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SERUM POLYCLONAL AND SPECIFIC IMMUNOGLOBULIN E LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

by
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AN ABSTRACT OF THE THESIS OF

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Title: Serum Polyclonal and Specific Immunoglobulin E Levels in Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis

Both IgE-mediated allergy and autoimmune diseases are thought to have similar causes; environmental factors that provoke the disease and predisposing genetic factors. Previous reports indicate that in some autoimmune diseases elevated polyclonal IgE levels were detected.

Patients with Rheumatoid Arthritis (RA) or Systemic Lupus Erythematosus (SLE) were included in this study. Their serum polyclonal IgE levels were determined by ELISA and attempts were made to detect allergen specific IgE antibodies using specific IgE Mediterranean allergen kit.

The aim of this investigation was to see if there was a correlation between serum polyclonal IgE, specific IgE levels and RA or SLE.

The results indicated that serum polyclonal IgE levels were not significantly different when levels in RA patients were compared to controls. Whereas the specific IgE levels detected against Mugwort Pollen, Alt. alternata, plantain pollen, Dog epithelia, Aspergillus fumigates were positively correlated with RA, and specific IgE levels against Timothy Grass pollen and D. pteronyssinus were positively correlated with SLE.

Conclusions that are suggested are based on a relatively small number of subjects included in the study. More patients must be studied in order to make concrete conclusions.

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ABBREVIATIONS

1. IgE: Immunoglobulin E
2. SLE: Systemic Lupus Erythematosus
3. RA: Rheumatoid Arthritis
4. ELISA: Enzyme-Linked Immunosorbent Assay
5. Anti-SSA/Ro: Anti-Sjögren's-syndrome-related antigen A, also called anti-Ro, or the combination *anti-SSA*/*Ro* or anti Ro/SSA *auto-antibodies*
6. *Anti-SSB/La: Mostly associated with Anti-SSA/Ro in* Sjögren's-syndrome
7. IgG: Immunoglobulin G
8. ANA: anti-nuclear antibody
9. Anti-Sm: Anti Smith antibodies and are very specific markers for SLE
10. Anti-dsDNA: anti double stranded deoxyribonucleic acid
11. RF: Rheumatoid factor
12. NK: natural killer cells
13. ACPA: anti-citrullinated protein antibodies
14. RR: Relative Risk
15. CI : Confidence Interval

CHAPTER I

INTRODUCTION

Both IgE-mediated allergy and autoimmune diseases are thought to have similar causes; environmental factors that provoke the disease and predisposing genetic factors. Previous reports indicate that in some autoimmune diseases elevated polyclonal IgE levels were detected.

Patients with Rheumatoid Arthritis (RA) (seropositive and seronegative types of RA) or Systemic Lupus Erythematosus (SLE) were included in this study. Serum polyclonal IgE levels were determined and attempts were made to detect allergen specific IgE antibodies in these patients.

The aim of this investigation was to see if there was a correlation between serum polyclonal IgE, specific IgE levels and RA or SLE.

CHAPTER II

LITERATURE REVIEW

A. Autoimmune Diseases

An autoimmune disorder is a condition in which a person induces a detrimental immune response against his/her own body constituents. The immune response can be cell- and/or humoral-mediated. (1)

Two autoimmune disorders that will be dealt with are: Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). In both diseases a humoral factor is thought to be involved. In the former, antibodies to self-IgG and in the latter antibodies to double stranded DNA and other nuclear factors are present in serum of patients. (2)

B. Systemic Lupus Erythematosus (SLE)

SLE is a chronic inflammatory autoimmune disease characterized by a Th2 response resulting in intense polyclonal B lymphocyte activation and vigorous production of auto-antibodies and inflammation (3,4). A Th2immune response also occurs in IgE mediated allergies. This response is characterized by a significant increase of serum IL-4, IL-10 and IgE-class immunoglobulins. (5) SLE is associated with courses of chronic systemic inflammation mainly due to the immune complex deposits in different organs and the production of auto-antibodies specific to different nuclear antigens. Some antinuclear auto-antibodies contribute to the clinical effects of SLE, such as anti-dsDNA antibodies, anti-SSA/Ro (anti-Sjögren's-syndrome-related antigen A, also called anti-Ro), and *anti-SSB/La (anti-* Sjögren's-syndrome-related antigen B) (6).In addition, the occurrence of

antiIgG antibodies belonging to the IgE classhas been detected in SLE patients with articular involvement, lymphadenopathy and anti-DNA antibodies. (6,7)

Studies have shown that SLE is eight to ten times more common in women than men, it can occur at any age but more often in people between 10 and 50 years of age, and it can affect the kidneys, the joints, the skin, the brain, and other organs. Moreover, for unknown reasons Afro-Americans and Asians are mostly affected by this disease. (7,8)

The causes of SLE are not fully known. Genetic predisposing factors are thought to be involved and certain drugs such as hydralazine, procainamide, quinidine, isoniazid, and minocycline, and viruses induce the disease (9).

Most SLE patients develop pain in the joints of the hands and knees) and some may develop arthritis. Symptoms are diverse and depend on the site of the inflamed area as shown in table 1(7,8, 10, 11).Blood tests that are done as an aid to diagnosis include the detection of antinuclear antibodies (ANA),antibodies to double stranded DNA (dsDNA), antibodies to Smith(anti-Sm)and anti-phospholipid antibodies. Some Complement protein levels are also determined (12,13, 14).

