and these were mostly during the autumn and winter months. Higher serum IgE has been associated with lower

FEV_1 and FEV_1/FVC ratios [121], which in turn increased the risk of asthma exacerbations. These findings suggested that the seasonal patterns in serum IgE found in this study, coincided with the seasonal variation in asthma exacerbations in this country.

5.1.3.1.2 Variations in Total and specific IgE levels in parents

One needs to take into consideration that the parents who participated in this study were not part of a case control study, as was the case for the children. They were selected as being their parents and were thus a mixed group. There were no significant differences in mean total IgE levels for parents of case children when compared to those of control children. On the other hand, fathers of case children had higher mean specific IgE levels to two seasonal allergens, olive and *Parietaria*. Mothers of case children also had higher specific olive IgE levels when compared to their control counterparts, but they also had higher specific IgE levels to dog, *Cladosporidium* and HDM. Hence, parents of case children had a higher tendency to having allergy to olive pollen. Mothers of cases tended to have higher specific IgE levels to more aeroallergens, pointing to the possibility of them having a different allergic profile to fathers. This is an interesting finding, in the context of the fact that mothers of case children had a higher tendency to have allergic rhinitis to mothers of control children. Perhaps, the fact that mothers of case children developed more atopy to more aeroallergens, led them to have symptoms of allergic rhinitis. It was also interesting to note that mothers of case children had significantly higher serum specific HDM levels compared to control children, the same specific IgE which was five times higher in the serum of case children when compared to control children ($p = 0.001$). This could have been due to a similar pattern of genetic inheritance of atopy

from the mothers to case children. It is unlikely that this was due to similar exposure to the allergen, as the results from house dust samples showed a lower concentration of Der p 1 in dust collected from the house of case children when compared to those of control children.

As was the case for children who participated in this study, both fathers and mothers had significantly higher total IgE levels during the autumn season when compared to the spring season ($p = 0.006$ and $p = 0.009$ for the respective groups).
When the specific IgE levels were analysed in fathers who participated in the study, only specific goldrenrod IgE varied significantly by season. This was completely different for the mothers, who had significant seasonal variability in specific serum IgE levels for all the aeroallergens investigated. This indicated a different pattern of atopy in adult females when compared to adult males in this cohort.

The most interesting findings were noted when the seasonal variances in total and specific serum IgE levels were compared between case children and parents who had doctor-diagnosed asthma. Both children and doctor-diagnosed asthmatic fathers had their lowest serum total IgE levels in spring (114.861U/mL ±261.660U/mL vs 61.8U/mL ±99.664U/mL). Fathers who had doctor-diagnosed asthma had the highest serum total IgE in winter (148.02U/mL ±129.953U/mL, followed by autumn 118.075U/mL ±28.167U/mL). Case children had the highest serum total IgE in autumn (321.16U/mL ±388.282U/mL followed by winter 230.359U/mL ±397.144U/mL). On the other hand, mothers who had doctor-diagnosed asthma, contrary to case children and doctor-diagnosed fathers, had their lowest mean serum

total IgE levels in autumn (12.733U/mL ± 13.008U/mL), while their highest were recorded during the winter season (254.080U/mL ±239.239U/mL). Interestingly, opposite to the relatively low spring serum total IgE levels in case children and doctor-diagnosed fathers, mothers had a higher mean total IgE level (201.780U/mL ±350.542U/mL) which was close to that obtained in the winter season.

The same similarities in seasonal variation between case children and doctor-diagnosed fathers was observed for serum specific HDM IgE. Doctor-diagnosed asthmatic fathers and case children had the highest serum specific IgE levels in autumn (4.413U/mL ±5.422U/mL and 8.797U/mL ± 18.638U/mL respectively) followed by winter (3.995U/mL ±6.475U/mL and 8.409U/mL ± 12.816U/mL) while these groups exhibited the lowest serum specific HDM IgE levels in spring (3.01U/mL ±5.333U/mL for doctor-diagnosed fathers and 6.539U/mL ±13.644U/mL for case children). On the other hand, doctor-diagnosed mothers had very low serum specific HDM IgE levels in spring and autumn (0.230U/mL ±0.080U/mL and 0.155U/mL ±0.049U/mL) while the highest levels were detected in winter (6.535U/mL ±7.057U/mL).

These results may indicate that the mechanisms leading to doctor-diagnosed asthmatic fathers and case children having respiratory symptoms in context of an IgE-mediated inflammatory pathway may have been related in these two groups. In fact, having a father who had a history of asthma increased the risk of a child to be part of the case group. Doctor-diagnosed mothers seemed to have different trends in serum IgE seasonal variability which could have been due to these mothers having a different inflammatory pathway or different causes to their respiratory symptoms. A clue to an explanation to this phenomenon could be found in the various papers on

phenotyping through cluster analysis which have been mentioned in this thesis. Haldar et al described an obese non-eosinophilic cluster, who were predominantly female, and had a late-onset asthma[109]. Moore et al, as part of the SARP study, described two clusters which both had predominantly female members, who mostly had a late onset-asthma, and which had a high BMI. Both of these groups had significant respiratory symptoms[121]. Finally Schatz et al as part of the TENOR study describe four of the five clusters who they identified in adult and adolescent asthma to be predominantly female (while the asthmatic children who were less than 12 years old were predominantly boys). Of these groups, two had predominantly late-onset asthma, and a high rate of obesity. One of these groups had less atopy, while the other group exhibited aspirin sensitivity [111]. Given the fact that these studies had indicated a trend towards a high weight, the mean BMI of the doctor-diagnosed asthmatic mothers was checked, and this was 28.39kg/m^2. 37.5% (6/16) had a BMI >30kg/m^2.

The findings described in these studies indicate that the females who had late-onset asthma had a different phenotype to adult males and asthmatic children, and this could explain the different seasonal patterns in mean total and specific serum IgE exhibited by the doctor-diagnosed mothers when compared to case children and doctor-diagnosed asthmatic fathers.

5.1.4 Clusters in the studied groups

Cluster analysis has been broadly used in the past years, since the first landmark paper by Halder et al[109] in 2008. The recognition of separate groups in a heterogenous condition which asthma is, increased the understanding of the different

phenotypes, the pathogenesis involved (including which inflammatory pathways are involved in the separate groups) and how treatment response varies.

5.1.4.1 The child clusters

The children who were included in the case group in this study, were highly symptomatic children, as they needed to report at least two of the following three symptoms: wheezing in the last 12 months, exercise induced wheezing in the last 12 months and/or nocturnal cough in the last 12 months. They were clustered according to their clinical results – predicted FEV_1, FEV_1/FVC ratio, Exhaled FeNO and serum Total IgE. Two distinct clusters were identified through K-means clustering, one which had 56/63 members (88.9%) who had lower serum Total IgE, lower exhaled FeNO and no airway obstruction, and a smaller group of 7/63 (11.1%) children who had very high total IgE levels, higher exhaled FeNO and lower FEV_1 and FEV_1/FVC ratios. It was interesting that 6 out of the 7 members (85.7%) of the latter group were boys, indicating a higher male predominance in this group who tended to have symptoms driven by high serum total IgE and with higher airway inflammation. This statistic did not reach statistical significance ($p = 0.265$) yet this was likely due to the fact that this was such a small group.

