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The Immunological Profile of Allergic Rhinitis

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ABSTRACT

Allergic rhinitis frequently occurred ,Many types of inflammatory cells and mediators are involved in this disorder. Of particular note is that the pro-inflammatory mediators (e.g. cysteinyl leukotriens, cytokines, and histamine) are involved in nasal and bronchial inflammation, in patients with rhinitis.

Aim

The aim of the study was to define the immunological profile of allergic rhinitis cases in regards to : Eosinophil count , total IgE levels , specific IgE levels , serum immunoglobulin's levels , serum complement component(C3,C4)levels , interleukin 4 and interleukin 10 levels.

Materials and methods:

This is a cross sectional study which was carried out on 100 patients with allergic rhinitis) at the ***Asthma and Allergy Institute*** in Baghdad, from February 2007 to July 2007, and fifty participants as a control group. Spirometry was performed with the goal of recording as precise as possible of spirometry measures forced expiratory volume in one second (FEV1) and forced vital capacity (FVC).

Samples of blood were drawn for serological study from all the participants.

Results and Discussion:

The age distribution of Allergic rhinitis showed the majority of cases occurred between the age of 5 and 35 years, but

The gender distribution showed female predominance in allergic rhinitis.

The mean total serum IgE level in AR was significant higher than its level in the control group (P<0.01).The mean serum concentration of IgG, IgM and C3 complement component were higher in rhinitis patients.

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Introduction:

The Greek term, Allergy, was regionally intended to mean, deviation from the original state **[1]**.

Allergic diseases results from an exaggerated response of the immune system to external innocuous substances. They are increasing cause of illness affecting about 1-6% of the population **[2]**.

Definition: AR is the inflammation of the nasal mucous membrane resulting from exposure to specific allergen, characterized by sneezing, rhinorrhoea, obstruction of the nasal passages, conjunctival, nasal and pharyngeal itching and lacrimation, all occurring in temporal relationship to allergen exposure.

AR can be perennial, occuring in response to allergens that are present throughout the year such as: house dust mite, animal danders, mould and cockroach. In up to one half of patients with perennial rhinitis, no clear cut allergen can be demonstrated as a cause **[3]**. Relatively small number of weeds that depend on wind rather than insects for cross pollination, as well as grasses and some trees will elicit a seasonal allergic rhinitis **[4]**. The ability of allergen to cause rhinitis rather than lower respiratory symptoms may be attributed to their large size (10-100um), and retention within the nose **[5]**.

Pathophysiology: The nose presents a large mucosal surface area through the folds of the turbinates and serves to adjust the temperature and moisture content of the inhaled air and to filter out particulate materials, about 10um in size, by impingement in a mucous blanket, cilliary action moves the entrapped particles to ward the pharynx **[6]**. The initial interaction occurs between the allergen (generally of 10,000 – 40,000 M.W) and intraepithelial mast cells and then proceeds to involve deeper perivenular mast cells, both of which are sensitized with specific IgE.

In sensitive individuals, the mast cells of the nasal mucosa and

submucosa generate and release mediators through IgE dependant reactions following the introduction of allergen into the nose **[7]**.

Rapid acting mediators, like histamine and prostaglandin D2, produce early phase reaction by increasing vascular permeability and tissue edema, while there are a late phase reactions in the nose produce obstruction and are due to cellular infiltration and were suggested to be the cause of symptoms of chronic rhinitis **[8]**. After challenging the nose with ragweed pollen , there is significant increase in recovery of histamine, leukotriens C4 ,D4 , E4 , prostaglandin D2 , Kinine, Kininogenase, and tosyl-L-argenine Methyl ester (TAME) esterase in nasal lavage fluid**[7]**.

In the late phase reaction, which begins within 2-4 hours following allergen exposure these mediators are also recovered with the exception of PGD2. This prostaglandin is released by mast cells but not by basophiles. This suggests that basophiles may play an important role in late phase reaction. Increasing evidences indicate that leukotriens act as important mediators of nasal inflammation **[9]**. The amount of antigen required to elicit symptoms of AR varies in a given individual at different times of the year. For subject sensitive to pollen, greater amount of antigen is required to elicit symptoms out of the season than in the height of the season. This phenomenon is termed (priming), and it is associated with accumulation of inflammatory cells at the site of nasal challenge **[10]**.

Diagnosis: The diagnosis of AR depends largely on accurate history and physical examination of head, neck and adjacent area. The primary objective of the history is to discover cardinal symptoms of sneezing paroxysms, clear rhinorrhoea, nasal and palatal itching which are characteristics but not diagnostic of AR **[11]**. One should ask about allergens such as pollen dust, molds and animal derived proteins as precipitating factors.