Treatment of SLE is directed toward decreasing inflammation and/or the level of autoimmune activity since there is no curative treatment. Medications used for treatment depends on the severity of the disease and includes immunosuppressive agents including corticosteroids and hydroxychloroquine (9).

C. Rheumatoid Arthritis (RA)

RA involves inflammation of the synovium due to infiltration of CD4+ T cells and NK cells into synovial joints. .The development of RA seems to result from a series of multi-factorial events with the involvement of both T and B cell-dependent pathways(15).

Specific auto-antibodies directed against antigenic determinants on the Fc fragment of IgG molecules have been described and are called rheumatoid factors (RF). However, the role of these factors in the pathogenesis of RA is not yet established (15,16).

RA primarily affects joints where the immune system mistakenly attacks the joints leading to deformities and pain which can lead to loss of function. It may also affect other organs and it can affect people of all ages. RA is three times more common in women than men. Mostly in women, it starts between 30 and 60 years of age; while in men, it often occurs later in life. The main cause of this disease and its pathogenic mechanism are not known yet (15,16).

RA symptoms vary. Patients might have flares which are sudden increases in symptoms and illnesses. The key RA symptoms are pain, fatigue, and warm, swollen, reddish joints. Long periods of joint stiffness are also common in the mornings. Also a typical symptom includes the inflammation of small joints in the wrist and hand. Most studies indicate that multiple joints are usually, but not always, affected by a symmetric manner which means if the joint of one side of the body is affected, the same joint of the other side of the body is also affected. Moreover, joint damage can occur early and do not contribute to the severity of illness. (16)

X-ray of the hands and feet are helpful in the diagnosis of Rheumatoid Arthritis. X-rays may not demonstrate any changes during early RA stages or may demonstrate juxta-articular osteopenia, soft tissue swelling and loss of joint space. As the disease progresses, bony erosions can be demonstrated (16). Moreover, Magnetic Resonance Imaging (MRI) and ultrasound can also be used for RA diagnosis.

Results of certain blood tests are also used as an aid to diagnosis of RA. These include the detection of rheumatoid factor and anti-citrullinated protein antibodies (ACPA). (16)

Treatment of RA includes the use of immunosuppressive drugs. Agents that neutralize the effect of Tumor Necrosis Factor-alpha have been reported to improve the status of the patient (17).

D. Immunoglobulin E (IgE)

Type I or Immediate Type Hypersensitivity is mediated by IgE antibodies. IgE produced upon first exposure to the allergen will bind to Fc receptors on mast cells and basophils, Upon second exposure the allergen will cross link two mast cell/basophil-bound IgE. This is followed by intracellular signaling resulting in degranulation and release of histamine and tryptase, followed later by leukotrienes, prostaglandins, cytokines and an eosinophil chemotactic factor,(18,19, 20)

Serum levels of IgE have been reported to be significantly elevated in parasitic diseases and patients with allergic diseases such as extrinsic asthma, hay fever, and atopic eczema (20, 21, and 22). Although IgE is the least abundant immunoglobulin isotype in blood; it is capable of triggering the most serious inflammatory reactions.

IgE was discovered in 1966 by two different groups: the first group consisted of Dr. Lawrence Lichtenstein and Dr. Philip Norman in the Johns Hopkins Department of Medicine's Division of Allergy and Infectious Diseases, and the second group included Dr. Kimishige Ishizaka and Dr. Margaret M. Hornbrook in the Children's Asthma Research Institute and Hospital in Denver. (23)

Millauer et al. (24) reported that high levels of serum IgE were detected in both RA and allergic patients. In this study, they detected significantly high specific IgE levels against Alternaria (specific allergen) in the RA patients. The results revealed that anti-IgG autoantibodies (rheumatoid factors) of different isotypes were detected only in RA patients sera and not in the controls' sera. However, they found increased levels of anti-IgE auto-antibodies in all RF positive groups and in the allergic group. From these findings they concluded that despite the elevated levels of serum IgE, there is a decreased prevalence of allergic diseases among RA patients.

A.M Atta et al.(25)reported a study done on 21female patients with SLE. Although these patients had elevated polyclonal IgE levels and IgE class anti-nuclear antibodies, they did not suffer from symptoms of allergy. They excluded a possible association between SLE and allergy.

Rebhun J et al. (26) reported that serum IgE levels were higher in patients with active SLE than in patients with SLE in remission.

Bernadete L Liphaus et al. (27) reported that the high levels of IgE in patients with Juvenile SLE were not related to parasitic infections nor to any allergic reactions.

Tea Skaaby et al. (28) reported after studying the specific IgE against certain inhalant allergens that there was no significant association between atopy and autoimmune diseases.

Magen E et al. (29) reported that undetectable serum total IgE may serve as marker of immune dysregulation and autoimmunity.

Barbara Dema et al. (30) reported that IgE autoantibodies independent or in combination with IgG autoantibodies may serve as indicators of active SLE disease. In

addition to that, they reported that there was a strong association of IgE autoantibodies with active nephritis in patients with SLE.