Fitzpatrick et al[110], described similar results when they performed cluster analysis in a cohort of 273 children from the Severe Asthma Research Program (SARP). In this analysis, they produced four clusters, as they included onset of asthma as a factor to differentiate the groups. The High Total IgE group which is described in this thesis was very similar to the fourth cluster described in the study by Fitzpatrick et al. These authors described the members of this group as Early-onset atopic asthma with advanced airflow limitation. This group consisted of 29/161 (18%) of the cohort

which was derived from the SARP. They were also predominantly male (19/29, 66%) and had relatively high median serum IgE [361kU/L (7-1800kU/L)]. They also had the highest FeNO [30ppb (94-1690ppb)]. The children described in this cluster were also on the higher inhaled corticosteroid doses, had a higher use of rescue short-acting inhaled bronchodilators and almost half of them reported asthma symptoms which were affecting their activities of daily living. In table 121, the fourth cluster of children with severe asthma described by Fitzpatrick et al was compared to the children who were members of the High Total IgE cluster in this study.

Table 121: Comparing High Total IgE Cluster to Cluster 4 described by Fitzpatrick et al and Cluster

	Cluster 4 Fitzpatrick et al	**High Total IgE Cluster**
No. of patients (%)	29 (18%)	7 (11.1%)
Boys (%)	19 (66%)	6 (85.7%)
Parental history of asthma (%)	22 (76%)	2 (28.6%)
Mean (range) serum IgE	361 (7-1800)	1133.2 (813.60 - 1250)
Mean (SD) FEV1/FVC	0.79 (±0.11)	0.77 (±6.40)
Exhaled Nitric oxide (range)	30 (4-169)	54.86 (20 - 131)

As described in table 121, the subjects in both clusters were predominantly male. The members of the High Total IgE cluster presented in this study had higher mean serum total IgE and exhaled FeNO, when compared to cluster 4 in the Fitzpatrick et al cohort, which could indicate that the children in this cluster had even higher airway

inflammation than the latter children. In fact, the mean FEV_1/FVC was marginally lower in the members of the High Total IgE cluster described in this thesis, possibly indicating that subjects had more obstructed airways.

Following the results presented from the SARP study, The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study published data on a much larger cohort of children and adolescents[111]. In our study, the children were divided in two groups: 518 children aged between 6 and 11 years old and 3612 adolescents who were 12 years or older, of which 1829 were over 18 years of age. Hence, it is not possible to directly compare the results obtained by the subjects who participated in this study, as they were aged between 11-14 years old and who would have fit somewhere between the two groups presented in the TENOR study. On the other hand, the two of the clusters presented in the TENOR study are particularly similar to the High Total IgE cluster found in the children who participated in this study. These were cluster 5 in the child group, which consisted of 14.1% of the child cohort, and who were all boys, exhibited High Total IgE, and had a low FEV_1/FVC ratio, and the fourth cluster in the adolescent group, which represented 16.5% of the adolescent group, consisted mainly by non-white members, and also had high total IgE levels and low FEV_1/FVC ratios. The authors, Schatz et al, made an interesting observation on the fourth cluster in the adolescent group, as they note that quality of life, socioeconomic and environmental factors due to the poorer conditions in which non-white families might be exposed to in this population might have had an impact on their asthma control. This observation will be discussed in more detail when discussing our findings related to the houses.

5.1.4.2 Clusters in fathers

K-means clustering produced similar groups to those identified in the children who participated in this study. The father high IgE cluster, similar to the one identified in the children cluster had a higher mean serum IgE and FeNO while having lower FEV_1 and FEV_1/FVC ratio. It was very interesting to note that only one out of the six fathers who were members of this group had been diagnosed as having asthma. Haldar et al had described a similar group to the one identified in the fathers who were members of the High IgE group. The authors described the group in their study as an Inflammation Predominant group, who had a later onset of symptoms, who were mostly male and had few daily symptoms while having active eosinophilic inflammation[109]. In the study presented in this thesis, sputum eosinophils were not measured, and instead FeNO was used as a marker of airway inflammation, and this was higher in the father High IgE group. The fact that most of the members of the High IgE father cluster were not diagnosed with asthma could be due to lack of respiratory symptoms, similarly to the group described by Halder et al. The TENOR study also described a group of adolescents and adults which was exclusively composed of males, who had been diagnosed with asthma, but had the least symptoms in their cohort. Although this group did not report respiratory symptoms and had good quality of life, 61.8% of the members of this group had a lower FEV_1/FVC ratio for their age, with a mean of 69.1 ±12.0 indicating airway obstruction, which was a similar trend to that seen in the father High IgE group in this study, who had more airway obstruction compared to the rest of the father cohort.

The main aeroallergen specific IgE which was found to be a risk factor for a father to be a member of the High IgE cluster was specific HDM IgE, with an OR of 1.208

(p = 0.016). This result corresponded to that obtained in the children case High IgE cluster, who had specific HDM IgE as the strongest predictor with an OR of 1.112 (p = 0.004). These findings continued to show a relationship between fathers and children, where it has been already shown that a child having a father who had doctor-diagnosed asthma was more likely to be part of the case group (OR 5.338), and that doctor-diagnosed asthmatic fathers and case children had similar seasonal variability in total and serum specific IgE levels.

5.1.4.3 Clusters in mothers

The groups identified through K-means clustering in the mothers was again identified with serum total IgE being the strongest factor which differentiated the groups. In this case, mothers who were part of their corresponding High IgE group had only slightly higher FeNO (17.67ppb ±9.785ppb vs 12.15ppb ±10.208ppb, p = 0.020 for the low IgE mother cluster), and practically no differences in FEV_1 and FEV_1/FVC.

Interestingly, while multivariate stepwise regression analysis in the High IgE clusters for both case children and fathers resulted in serum specific HDM IgE being the strongest predictor, this was not the case of the mother High IgE cluster. In fact the strongest predictor for this group was serum specific *Parietaria* IgE (OR 1.523, p = 0.011) followed by serum specific olive IgE (OR 1.897×10^3, p = 0.041). Both these aeroallergens are pollens which are typically associated with seasonal allergic rhinitis. This corresponds with the finding of a diagnosis of allergic rhinitis rather than asthma in mothers acting as a risk factor for the children being a member of the case group (OR 3.038, p = 0.037). These findings highlight the differences in the response of adult males and female to aeroallergens which has been presented in the

study, and the different heritability of atopic conditions from fathers and mothers to children.

5.1.4.4 Correlations between child and parent clusters

The children who were members of the High IgE cluster were more likely to have fathers in the corresponding High IgE cluster ($p = 0.043$) while this was not the case for having a mother who was a member of the mother High IgE cluster ($p = 0.076$). When a logistic regression was applied, having a father who was a member of the corresponding High IgE cluster made it 16.5 times more likely for the child to be a member of the case high IgE cluster ($p = 0.022$). These findings further indicate the possibility hereditary relationship in the atopic mechanisms between the fathers and the children, especially in the case of members of the respective High IgE clusters.

5.2 Variations and observations related to houses

Observations which have been reported in this thesis further prove that genetics alone cannot explain the presence of symptoms in a child predisposed to asthma, and interaction with environmental factors is of essential importance[85]. If one had to look at the local scene, it was extremely interesting to note in the ECRHS a slightly lower prevalence of asthma was noted in Mediterranean countries when compared to central European countries. It was a similar situation in younger individuals where the ISAAC study reported a lower prevalence of all allergy-related conditions in 13-14 year old from Mediterranean countries such as Italy and Spain when compared to Northern European counties such as the UK, the Republic of Ireland, Germany and Sweden[6]. On the other hand, Maltese children did not have the same low prevalence's reported by the other Mediterranean countries in this respect but had the

third highest prevalence in allergic rhinoconjunctivitis from all European countries which participated in ISAAC and ranked 15th in self-reporting of asthma. Malta had not participated in the ECRHS, yet if one had to extrapolate the findings from the ISAAC study, Malta might have presented similar differences when compared to other Mediterranean countries. this could be due to environmental differences to these countries which are independent of the climates which are similar to the local one. These findings might be due to indoor and outdoor environmental factors which are different to other Mediterranean countries.