Other irritants, such as tobacco smoke, air pollutant perfumes or ammonia, may cause irritant non-

allergic rhinitis **[12]**. Changes in environmental variables, like temperature, humidity, barometric pressure changes, exercise, emotion and food also induce rhinitis symptoms. The time course of symptoms is important. Year round symptoms may suggest Perennial allergic rhinitis or non allergic disease **[13]**. The seasonal symptoms of rhinitis in Iraq extended from March to September **[14]**.

Lab –tests for diagnosis of AR:

1- Skin test.

2- In vitro test (ALISA), for total and specific IgE levels.

3- Blood eosinophil level.

4- Nasal cytology.

5- Rhinomonometry (objective test of airway patency).

6- Rhinoscopy (flexible and rigid).

7- Imaging – radiology and CT scan.

8- Tests of mucocilliary clearance.

9- Nasal provocation challenge.

The aim of the study:

To compare the immunological profile of allergic rhinitis and non allergic groups

Objectives:

To define the immunological differences between allergic rhinitis and non allergic group in regard to:

1- Peripheral blood Eosinophil count

2-serum total IgE levels

3-serum specific IgE levels

4- Serum immunoglobulin's levels

5- Serum complement (C3, C4) levels

6-serum interleukin 4 and interleukin 10 levels

7- Skin test

8- Spiro metric analysis as a clinical parameter.

2. Patients, Materials and Methods:

The study was carried out on Iraqi people attending at the main consulting center of allergy and asthma in Baghdad during the period between February 2007 and July 2007.

2.1.1: Patients Group: Other 100 patients with AR were studied; their age rang between 5-55 years, also of mixed gender. The diagnosis of AR depends on history of rhinorrhoea, nasal obstruction, nasal itching and sneezing. These patients had family history of atopy and had positive results in

conducting skin test. Spirometry was done for them and patients with sign of airway obstruction even with no symptoms of asthma were excluded. Physical examination was done including: chest auscultation and the patients with signs of nasal septal deviations and nasal polyps were excluded.

A questionnaire containing personal details with type and duration of the disease was adapted from the latest guideline for the diagnosis and management of asthma published by (NHLBI 1997). The patients with late onset symptoms and of no history (personal or familial) of atopy, and with negative skin test are all excluded from this study.

2.1.2: Control Group:

Fifty apparently healthy individuals, of both gender matched with patients age, sex and ethnicity were used as control group, taken from the center employees. A detailed history of asthma and AR and other atopy in their families were questioned. The entire control group had negative skin test and normal pulmonary function test. The control subjects with positive findings were all excluded.

2.2: Specimen Collection:

From patient and control groups, venous blood samples were collected; 10 ML of blood were aspirated by venous puncture. 2 ml for Eosinophils count was separated and 8 ml were allowed to clot, centrifuged then separated serum immediately frozen at 2-8 ^{0}C, to be used for immunological assessment.

2.3: Materials:

2.3.1: Allergens Extracts:

Allergen extract types used in skin test are manufactured in Stallergen Company in France.(Appendix 3).

2.4: Methods:

2.4.1: Skin Test: was done for the patients and the control. It was performed according to the standard method described by: Pepys 1975, using aqueous allergen extracts supplied by: Stallergen (France), and by the Iraqi labs for the production of vaccine and sera in Baghdad. The panel of 14type of allergen was used.

(HDM, D1, D2, M1, M2, M3 ,M4 ,G2 ,G5, W6, W10, T1, T3, Control positive and negative.

The intardermal test was done with HDM, D1, and D2 only. On the volar aspect of the forearm. The skin must be clean, free of eczema or any other defect. After cleaning the forearm skin with alcohol, soaked cotton and letting it to dry. A grid is marked with a pen with 3 cm intervals in adult and 2cm in children. The pattern follows corresponding list of allergen extracts in concentration of (1x10 of index of reactivity IR), intracutaneously using 1ml syringe through 26 gauge needle. The syringe was placed at an angle of 45 degree to the forearm; needles bevel was downwards facing the skin and penetrating entirely not going deeper than the superficial layer of the skin. A volume of approximately 0.2ml of extract was gently injected to produce a small superficial bulb, the reaction was read after 15 min. a positive result is a skin weal more than 2 mm greater than that observed with the negative control solution. The result of the skin test should be interpreted in the light of the clinical history.[**15**]