CHAPTER III

MATERIALS AND METHODS

A. Patients And Controls

Five SLE patients and 34 RA patients (18-75yrs/old) of Lebanese nationality, fulfilling the American College of Rheumatology (ACR) classification criteria for SLE and RA were recruited for the purpose of the study after attaining the Institutional Review Board (IRB) approval. All patients were attending Dr. Imad Uthman Clinic at the American University of Beirut. A total of 39 sex and age matched controls reporting no immediate family history of SLE and RA, or any other autoimmune diseases were also studied. Consent forms were signed from all participants prior to enrollment in the study. Subjects on immunosuppressive therapy and patients suffering from known allergic reactions were excluded from the study. Description details of the population studied are defined in Table 2.

B. Sample Collection

After signing the consent form, 4 to 5ml of blood was collected from each participant in plain tubes. Blood samples were centrifuged at 1500rpm for 15min and serum was collected and stored at -20°C until sample size was complete.

C. Polyclonal IgE Level Determination

To assess for any correlation between IgE levels and disease pathogenesis in SLE and RA affected individuals, polyclonal IgE levels of all serum specimens of both controls and patients were determined using the Immunoglobulin E (IgE) Human Elisa Kit obtained from abcam (Cambridge, MA, USA). Standards, samples, control and blank were all run in duplicates for reliability of results. The kit was used according to the manufacturer's protocol.

Briefly;

1. Reagents And Kit Content

a- Streptavidin Coated Microplate (96 wells)

b- Stop Solution

c- IgE Biotin Conjugate

d- TMB Substrate Solution

e- 50x Washing Solution

f- IgE HRP Conjugate

g- IgE Standards

2. Procedure

- All materials and prepared reagents were equilibrated to room temperature prior to use.
- All reagents were ready to use except the 1x washing solution that was prepared by adding 1ml of washing solution to 49 ml of distilled water.

- Twenty five µLof standards, controls and samples were transferred into their respective wells and 100 µL of IgE biotin conjugate was added to each well and incubated for 30 min at room temperature.
- After incubation, the contents of the wells were aspirated and each well was washed three times with 300 µL of 1x Wash Solution.
- Hundred µL of IgE HRP conjugate was added into all wells and incubated for 30 minutes at room temperature.
- After incubation, the contents of the wells were aspirated and each well was washed as described above.
- Hundred µL of TMB Substrate Solution was added into all wells and incubated for 15 minutes in the dark.
- After enough incubation time, 100 µL stop solution was added to all wells until a yellow color was developed.
- Absorbance was measured at 450 nm using the Bio-Tek/ELx800 micro-plate reader within 30 minutes after the addition of the Stop Solution.

D. Specific IgE Determination

In order to assess for specific IgE levels of certain identified Mediterranean allergens, the Biocheck-Polycheck- Allergy Diagnostic- Mediterran-II Panel with 20 Inhalation Allergens kit (GmbH, Munster, Germany) was used. The kit was handled according to the manufacturer's protocol. All incubation steps were performed with continuous shaking to provide homogenous spread of reagents on the cassette. Briefly;

1. Reagents and Kit Content

- Polycheck Inhalation cassette (containing the prefixed allergens)

- Start Solution
- Ligand labeled anti-IgE antibody
- Enzyme-labeled anti-ligand
- Substrate Solution
- Wash Buffer
- Biocheck Imaging Software (BIS) for patient oriented analysis, calculation, and report

2. Procedure

- All test components were equilibrated to room temperature prior to use.
- All reagents were ready to use except the wash buffer that was dissolved in 1 liter demineralized water.
- The cassettes were overlaid with the wash solution to activate the membrane.
- Two hundred fifty µL of start solution was added to the allergen cassette and incubated for 60 seconds. The cassette was then tapped carefully on absorbent paper.
- Two hundred µL of controls and patients sera were added respectively onto the membrane and incubated for 60 minutes.
- After incubation, samples were decanted and washed three times with 1 ml of Polycheck wash buffer, 250µLof wash buffer was then added and incubated for 5 minutes. This step was repeated three times to insure proper washing of the cassettes.
- After enough washing, 250 µL of Enzyme-labeled Anti-Ligand was added and incubated for 20 minutes then decanted and washed three times as described above.

- Two hundred fifty µL of Polycheck substrate solution was then added and incubated for 20 minutes in the dark. Then the substrate solution was decanted and washed three times as described above.
- The cassettes were left to air-dry and were then evaluated using a scanner and the Biocheck Imaging Software for analysis.

E. Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics 19. Chi-square was calculated and the relative risks, P values, percentages and confidence intervals were determined.

CHAPTER IV

RESULTS

A. Serum IgE Levels

As demonstrated in **table 3**, serum polyclonal IgE levels that were tested by ELISA indicated that the prevalence of high polyclonal IgE levels was similar in both groups. Five of 26 RA patients (19.2%) and 5 of 26 Control subjects (19.2%) had elevated polyclonal IgE levels. The mean and standard deviation values of the controls were 104.071 IU/ml± 125.593 IU/ml which were higher than those of the RA patients 91.181IU/ml ±133.019 IU/ml. The two-tailed P value was 0.7485. The highest total IgE concentration observed was 566.745IU/ml among RA patients which was higher than that of controls, 491.0784 IU/ml.