One could argue that the presence of the same environmental triggers present in a household could affect more than one member of a family who dwell in it, and therefore would increase the likelihood for a relative to develop an allergic condition. This could also explain the increased prevalence of family members reporting these conditions in the case group. Hence, it is essential to look into environmental factors and variables shared by these individuals, this logically being the household. This would be in conjunction with the genetic predisposition found in members of the same family having an allergic predisposition which is triggered by the same environmental factors.

Although there were no significant differences, when considering the type of buildings inhabited by the case group, one could notice a trend indicating that these children tended to live in relatively smaller dwellings. 42.4% of the case children against 25.8% of control children lived in apartments, which tend to have a smaller living area when compared to a house or a villa. Interestingly, 56.5% of the control

children compared to 39.4% of the case group lived in a single-family house. These proportions did not reach statistical significance (p = 0.127), though this could have been due to a relatively small study population, which could have resulted in a type II error. Smaller dwellings would result in higher indoor concentration of allergens, as reported by Ronsefeld et al[86]. This study also reports variations in the presence of various allergens depending on the residence type. Rodent allergen tended to be higher in public housing. Differences in housing age and single-family units could also reflect socio-economic differences between cases and controls.

Two interesting findings showed that houses inhabited by cases were less likely to have a fireplace or an air conditioner (both significant p=0.05). This could indicate that their parents were actively avoiding installing such devices, as they would feel that these could affect their children's symptoms. If one had to browse through the Asthma UK website, one would find various comments indicating that asthmatics feel that fireplaces and air conditioners are detrimental to their health. Interestingly, no studies have shown a correlation between indoor combustion (as in fireplaces) and asthma control[87], which would disprove these beliefs. This could also reflect that cases might have been coming from families who could not afford having commodities such as air conditioners or a fireplace installed in a house (fireplaces in Malta tend to be more of a decorative addition to a household, as the local climate does not make their regular use necessary).

On the other hand, whilst the installation of certain features in a home are easier to control by the parents who own/rent the house, other characteristics can be more difficult to control. This can include the presence of dampness and mould in the

building. The Maltese climate is already relatively humid[88], yet building characteristics and maintenance could lead to higher indoor humidity and dampness which can lead to higher concentrations of allergens from mould and house dust mite.

While there were no reported differences between case and control homes as regards to general visible mould in the house and water leakage/damage, it was very interesting to note that 16 children's bedrooms from the case group were reported to have visible mould when compared to 6 having the same problem in the control group. This was statistically significant ($p = 0.034$). Also, the parents of 23 cases (36.5%) reported a smell of mould against only 5 controls (8.5%). This was a very significant finding ($p<0.001$), and while one could argue that these parents could be over-reporting these features as they would be concerned about them as opposed to control parents who could be naïve to such a problem, it could also be pointing to a cause for the cases reporting uncontrolled symptoms.

An important study to mention when reporting these results was the 3-HE study, which was carried out in Sweden, and studied 7,554 dwellings [100]. It looked into the relation of doctor-diagnosed asthma, doctor-diagnosed allergy, self-reported pollen allergy and self-reported eczema, associated with the following building characteristics: construction year, size, ventilation, heating, maintenance management, crowdedness, airing habits, recent redecoration, water or dampness damage and indication of high air humidity. The results of this study also showed that a smell of mouldy air was associated with doctor-diagnosed asthma and doctor-diagnosed allergy. An association was also found between eczema and water damage during the previous five years and mould odour in the building. One must again remember that our study compared these findings to doctor diagnosed asthma and

allergy. The fact that in the case control cohort being presented, these associations were relatively strong when compared to the findings reported by Norback et al ($p<0.001$ for a case house having a smell of mould and $p = 0.034$ for visible signs of mould in the child's bedroom) might indicate a stronger relationship between these finding and uncontrolled respiratory symptoms.

Another observation regarding the dwellings inhabited by the cases which stands out was their proximity to busy traffic when compared to the control houses. The parents of 54.3% of cases reported that their children were living in an area which was close to busy traffic when compared to 30.8% of control children ($p = 0.058$). The trend was similar when they were asked whether the children lived within 200m from heavy traffic (66% vs 50.9%, $p = 0.086$). Had the sample been larger, the results might have even reached statistical significance. As one delved deeper into these observations, and compared the children who lived closest to busy roads (within 50m) to those who lived further away from such roads (more than 50m), one found that 48.8% of the case children lived within 50m from a busy road, when compared to 27.8% of the control children ($p = 0.049$). These are important observations when one considers the high vehicular density in the Maltese islands, as this could have been contributing to the symptoms experienced by these individuals. In a study carried out in Sweden by Lindgren et al[101], exposure to traffic was associated with a higher prevalence of allergic asthma and allergic rhinitis, but not with asthma or rhinitis triggered by non-allergic factors such as cold weather, SHS, scents and dusty places. They reported an association between Nitric oxides (NO_X) and allergic asthma in the city of Malmo, but not outside urban areas. They also found a clear relation between exposure to traffic and asthma triggered by pollen or furred animals. A

similar study in Italy showed an associated between vehicular traffic and allergic rhinitis, and a potential influence by climatic conditions which could increase the effects of nitrogen dioxide NO_2 exposure[102]. Another possible mechanism may be due to exposure to diesel exhaust particles (DEP), which has been reported by Lee et al[103]. In this study mice were exposed to DEP, and then exposed to house dust mite extract. These then underwent bronchoalveolar lavage, this which then examined through liquid chromatography. It was found that DEP exposure induced the cysteine oxidative state in the epithelial lung fluid, which led to increase in TH2 cytokines following DEP and HDM co-exposure. Therefore, one could speculate that DEP could potentiate the effect of HDM allergens in patients with allergic asthma.

Clinical measures which are closely associated with allergic sensitization in children are fractional exhaled nitric oxide[90] (FeNO) and Immunoglobulin E[91] (IgE). Interestingly while there were no significant differences between the case and control group in FEV_1 and FEV_1/FVC, FeNO was almost twice as high in the case group when compared to the control group ($p<0.0001$). Total IgE was greater than double in the case group when compared to the control group ($p=0.027$). One could argue that as the cases and controls were chosen *a priori* with the above-mentioned characteristics for allergic conditions versus absolutely no such features, then such results were to be expected. On the other hand, one cannot ignore studies such as that of Norbäck et al[92], when a direct correlation was found between FeNO, respiratory symptoms and *Aspergillus versicolor* allergen levels in Malaysia. Given the findings found in this study, it would not be unreasonable to think that these is a similar situation locally.

Nasal patency also varied between the case and control group. The mean minimum area of the left nostril was significantly smaller in the case children when compared to the control children ($p = 0.011$). There were no significant differences when the right nostrils were compared between the two groups. Given the above findings confirming higher FeNO and serum total IgE levels in the case group, it would not be unreasonable to suspect that the differences in nasal patency were due to allergic rhinitis, which we know is highly prevalent in Maltese children and commonly associated with asthma[72].

If one had to summarise the above observations, the case children have a strong tendency to have a positive family history to an allergic condition, and thus might have a potential genetic predisposition to these conditions. They are also more commonly exposed to SHS which is known to increase the likelihood to develop sensitisation to allergens[89]. The case children also have a greater tendency to inhabit buildings which could expose them to mould (and hence present an environment which promotes mould growth). They also lived closer to busy roads, which could also expose these children to exhaust fumes which has also been associated with increased sensitisation to allergens. Clinical testing confirmed that is was highly likely that the cause for the respiratory symptoms which were being experienced by the case group was due to allergy-mediated process (given high FeNO, serum total IgE and decreased nasal patency).

5.3 General Conclusions

5.3.1 Conclusions from questionnaires on children

a) Exposure to SHS in the first year of life increased the risk of having uncontrolled asthma symptoms in adolescence ($p=0.001$, Chi-squared).

b) Current exposure to SHS increased the risk of having uncontrolled asthma symptoms in adolescence ($p=0.006$, Chi-squared).

c) Children who had uncontrolled asthma symptoms were more likely to have had bronchitis ($p=0.027$, Chi-squared), and/or asthmatic bronchitis ($p<0.001$, Chi-squared) in the first two years of life

d) A positive family history of allergies increased the likelihood of a child having uncontrolled asthma symptoms.

e) Having a sibling who was diagnosed with asthma was a strong predictor (OR 6.1) for a child to have uncontrolled asthma symptoms, followed by having a father with a history of asthma (OR 5.338), and having a mother who had a history of allergic rhinitis (OR 3.038).

f) Having a pet at home increased the likelihood of a child having uncontrolled asthma symptoms ($p=0.05$, Chi-squared).

g) The type of building in which a child lived did not change the likelihood of a child having uncontrolled asthma symptoms.

h) There was a signal showing that having a house which was closer than 50m to busy traffic might increase the likelihood of having uncontrolled respiratory symptoms (($p=0.049$, Chi-squared).

i) Having a smell of mould in the house increased the likelihood for a child to have uncontrolled asthma symptoms ($p<0.001$, Chi-squared).

j) Having visible mould in the child's bedroom increased the likelihood for a child to have uncontrolled respiratory symptoms (p=0.034, Chi-squared).

5.3.2 Conclusions from clinical tests

a) A child who had uncontrolled asthma symptoms was more likely to have lower FEV_1/FVC ratio ($p = 0.059$, t-test).
b) A child who had uncontrolled asthma symptoms was more likely to have a higher FeNO level ($p < 0.001$, Mann-Whitney-U).
c) A child who had uncontrolled asthma symptoms was more likely to have a higher serum total IgE level ($p = 0.027$, Mann-Whitney U).
d) Children who had uncontrolled asthma symptoms had lower mean AMCA per nostril when compared to asymptomatic and non-atopic individuals ($p = 0.011$, Mann-Whitney U).
e) The commonest atopy to an aeroallergen detected through skin prick testing in this group of Maltese children studied was that to house dust mite, followed by cat dander and olive tree pollen.
f) The commonest atopy to an aeroallergen detected through skin prick testing in the Maltese adult males (fathers) who participated in this study was to house dust mite, followed by *Parietaria* pollen and grass pollen mix.
g) The commonest atopy to an aeroallergen detected through skin prick testing in the Maltese adult females (mothers) who participated in this study was to house dust mite, followed by *Parietaria* pollen and olive tree pollen.

h) Maltese children who had uncontrolled asthma symptoms were more likely to have house dust mite atopy detected through skin prick testing ($p < 0.001$, Chi-Squared).

i) Mothers of children who had uncontrolled asthma symptoms were more likely to have house dust mite atopy detected through skin prick testing.

j) Children who had uncontrolled asthma symptoms were more likely to have higher serum Total IgE levels and serum specific IgE to HDM levels ($p = 0.25$, Mann-Whitney U).

k) Fathers of children who had uncontrolled asthma symptoms were more likely to have higher specific IgE levels to olive pollen ($p=0.018$) and *Parietaria* pollen ($p = 0.04$, Mann-Whitney U).

l) Mothers of children who had uncontrolled asthma symptoms were more likely to have higher specific IgE levels to olive pollen, dog dander and *Cladosporidium* spores.

m) Children who had uncontrolled asthma symptoms tended to have higher serum Total IgE levels in autumn when compared to spring ($p = 0.15$, Kruskall-Wallis).

n) Children who had uncontrolled asthma symptoms had higher specific IgE levels to HDM in winter with lower levels in spring and autumn $p = 0.24$). There was also significant seasonal variability in serum specific IgE levels to *Parietaria* ($p = 0.017$), olive ($p=0.005$), *Cladosporidium* ($p=0.013$), *Alternaria* ($p=0.02$) and goldrenrod pollen ($p=0.045$).

o) Maltese adult males (fathers) were more likely to have higher serum IgE levels in autumn when compared to winter (p=0.24, Kruskall-Wallis).

p) Maltese adult females (mothers) were more likely to have seasonal serum specific IgE variability to all the aeroallergens tested in this study.

q) Maltese adult females (mothers) who had doctor-diagnosed asthma were more likely to have a higher serum Total IgE level in winter when compared to autumn (p=0.036, Mann-Whitney U).

r) Children who had higher serum total IgE levels tended to have fathers and/or mothers who had higher total IgE levels.

s) Children who had higher serum specific HDM IgE levels tended to have fathers and/or mothers who had higher specific HDM IgE levels.

t) Children who had uncontrolled asthma symptoms and fathers who had doctor-diagnosed asthma seemed to have similar responses to allergens. Mothers who had doctor-diagnosed asthma seemed to have different responses to these groups and had seemed to correspond to late onset female asthmatic cohorts reported in the literature.

5.3.3 Groups detected in this study

a) The children who had current asthma symptoms could be divided into two groups, a smaller one with very high serum Total IgE levels and high FeNO levels (High IgE cluster) and another bigger group with relatively low serum Total IgE levels and low FeNO levels (Low IgE cluster).

b) Children who experienced uncontrolled asthma symptoms, and who also had very high serum total IgE were more likely to have higher serum specific HDM IgE level and specific *Parietaria* IgE level than the other children who also had uncontrolled asthma symptoms but did not have high total IgE.

c) A high serum specific IgE level to HDM increased the likelihood of a child who had uncontrolled asthma symptoms to be part of the same group children who had a high serum IgE and a high FeNO.

d) In the group of fathers who participated in this study, the cohort of these who had very high serum total IgE and high FeNO had higher serum specific IgE to HDM, olive pollen and cat dander when compared to the rest of the fathers.

e) A high serum specific IgE level to HDM increased the likelihood of a father to have a high serum IgE and a high FeNO.

f) In the group of mothers who participated in this study, the cohort of these who had very high serum total IgE and high FeNO had higher serum specific IgE to HDM, olive pollen and *Parietaria* when compared to the rest of the mothers.

g) A high serum specific IgE level to olive pollen or *Parietaria* increased the likelihood of a mother to have a high total serum IgE and a high FeNO.

h) Children who had uncontrolled asthma symptoms and were a member of the high IgE group were more likely to have a father who was a member of the corresponding high IgE group.

5.3.4 Conclusions from houses

a) Mean endotoxin levels were higher in houses in which the inhabitants had reported indoor mould growth (p=0.024, Mann Whitney U).

b) Mean endotoxin levels were higher in houses in which the inhabitants reported mould growth in the child's bedroom (p=0.011, Mann Whitney U).

c) Children who had uncontrolled asthma symptoms and who were part of the subgroup who had higher total serum IgE levels were more likely to live in houses which had higher mean HDM allergen detected in the dust present.

d) We observed the presence of mould and humidity in most of the houses which housed individuals who had high serum IgE and high FeNO.

5.4 Limitations of the study

There were various limitations in this study. The number of participants who participated in this study was small when compared to other studies, such as the ISAAC, TENOR and SARP.