B. Specific IgE Levels

1. Percentage (RA and Controls)

As detailed in **Table 4** and shown in **Figure 1**, 5 allergens had higher prevalence in RA patients than controls, these included: Mugwort pollen (7.69% of RA patients compared to 3.8% of controls), Plantain pollen (11.53% of RA compared to 3.8% of controls), Dog epithelia (34.61% of RA patients compared to 15.38% of controls), *Aspergillus fumigatus* (15.38% of RA patients compared to 11.53% of controls), and Alternaria Alternata (Alt. Alternata) (19.23% of RA patients compared to 11.53% of controls). However, there are 8 other allergens that showed increased specific IgE in controls rather than in RA patients, these included: Alder pollen (RA patients 3.8%, controls 7.69%), Hazel pollen (RA patients 0%, controls 3.8%), Oak pollen (RA patients

0%, controls 7.69%), Timothy Grass pollen (RA patients 0%, controls 3.8%), Rye pollen (RA patients 3.8%, controls 7.69%), Dermatophagoides pteronyssinus (D. pteronyssinus) (RA patients 15.38%, controls 19.23%), Cat epithelia (RA patients 11.53%, controls 15.38%), and Penicillium notatum (Pen. notatum) (RA patients 15.38%, controls 19.23%). Some allergens had similar percentages in both RA patients and controls and these included: Birch pollen (3.8%), Horse epithelia (11.53%), Guinea Pig epithelia (7.69%), Hamster epithelia (7.69%), Rabbit epithelia (11.53%), Dermatophagoides farina (D. farinae) (30.76%), and Cladosporium herbarum (Cladosp. herbarum) (15.38%).

2. Incidence (RA and Controls)

Table 5 and **Figure 2** show the incidence of RA among allergic and non-allergic individuals. Five allergens had higher incidence of RA; these included Mugwort pollen, Plantain pollen, dog epithelia, Aspergillus fumigatus, Alt. Alternata; 66% of individuals who were allergic to Mugwort pollen had RA compared to 49% of those who were not allergic and had RA(RR=1.361, CI=0.582-3.183); 75% of those allergic to Plantain pollen had RA set against 48% of the ones who were not allergic and had RA (RR=1.565, CI=0.827-2.963), 69% of subjects who were allergic to dog epithelia had RA compared to 44% of those who were not allergic and had RA (RR=1.548, CI=0.933-2.567), 57% of those allergic to *Aspergillus fumigatus* had RA in comparison to 49% who were not allergic to it and had RA (RR=1.169, CI=0.576-2.372), and 62% of people allergic to Alt. alternata had RA while 47% of those non allergic had RA (RR=1.310, CI=2.705-2.433). Other allergens had lower incidence of RA; these included Alder Pollen, Rye Pollen, D. pteronyssinus, Cat epithelia and Pen. notatum; indicating that the percent of individuals allergic to any of these allergens and had RA was lower than 50% compared to >50% of

individuals who were not allergic to any them and had RA; generally (RR<1, CI=0.129-3.313). Some allergens showed no difference in the incidence of developing RA or not if allergic to any of them, these included: Birch pollen, D. farinae, Horse epithelia, Guinea Pig epithelia, Hamster epithelia, Rabbit epithelia and Cladosp. herbarum; incidence was 50% (RR=1; generally CI=0.243-4.11). Three allergens didn't show any relative risk value, none of the RA patients showed specific IgE levels against them; these included: Hazel, Oak and timothy grass pollen.

3. Percentages (SLE and Controls)

Concerning percentages of allergies among SLE patients versus their matched controls shown in **Table 6 and Figure 3**; none of the SLE patients were allergic to Alder pollen, Birch pollen, Hazel pollen, Oak pollen, Rabbit epithelia, Cladosp. herbarum, and Pen. notatum compared to 20% of their matched controls who were allergic to these allergens. Moreover, none of the SLE patients or their matched controls were allergic to Mugwort pollen, Plantain pollen, Asp. fumigatus, Guinea Pig epithelia, Hamster epithelia, and Alt. alternata. Only the SLE patients were allergic to Timothy Grass pollen and D. pteronyssinus (100% SLE patients allergic). Identical allergic patterns were observed between SLE patients and Controls for Rye pollen (20%), Dog epithelia (40%), Cat epithelia (20%), and Horse epithelia (20%). Whereas the percentage of SLE patients allergic to D. farinae was 20% which is lower than that of the controls (40%).

4. Incidence (SLE and Controls)

As demonstrated in **Table 7** and **Figure 4**, the incidence of SLE among allergic and non allergic individuals showed that 100% of those allergic to D. pteronyssinus and Timothy Grass pollen had SLE compared to 44% who were not allergic to them and had SLE (RR=2.25, CI=1.084-4.671). Additionally, none of those allergic to Birch pollen, Hazel pollen, Oak pollen, Rabbit epithelia, and Cladosp. herbarum had SLE whereas 55% of those non allergic to these allergens had SLE. Furthermore, some allergens showed no difference in the incidence of developing SLE or not if allergic to any of them, these included: Rye pollen (RR=1, CI=0.212-4.709), Cat epithelia (RR=1, CI=0.212-4.709), Dog epithelia (RR=1, CI=0.282-3.544), and Horse epithelia (RR=1, CI=0.212-4.709). No positive or negative incidence for SLE was observed with the following allergens Mugwort pollen, Plantain pollen, Guinea Pig epithelia, Hamster epithelia, Asp. fumigatus, Pen. notatum, and Alt. alternata. Finally, 33% of those allergic to D. farinae had SLE in contrast to 57% of those non allergic and had SLE (RR=0.583, CI=0.104-3.271).