As there were limited funds, not all participants could have a full panel of specific IgE levels analysed from the serum. For the same seasons, not all the children's houses could have their homes analysed, particularly to dust. Ideally, rather than collecting dust samples from the living room, as a representation of the dust allergens to which the members of the family were exposed, it would have been more accurate to collect two samples, one from the parents' bedroom, and another from the child's bedroom. It would also have been ideal to vacuum samples from the beds and bed linen, rather than floor and surfaces, as this would have decreased the effects which daily floor cleaning could have on the dust samples.

Ideally, dust could have also been tested for aspergillus and penicillum species, which are indoor moulds, rather than Alternaria which tends to be more of an outdoor mould, which grows preferentially on plants. The same could be said for serum specific IgE analysis, where it would have been idea to add panels for penicillium and aspergillus species. *Urticacae* family pollen could have been another allergen which could have been tested for, from an atopy perspective.

Measuring serum eosinophils would have added value, as serum eosinophils have been shown in the literature to be a marker for eosinophilic inflammation in the airways. A more direct measure of eosinophilic inflammation rather then FeNO, could have been induced sputum for microscopic examination of the cells present in these samples.

Given these limitations, the study did not have enough power to detect specific findings in the houses or pinpoint house characteristics which determine respiratory and atopy related symptoms. Perhaps, one could use the cluster model described in this study and focus testing in houses dwelled by specific phenotypes, in more detail and involving more rooms (e.g. all bedrooms) in order to identify potential indoor causes of respiratory problems.

5.5 Future research proposed

The finding of this research shows primarily that there are household features being reported in the houses of children with respiratory symptoms which point to traffic pollution, and to indoor mould.

It also shows that there are objective differences in the clinical measures of children who have respiratory symptoms, namely higher serum IgE and airway inflammation (as measured through FeNO).

I would suggest that a more detailed research programme is carried out locally to investigate the environmental characteristics of houses which home children who have respiratory symptoms. One could do this by recruiting a cohort of children who have active respiratory symptoms, and taking repeated measurements of indoor dust, sample these for house dust mites (different species such as *Dermatophagoides pteryonyssinus* and *farina*e), moulds (*Aspergillus spp, Penicillium spp, Cladosporidum spp*). Samples could also be taken from walls and ceiling which are

showing signs of dampness. A one-year measurement of relative humidity in these homes would also be beneficial, together with measurements pertaining to traffic pollution such as $PM_{2.5}$.

These measurements can then be correlated to clinical measurements similar to the ones presented in this thesis, and ideally repeated in the four different seasons of the year, in order to study both how indoor environments vary throughout a one year period, and how these effect respiratory function and inflammation throughout this timespan.

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Appendix 1. Ethical approvals

L-UNIVERSITÀ TA' MALTA
Msida – Malta
Skola Medika
Sptar Mater Dei

UNIVERSITY OF MALTA
Msida – Malta
Medical School
Mater Dei Hospital

Ref No: ~~72/2011~~ 77 / 2011

21st February, 2012

Dear Dr. Christopher Zammit

Please refer to your application submitted to the Research Ethics Committee in connection with your research entitled:

IMPACT OF SCHOOL AND INDOOR AIR QUALITY ON CHILDREN'S RESPIRATORY HEALTH IN MALTESE SECONDARY SCHOOLS'

The University Research Ethics Committee granted ethical approval for the above mentioned protocol.

Yours sincerely,

Dr. Mario Vassallo
Chairman
Research Ethics Committee

Email: umme@um.edu.mt • Web: http://www.um.edu.mt/ume

DIRETTORAT GHAL KWALITA' L STANDARDS FI-EDUKAZZJONI
FURJANA VLT 2010
MALTA

DIRECTORATE FOR QUALITY AND STANDARDS IN EDUCATION
FLORIANA VLT 2000
MALTA

Request for Research in State Schools

A. (Please use BLOCK LETTERS)

Surname: BILOCCA Name: DAVID

I.D. Card Number: 434482M

Telephone No: 21411905 * Mobile No: 99891569 *

Address: 1, "ANTONIA", TRIQ IL-GARRIEB, 3

Locality: MOSTA Post Code: MST 1784

E-mail Address: dbilocca@yahoo.com

Faculty: MEDICINE AND SURGERY Course: PhD Year Ending: 2015

Title of Research: ASSESSING AND COMPARING THE IMPACT OF SCHOOL AND HOME INDOOR AIR QUALITY ON SECONDARY SCHOOL CHILDREN

Aims of research: o Long Essay o Dissertation ✓ Thesis ✓ Publication

Time Frame: 2 years Language Used: ENGLISH

Description of methodology: PART OF RESPIRA PROJECT FUNDED BY EU

Schools where research is to be carried out: TO BE DECIDED

Years / Forms: 1-4 Age range of students: 4-14

* Telephone and mobile numbers will only be used in strict confidence and will not be divulged to third parties.

I accept to abide by the rules and regulations re Research in State Schools and to comply with the Data Protection Act 2001.

Warning to applicants - Any false statement, misrepresentation of concealment of material fact on this form or any document presented in support of this application may be grounds for criminal prosecution.

Signature of applicant: Date: 4/10/11

B. Tutor's Approval (where applicable)

The above research work is being carried out under my supervision

Tutor's Name: PROF MONTEFORT Signature: _____

Faculty Medicine and Surgery Faculty Stamp _____

C. Directorate for Quality and Standards in Education - Official Approval

The above request for permission to carry out research in State Schools is hereby approved according to the official rules and regulations, subject to approval from the University of Malta Ethics Committee.

Raymond Camilleri
Director, RDD

Director Date: 06 / 10 / 2014 Official Stamp

(Research and Development Department)

Conditions for the approval of a request by a student to carry out research work in State Schools

Permission for research in State Schools is subject to the following conditions:

1. The official request form is to be accompanied by a copy of the questionnaire and / or any relevant material intended for use in schools during research work.

2. The original request form, showing the relevant signatures and approval, must be presented to the Head of School.

3. All research work is carried out at the discretion of the relative Head of School and subject to their conditions.

4. Researchers are to observe strict confidentiality at all times.

5. The Directorate for Quality and Standards in Education reserves the right to withdraw permission to carry out research in State Schools at any time and without prior notice.

6. Students are expected to restrict their research to a minimum of students / teachers / administrators / schools, and to avoid any waste of time during their visits to schools.

7. As soon as the research in question is completed, the Directorate for Quality and Standards in Education assumes the right to a full copy (in printed C.D.) of the research work carried out in State Schools. **Researchers are to forward the copies to the Assistant Director, International Research, Directorate for Quality and Standards in Education.**

8. Researchers are to hand a copy of their Research in print or on C.D. to the relative Schools.

9. In the case of video recordings, researchers have to obtain prior permission from the Head of School and the teacher of the class concerned. Any adults recognisable in the video are to give their explicit consent. Parents of students recognisable in the video are also to be requested to aprove that their siblings may be video-recorded. Two copies of the consent forms are necessary, one copy is to be deposited with the Head of School, and the other copy is to accompany the Request Form for Research in State Schools. Once the video recording is completed, one copy of the videotape is to be forwarded to the Head of School. The Directorate for Quality and Standards in Education reserves the right to request another copy.

10. The video recording's use is to be limited to this sole research and may not be used for other research without the full consent of interested parties including the Directorate for Quality and Standards in Education.

Statement of Consent

I hereby give my consent to the Directorate for Quality and Standards in Education to process and record personal and sensitive data being given herewith in order to be able to render me with the service I am applying for.
I fully understand that:

a) by not giving out my application cannot be processed;
b) authorised personnel who are processing this information may have access to this data in order to supply me with the service being applied for
c) edited information, that would not identify me, may be included in statistical reports

I know that I am entitled to see the information related to me, should I ask for it in writing.
I am aware that for the purpose of the Data Protection Act, the Data Controller for this Directorate is:
The Directorate for Quality and Standards in Education
Floriana, VLT 2000

I have read and understood this statement of consent myself

This statement of consent was read and explained to me

Signature: _____ ID number: _____ (Data subject)

Signature: _____ ID number: _____ (Reader if applicable)

Date: 4/10/11

Data Protection Policy

The Data Protection Act, 2001 regulated the processing of personal data held electronically and in manual form. The Directorate for Quality and Standard in Education is set to fully comply with the Data Protection Principles as set out in the Act.

a) The Directorate will hold information you supply in accordance to your request to carry out research in State Schools and / or Directorates' documents.
b) The information you give may be disclosed to other Departments of the Directorate for Quality and Standards in Education, who may also have access to your data.

Your rights:
You are entitled to know what information the Directorate holds and processes about you and why, who has access to it, how it is kept up to date, what the Directorate is doing to comply with its obligations under the Data Protection Act, 2001.

The Data Protection Act, 2001 sets down a formal procedure for dealing with data subject access requests which the Ministry of Education, Culture, Youth and Sport follows.

All data subjects have the right to access any personal information kept about them by the Directorate either on computer or in manual files. Requests to access to personal information by data subjects must be made in writing and addressed to the Data Controller of the Ministry of Education, Culture, Youth and Sport. An identification document such as a photocopy of the Identity Card, photocopy of passport etc. of the data subject making the request must be submitted with the request. Such identification material will be returned to the data subject.

The Directorate aims to comply as quickly as possible with requests for access to personal information and will ensure that it is provided with in reasonable time, the reason will be explained in writing to the data subject making the request.

All data subjects have the right to request that their information be amended, erased or not used in the event the data is incorrect.

MINISTRY FOR HEALTH,
THE ELDERLY and COMMUNITY CARE

RESPIRA PROJECT Code A1.2.3-72

RESPIRA

Information Sheet

And

Consent Form

If you have any questions, comments or concerns you may discuss them with the researcher present at the time. Alternatively you can contact us during normal working hours (8am to 8pm) on the following numbers:

Mobile: 79847289; 79303009;

2 copies required per consent:
1 copy for the parent/guardian; 1 copy kept for research documentation.

Appendix 2. Questionnaires

MINISTRY FOR HEALTH,
THE ELDERLY and COMMUNITY CARE **RESPIRA PROJECT Code A1.2.3-72**

RESPIRA

QUESTIONNAIRE ON RESPIRATORY AND ALLERGIC HEALTH OF SCHOOLCHILDREN AND HOME ENVIRONMENT
(To be completed by the Children)

Name and Surname of Child _____

Name of parent/gaurdian _____

Address _____ Tel. Number _____

_____

MINISTRY FOR HEALTH,
THE ELDERLY and COMMUNITY CARE **RESPIRA PROJECT Code A1.2.3-72**

RESPIRA

QUESTIONNAIRE ON RESPIRATORY AND ALLERGIC HEALTH OF SCHOOLCHILDREN AND HOME ENVIRONMENT
(To be completed by the Parents/Gaurdians)

Name of Child _____

Name and Surname of parent/gaurdian _____

Parent/gaurdian Identity card number _____

Address _____ Tel. Number _____

_____

Appendix 3. Clinic and House fieldwork booklets

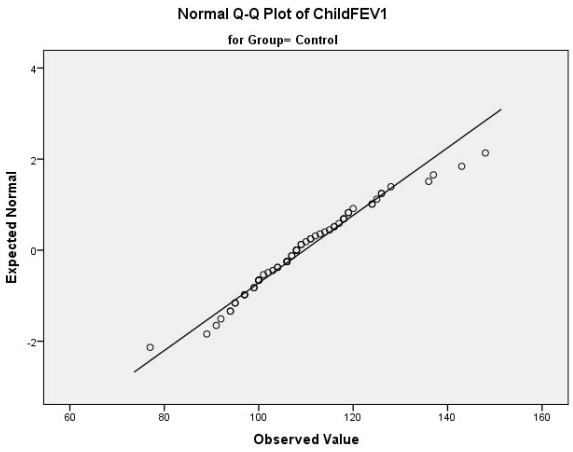
Appendix 4. Normality Tests and Tests on Log_{10}

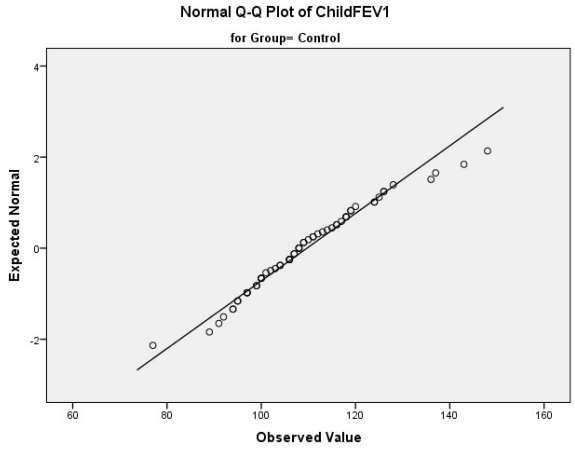
FEV1

The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to assess the normality assumption of Child's FEV_1 for each case and control group separately. The null hypothesis specified that the score distribution was normal, and was accepted if the p-value exceeded the 0.05 level of significance. The alternative hypothesis specified that the score distribution was not normal, and was accepted if the p-value is less than 0.05 criterion.

Tests of Normality for Child's FEV1

		Kolmogorov-Smirnov			Shapiro-Wilk		
	Group	Statistic	df	p-value	Statistic	df	p-value
ChildFEV1	Case	.065	65	.200*	.987	65	.706
	Control	.087	60	.200*	.974	60	.223





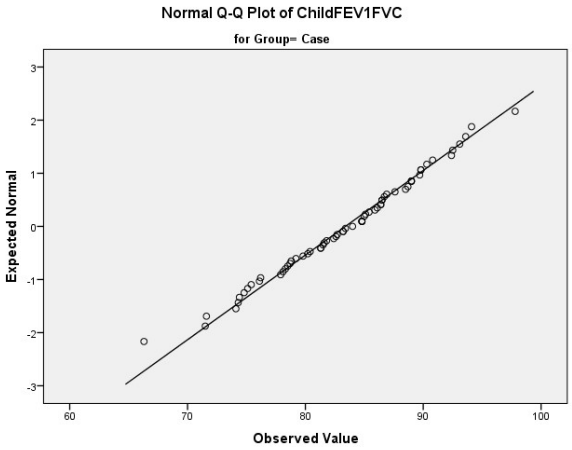
Both tests reveal that the Child's FEV_1 score distribution was normal as they exceeded the p=0.05 level of significance. For this reason, parametric tests were be used to analyse the Child's FEV_1 scores.