Table 1. Site of Inflammation versus Symptoms in SLE Patients

Site of Inflammation	Symptoms
Brain and Nervous System	seizures, headache, and personality changes
Heart	myocarditis, pericarditis
Gastro intestine	Nausea, vomiting
Joints	pain, swelling, and/or arthritis
Skin	malar rash or butterfly rash on the cheeks and the nose, photosensitivity
Lungs	pneumonitis, interstitial lung disease, and chest pain during deep breath
Kidneys	acute or chronic renal failure and acute nephritic disease
Bone marrow and blood	cytopenia, leukopenia, anemia, or thrombocytopenia
Muscle	Avascular necrosis, arthropathy, frank arthritis

Table 2.Tests done to confirm the Diagnosis of RA and SLE Patients Recruited

patients	Gender	High ESR	High CRP	Anti-CCP +ve	RA latex +ve	Anti-dsDNA +ve	Anti-ANA+ve
34 RA	26 Females	11	12	1	6	-	-
	8 Males	4	4	-	1	-	-
5 SLE	5 Females	5	2	-	-	2	3

Table 3. Polyclonal IgE Levels among RA patients and Controls

Groups	RA Patients	Controls
Number of individuals having polyclonal IgE level >150 IU/ml	5	5
Number of individuals having polyclonal IgE level <150 IU/ml	21	21
Total	26	26
Polycolonal IgE levels Mean IU/ml	91.1811 (IU/ml)	104.071 (IU/ml)
Standard Deviation	133.0192	125.5936
Two-tailed P value	0.7485	

Table 4: Percent of Allergies among RA and Controls

Allergens	% of Allergies among RA Patients	% of Allergies among Controls
Mugwort Pollen	7.69	3.8
Pllantain Pollen	11.53	3.8
Dog Epithelia	34.61	15.38
Asp.fumigatus	15.38	11.53
Alt.alternata	19.23	11.53
Alder Pollen	3.8	7.69
Hazel Pollen	0	3.8
Oak Pollen	0	7.69
Timothy Grass Pollen	0	3.8
Rye Pollen	3.8	7.69
Pen.notatum	15.38	19.23
D. pteronyssinus	15.38	19.23
Cat Epithelia	11.53	15.38
Cladosp. herbarum	15.38	15.38
Birch Pollen	3.8	3.8
D. farinae	30.76	30.76
Horse Epithelia	11.53	11.53
Guinea Pig Epithelia	7.69	7.69
Hamster Epithelia	7.69	7.69
Rabbit Epithelia	11.53	11.53

Table 5. Incidence of RA among Allergic and Non Allergic Individuals

Allergen	% of RA positive & allergic	% of RA positive & Non allergic	P value	RR	Confidence Interval
Mugwort Pollen	66	49	0.5	1.361	0.582-3.183
Pllantain Pollen	75	48	0.3	1.565	0.827-2.963
Dog Epithelia	69	44	0.11	1.548	0.933-2.567
Asp.fumigatus	57	49	0.5	1.169	0.576-2.372
Alt. alternata	62	47	0.35	1.31	0.705-2.433
Alder Pollen	33	51	0.5	0.653	0.129-3.313
Hazel Pollen	0	51	0.5	NA	NA
Oak Pollen	0	52	0	NA	NA
Timothy Grass Pollen	0	51	0.5	NA	NA
Rye Pollen	33	51	0.5	0.653	0.129-3.313
Pen. notatum	44	51	0.5	0.869	0.396-1.908
D. pteronyssinus	44	51	0.5	0.829	0.396-1.908
Cat Epithelia	43	51	0.5	0.839	0.340-2.066
Cladosp. herbarum	50	50	0.64	1	0.471-2.124
Birch Pollen	50	50	0.7	1	0.243-4.11
D. farinae	50	50	0.61	1	0.555-1.802
Horse Epithelia	50	50	0.66	1	0.427-2.341
Guinea Pig Epithelia	50	50	0.69	1	0.361-2.773
Hamster Epithelia	50	50	0.69	1	0.361-2.773
Rabbit Epithelia	50	50	0.66	1	0.427-2.341

Table 6. Percent of Allergies among SLE and Controls

Allergen	% of allergies among SLE Patients	% of allergies among Controls
Alder Pollen	0	20
Birch Pollen	0	20
Hazel Pollen	0	20
Oak Pollen	0	20
Timothy Grass Pollen	20	0
Rye Pollen	20	20
Mugwort Pollen	0	0
Pllantain Pollen	0	0
D. pteronyssinus	20	0
D. farinae	20	40
Dog Epithelia	40	40
Cat Epithelia	20	20
Horse Epithelia	20	20
Guinea Pig Epithelia	0	0
Hamster Epithelia	0	0
Rabbit Epithelia	0	20
Asp. fumigatus	0	0
Cladosp. herbarum	0	20
Pen. notatum	0	20
Alt. alternata	0	0