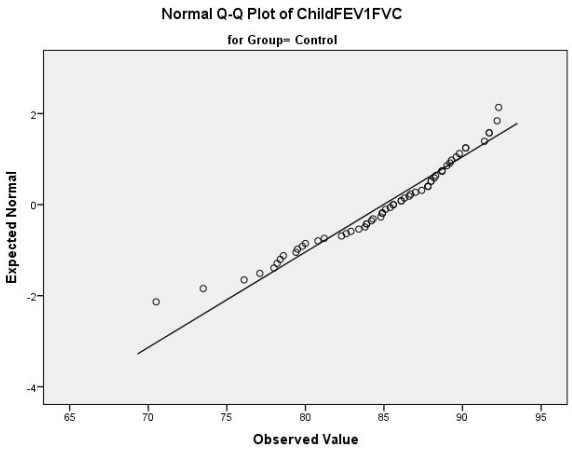
FEV_1/FVC ratio

The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to assess the normality assumption of Child's FEV_1/FVC ratio for each case and control group separately. The null hypothesis specified that the score distribution was normal, and was accepted if the p-value exceeded the 0.05 level of significance. The alternative hypothesis specified that the score distribution was not normal, and was accepted if the p-value is less than 0.05 criterion.

Tests of Normality for Child's FEV1/FVC ratio

		Kolmogorov-Smirnov			Shapiro-Wilk		
	Group	Statistic	df	p-value	Statistic	df	p-value
ChildFEV1FVC	Case	0.081	65	0.200*	0.991	65	0.931
	Control	0.103	60	0.182	0.951	60	0.018





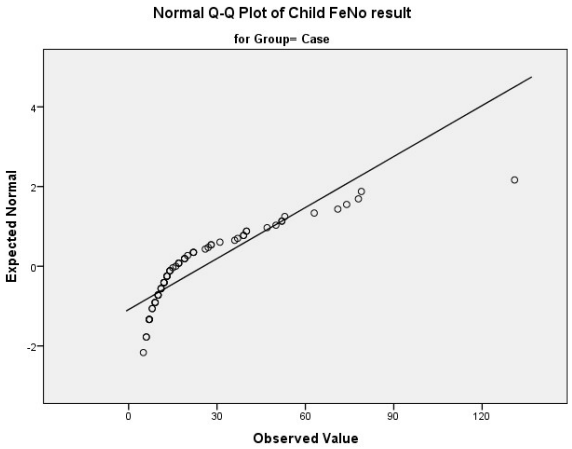
Both tests reveal that the Child's FEV_1/FVC ratio score distribution was normal as they exceeded the p 0.05 level of significance. For this reason, parametric tests were be used to analyse the Child's FEV_1 scores.

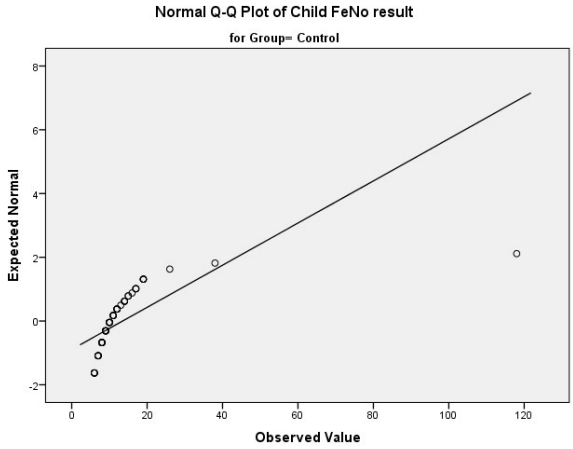
Child's FeNO

The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to assess the normality assumption of Child's FeNO for each case and control group separately. The null hypothesis specified that the score distribution is normal, and was accepted if the p-value exceeded the 0.05 level of significance. The alternative hypothesis specified that the score distribution was not normal, and was accepted if the p-value is less than 0.05 criterion.

Tests of Normality for Child's FeNO

		Kolmogorov-Smirnov			Shapiro-Wilk		
	Group	Statistic	df	p-value	Statistic	df	p-value
Child FeNo result	Case	0.221	65	0.000	0.759	65	0.000
	Control	0.310	57	0.000	0.371	57	0.000





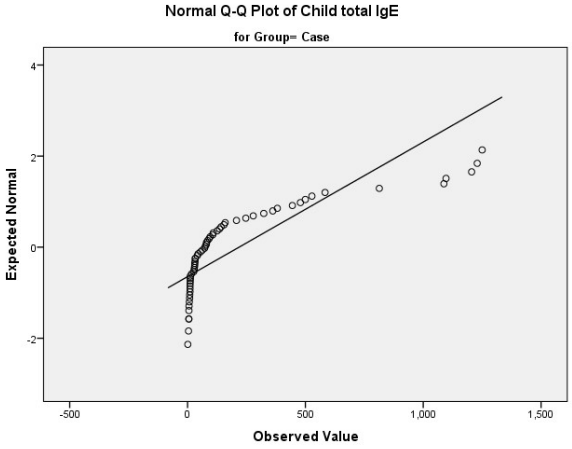
Both tests reveal that the FeNO score distribution was right skewed and did not satisfy the normality assumption. For this reason, non-parametric tests were be used to analyse the FeNO scores.

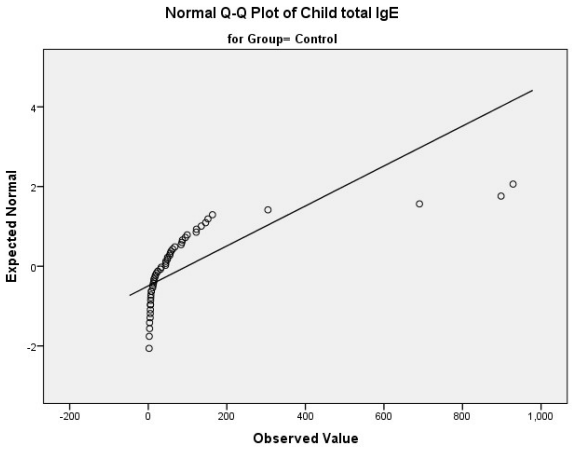
Child's Total IgE

The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to assess the normality assumption of the Child's total serum IgE distribution for each case and control group separately. The null hypothesis specified that the score distribution is normal and was accepted if the p-value exceeded the 0.05 level of significance. The alternative hypothesis specified that the score distribution was not normal and was accepted if the p-value is less than 0.05 criterion.

Tests of Normality for Child's Total Serum IgE

		Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	p-value	Statistic	df	p-value
Child total IgE	Case	.286	60	0.000	.656	60	0.000
	Control	.314	50	0.000	.479	50	0.000





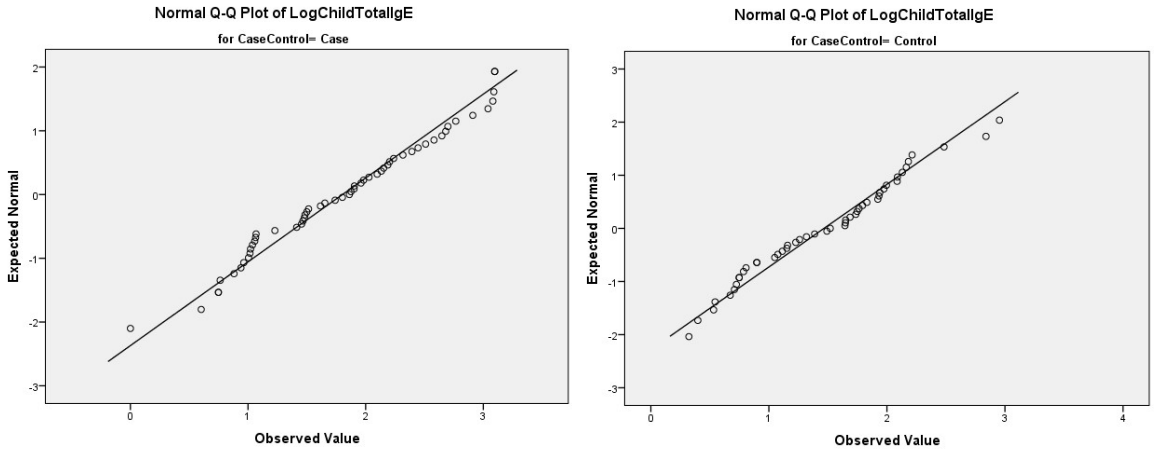
Both tests reveal that the Child's total serum IgE score distribution was right skewed and did not satisfy the normality assumption. For this reason non-parametric tests were be used to analyse the total serum IgE scores.