Table 7. Incidence of SLE among Allergic and Non Allergic Individuals

Allergen	% of SLE positive & Allergic	% of SLE positive & Non Allergic	P value	RR	Confidence Interval
Alder Pollen	0	55	0.5	NA	NA
Birch Pollen	0	55	0.5	NA	NA
Hazel Pollen	0	55	0.5	NA	NA
Oak Pollen	0	55	0.5	NA	NA
Timothy Grass Pollen	100	44	0.5	2.25	1.084-4.671
Rye Pollen	50	50	0.778	1	0.212-4.709
Mugwort Pollen	0	0	NA	NA	NA
Plantain Pollen	0	0	NA	NA	NA
D. pteronyssinus	100	44	0.5	2.25	1.084-4.671
D. farinae	33	57	0.5	0.583	0.104-3.271
Dog Epithelia	50	50	0.73	1	0.282-3.544
Cat Epithelia	50	50	0.77	1	0.212-4.709
Horse Epithelia	50	50	0.77	1	0.212-4.710
Guinea Pig Epithelia	0	0	NA	NA	NA
Hamster Epithelia	0	0	NA	NA	NA
Rabbit Epithelia	0	55	NA	NA	NA
Asp. fumigatus	0	0	NA	NA	NA
Cladosp. herbarum	0	55	NA	NA	NA
Pen.notatum	0	55	NA	NA	NA
Alt. alternata	0	0	NA	NA	NA

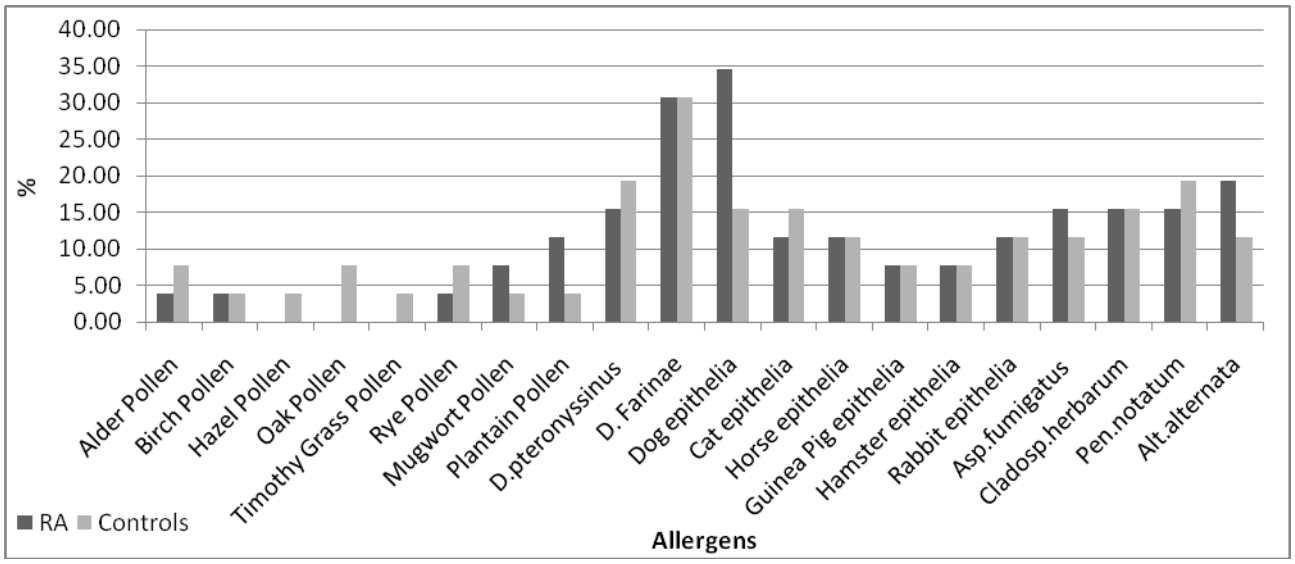

Figure 1. Percent of Allergies among RA Patients and Controls were detected using a Specific IgE ELISA kit against 20 Mediterranean allergens. RA patients had higher specific IgE levels against Mugwort pollen, Plantain pollen, Dog epithelia, Asp. fumigatus, and Alt. alternata compared to their sex and age matched controls.

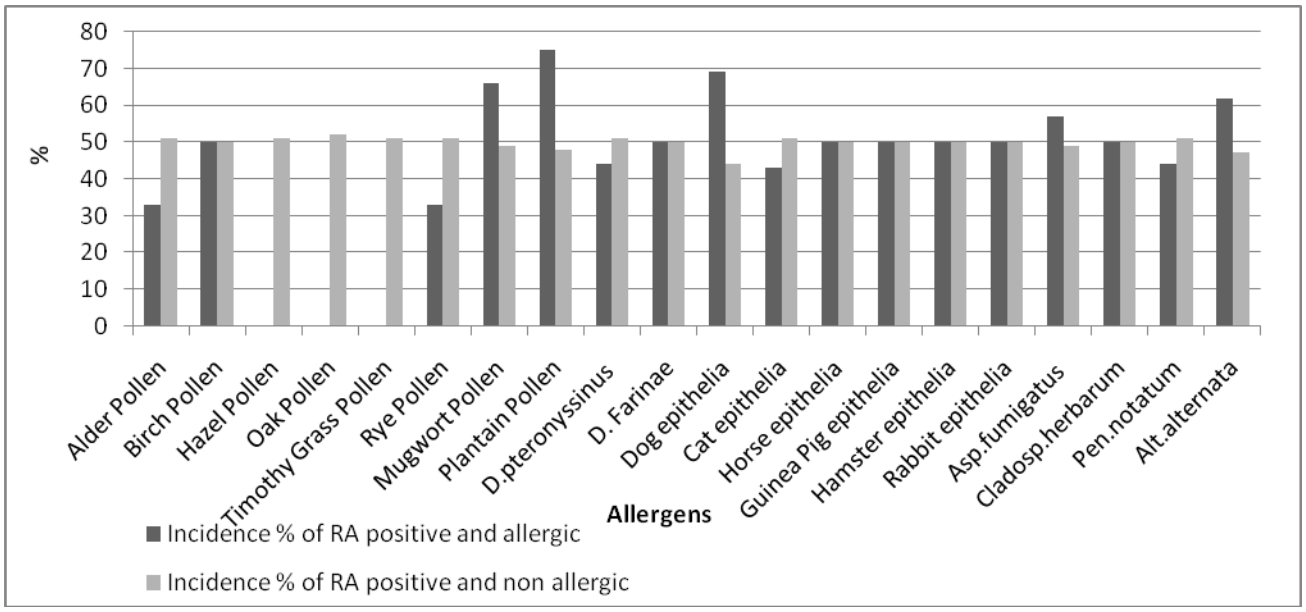

Figure 2. Incidence of RA among Allergic and Non Allergic Individuals were calculated. Higher incidence of individuals allergic to Mugwort pollen, Plantain pollen, Dog epithelia, Asp. fumigatus, and Alt. alternata had RA compared to those non allergic to them.

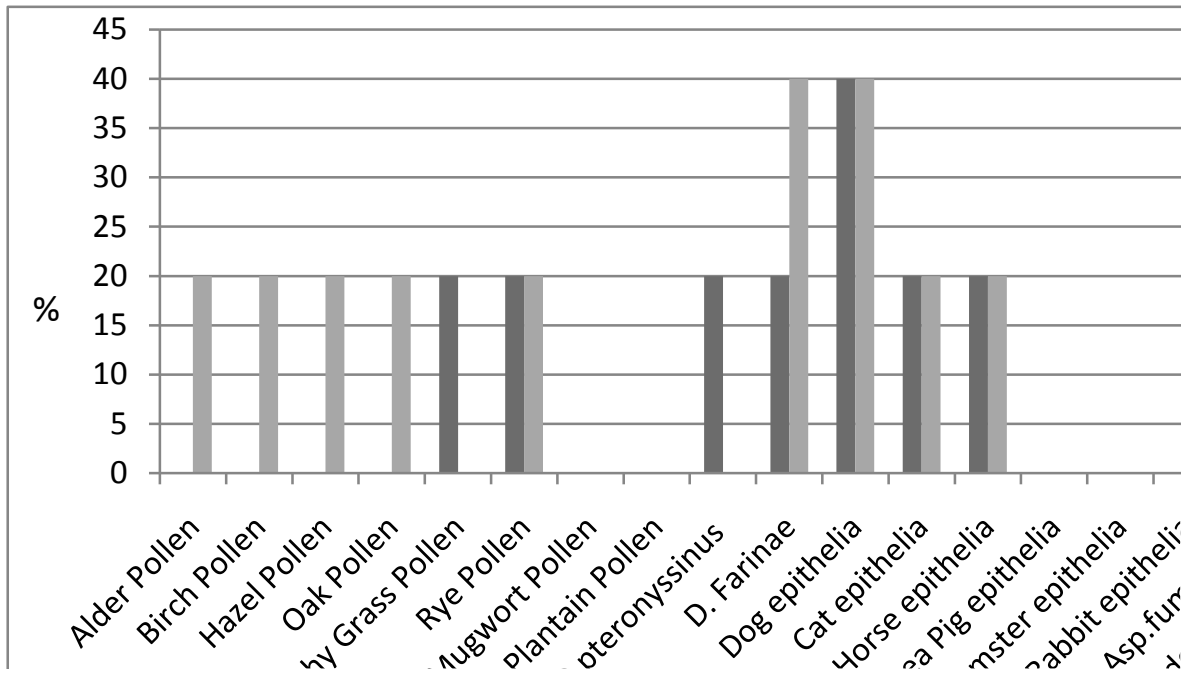

Figure 3. Percent of Allergies among SLE and Control were detected using a Specific IgE ELISA kit against 20 Mediterranean allergens.SLE patients had higher specific IgE levels against Timothy Grass pollen and D. pteronyssinus.