Output for Log10 IgE Data

The data for Child's Total Serum IgE underwent a Log_{10} transformation and was assessed for normality. $Log10_{10}$ total serum IgE was then tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results for these tests exceeded the p-value of 0.05 significance, and therefore Log_{10} total IgE achieved a normal distribution.

Tests of Normality for Child's Total Serum IgE

		Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	p-value	Statistic	df	p-value
Child total IgE	Case	0.106	55	0.191	0.970	55	0.194
	Control	0.098	47	0.200	0.971	47	0.300



The data for all the children's various specific IgE tests underwent a Log_{10} transformation and was assessed for normality. $Log10_{10}$ specific serum IgE was then tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results for these tests did not exceed the p-value of 0.05 significance, and therefore Log10 specific IgE had a non-normal distribution and these results needed to be analysed using non-parametric tests. Given the fact that the analysis was performed comparing the total IgE to the specific IgE, it was decided to keep the data without a Log_{10} transformation and using non-parametric tests on this data.

Tests of Normality

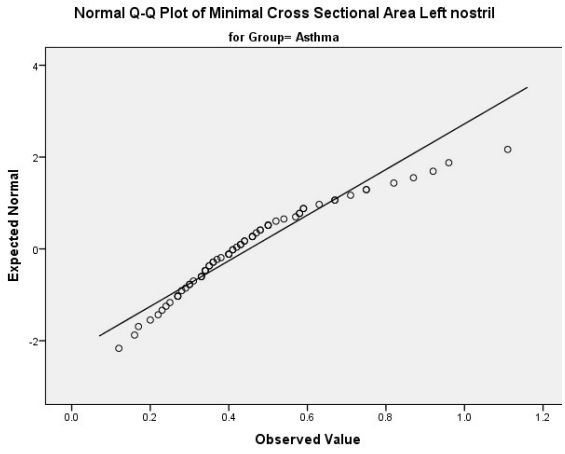
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	p-value	Statistic	df	p-value
Log_{10} Child HDM IgE	Case	0.298	55	0.000	0.800	55	0.000
	Control	0.322	47	0.000	0.604	47	0.000
Log_{10} Child *Parietaria* IgE	Case	0.407	55	0.000	0.526	55	0.000
	Control	0.242	47	0.000	0.720	47	0.000
Log_{10} Child Olive IgE	Case	0.247	55	0.000	0.763	55	0.000
	Control	0.296	47	0.000	0.479	47	0.000
Log_{10} Child Cat IgE	Case	0.303	55	0.000	0.683	55	0.000
	Control	0.233	47	0.000	0.776	47	0.000
Log_{10} ChilddogIgE	Case	0.257	55	0.000	0.688	55	0.000
	Control	0.232	47	0.000	0.769	47	0.000
Log_{10} Child *Cladosporidium* IgE	Case	0.163	55	0.001	0.879	55	0.000
	Control	0.179	47	0.001	0.850	47	0.000
Log_{10} Child *Alternaria* IgE	Case	0.234	55	0.000	0.668	55	0.000
	Control	0.206	47	0.000	0.897	47	0.001
Log_{10} Child Goldenrod IgE	Case	0.127	55	0.028	0.961	55	0.069
	Control	0.159	47	0.004	0.934	47	0.011

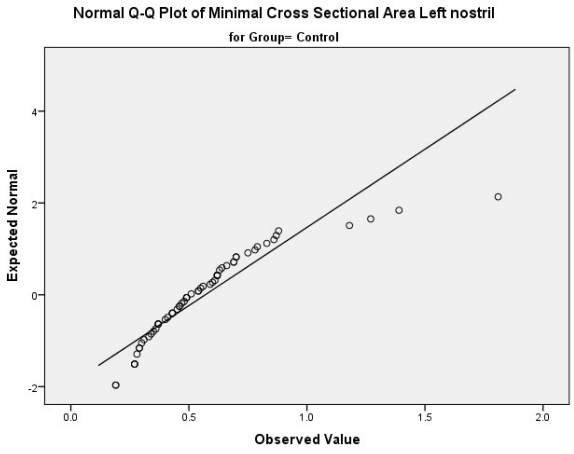
Child's acoustic rhinometry

The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to assess the normality assumption of the Child's right and left minimal cross-sectional area (MCA) distribution, for each case and control group separately. The null hypothesis specified that the score distribution is normal and was accepted if the p-value exceeded the 0.05 level of significance. The alternative hypothesis specified that the score distribution was not normal and was accepted if the p-value is less than 0.05 criterion.

Tests of Normality for Left Minimal Cross Sectional Area

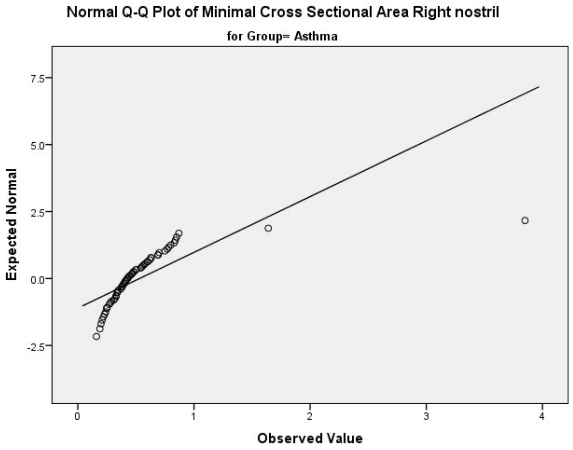
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Group	Statistic	df	p-value	Statistic	df	p-value
Minimal Cross Sectional Area Left nostril	Asthma	0.129	65	0.009	0.928	65	0.001
	Control	0.144	60	0.003	0.841	60	0.000

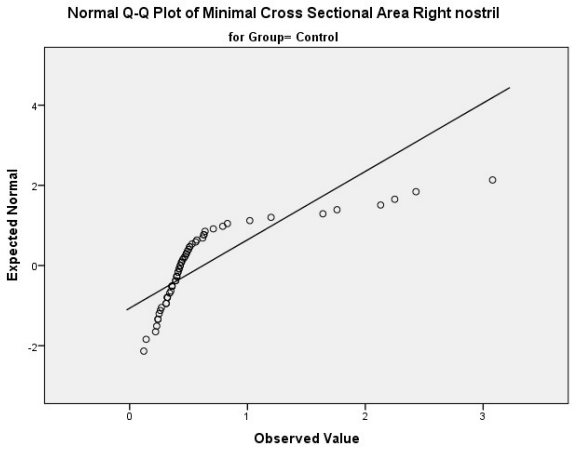




Tests of Normality for Right Minimal Cross Sectional Area

		Kolmogorov-Smirnov			Shapiro-Wilk		
	Group	Statistic	df	p-value	Statistic	df	p-value
Minimal Cross Sectional Area Right nostril	Asthma	0.223	65	0.000	0.489	65	0.000
	Control	0.306	60	0.000	0.628	60	0.000





Both tests reveal that the Child's left and right minimal cross-sectional area score distribution was right skewed and did not satisfy the normality assumption. For this reason non-parametric tests were be used to analyse the left and right minimal cross sectional area score distribution scores.