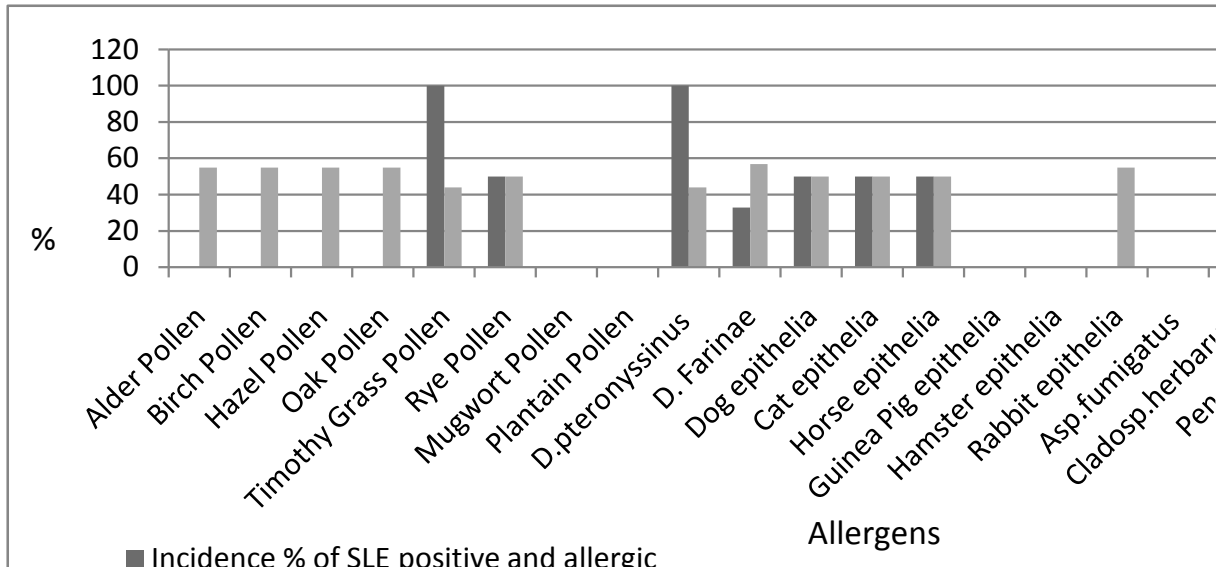

Figure 4. Incidence of SLE among Allergic and Non Allergic Individuals were calculated. Higher incidence of individuals allergic to Timothy Grass pollen and D. pteronyssinus compared to those non allergic to them.

CHAPTER V

DISCUSSION

Systemic Lupus Erythematosus and Rheumatoid Arthritis are two chronic inflammatory autoimmune diseases that can affect any age group and any organ in the patient's body (3,4, 15).The causes of most autoimmune diseases have been related to genetic and environmental factors. For example, in the former case, the association of HLA alleles with certain autoimmune diseases has been reported. In the latter case drugs, microbial agents, smoking and radiation are among the predisposing factors.(3,9,31,32). In a similar but not identical manner, IgE-mediated allergic diseases have predisposing genetic factors and provoking environmental factors.

Hence, the objective of this study was to determine serum polyclonal IgE levels, and when possible determine the presence and levels of specific IgE levels in patients with RA, SLE and normal controls to check if there is any correlation between these parameters and the autoimmune disease.

There was no significant difference between the number of RA patients having high polyclonal IgE levels and that of the controls. Therefore, it appeared that there was no correlation between high polyclonal IgE levels and RA which may confirm the results of the study reported by Magen et al. (29) that were mentioned in the literature review.

On the other hand, when studying the different allergens and their correlation with RA, it was found that Mugwort allergies would increase the risk of developing RA by 66% as opposed to the 49% of not having the allergy and developing RA. 66% of patients with RA were allergic to Mugwort pollen while 49% of those who were not allergic to Mugwort pollen had RA with a relative risk above 1. Similarly, higher percentages of individuals who were allergic to Plantain pollen, Dog epithelia, Asp. fumigatus, and Alt. alternata had

RA whereas lower percentages of individuals who were not allergic to the same allergens had RA with a relative risk more than 1 as well. Since the relative risk was >1 in these allergens, this means that these allergens can be considered as risk factors for RA patients. In comparison to other allergens like Alder pollen, Hazel pollen, Oak pollen, Timothy Grass Pollen, Rye pollen, D. pteronyssinus, Cat epithelia, and Pen. notatum where a low number of individuals allergic to them had RA in comparison to higher number of participants not allergic to them and have RA with a relative risk below 1. Therefore, this group of allergens might be considered as protective factors against RA. Moreover, the percentages of subjects allergic and non allergic to Birch pollen, D. farinae, Horse epithelia, Guinea Pig epithelia, Hamster epithelia, Rabbit epithelia, and Cladosp. herbarum having RA with a relative risk equals to one. Hence, this bundle of allergens appears to be neither protective nor risky in relation to having RA.

In addition, 100% of individuals who were allergic to Timothy Grass pollen and D. pteronyssinus had SLE while 44% of those non allergic to these allergens had SLE (RR=2.25 >1). We can conclude that these 2 allergens might be risk factors for SLE. Moreover, none of individuals who were allergic or non-allergic to Mugwort pollen, Plantain pollen, Guinea Pig pollen, Hamster pollen, Asp. fumigatus, and Alt. alternata had SLE. Thus, these allergens cannot be protective nor risky in relation to having SLE. Whereas 0% of those allergic to Alder pollen, Birch pollen, Hazel pollen, Oak pollen, Rabbit epithelia, Cladosp. herbarum, and Pen. notatum had SLE compared to 55% of those non allergic to these allergens and had SLE. This might indicate that these allergens can be protective against SLE. To add up, the incidence among individuals allergic and non allergic to Rye pollen, Dog epithelia, Cat epithelia, and Horse epithelia and have SLE was the same. In conclusion, these allergens are neither protective factors nor risk factors to SLE. Last but not least, 33% of those allergic to D. farinae had SLE while 57% of those

non allergic to D. farinae had SLE (RR<1). Therefore it appears that D. farinae is a protective factor against SLE. It can be noted that all the P values of all allergens were above 0.05 due to the small size of samples.

In conclusion, certain allergens can be considered as environmental risk factors for both RA and SLE. Mugwort pollen, Plantain pollen, Dog epithelia, Asp. fumigatus, and Alt. alternata can be environmental risk factors for RA. In addition,, Timothy Grass pollen and D. pteronyssinus can be environmental risk factors for SLE which may confirm the results of the study reported by Barbara Dema et al. (30) that were mentioned in the literature review. Other allergens can be either protective or not protective and not risk factors. Finally, conclusions suggested were based on a small number of subjects included in the study. More patients must be studied in order to make concrete conclusions.

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