Prick skin test: was performed by placing a drop of allergen extract of a concentration (1x10 IR), on the volar aspect of the forearm. The next drop was placed approximately 3 cm apart. A disposable hypodermic needle (25-27 gauge) , was passed through the drop and inserted into the epidermal surface at a low angle with the bevel facing upwards, the needle tip was gently lifted upwards to elevate a small portion of the epidermis without inducing bleeding , the needle was then withdrawn and the solution gently wiped away. The result was also read after 15 min according to the diameter of the reaction zone. In both types of skin test a positive control (histamine hydrochlorate 10mg/ml and a negative control phenol were used). [16]

2.4.2: ELISA for the Quantitative Determination of Total IgE:

Principle: total IgE micro plate ELISA kit is a two site enzyme linked immunosorbant assay, for the quantitative determination of IgE. First

mouse monoclonal antibody is immobilized in to the plastic wells. The diluted sample is added to the solid phase Abs coated well with incubation. After washing, only the antigen from the patients sample remains bound on to the well. Then a conjugate labeled Abs is added to form a (sandwich) complex, if IgE is present in the sample. After incubation and washing to remove unbound conjugate and other unbound components, a third incubation with chromogen, Para nitro phenol phosphate (PNPP) is performed. The reaction is stopped with NaOH, and the plate is read at 405 nm in a spectrophotometer. The color intensity was directly proportional to the amount of IgE in the sample, and the level of unknown IgE was then determined by comparing the optical density with data established using known IgE standards in the same assay system.[17]

2.4.3. EIA Estimation of Specific IgE Abs:

The procedure uses enzymatic immunoassay (EIA) method, for the semi-quantitative determination of allergen specific IgE concentration in human serum or plasma, which is incubated in the first step with allergen covalently bound to the solid support of a paper disk. If the sample contains Abs, directed against this allergen, it will be bound to the solid phase. Washing the support with a washing solution removal of Abs having not reacted with the disk. Incubation of the washed paper disk with enzyme linked antisera in human IgE allows the antisera to fix the allergen specific IgE Abs, to be bound to the solid support. After a second washing of the disk, a chromogenic solution is added and incubated. This result in the development of yellow color which is measured spectrophotometrically at 405 nm. After stopping the reaction and measuring with a color meter, the level of allergen specific IgE is calculated. [18]

In this study , specific IgE against specific antigens were used , such antigens were : D1 (Dermatophagoids Ptronesyines), D2 (Der.Farinae) , D71(Lepidoglyphus

Destructor), G2(Bermoda Grass), G4(Meadow Fescue), M1 (Pencillium Notatum), M2 (Cladosporum Herbarum), M3 (Aspergillus Fumegatus), M4 (Mucor Racemosus), M5(Candida Albican), M6 (Alternaria Tenuis), T1(Maple Box Elder), T3 (Birch), W6 (Mugwort Sagebrush), W10 (Lambs Quarters).

2.4.4. Blood Eosinophil Counts:

Routine blood film was stained with lishman's stain, 100 leukocytes were counted and the percentage of Eosinophils was obtained accordingly, and then multiplies this percentage with the total WBC count to gate eosinophil count /ml. [19]

2.4.5: Estimation of Serum Interleukin4 by ELISA:

Principle: the concentration of IL4 in the sera of patients was measured by double sandwich enzyme linked immunosorbant assay, according to the manufacturer instructions.[20]

2.4.6: Estimation of Serum IL10 by ELISA:

Principle:

Human IL10 kit is a sandwich type of enzyme immunoassay in which a monoclonal antihuman IL10 Abs are bound on to polystyrene microtiter wells. [20]

2.4.7: Estimation of Serum IgG, IgM and IgA Levels:

Serum immunoglobulins were quantitated by single radial immunodiffusion technique under standardized conditions with commercially available endplates. 5uL of reference and test sera were dispensed into the endoplate wells , using a 5ul aspirator fix .the endoplate incubated at room temperature for 24h (72h for IgM) , Diameter of precipitating ring were measured using a millimeter graded viewer. The diameter of the reference sera was compared to their corresponding values in the stastical control report. If they fail in the reference sera diameter range recorded in the stastical control report, the table for conversion of end point ring diameter to concentrations was used to convert the test sera diameters to concentrations (mg-dl), reference sera used only in one endoplate per

kit.[19]

2.4.8: Serum Complements Components (C3 and C4):

A single radial immunodiffusion test similar to that applied to serum immunoglobulins determination was used with a specific endplate . C3 and C4 concentration were determined as same as immunoglobulins test. [21]

Statistical Analysis:

The suitable statistical methods were used in order to analyze and assess the results, these include the followings:

1- Descriptive Statistics:

A) Statistical tables including observed frequencies with their percentages.

B) Summary statistic of the readings distribution (mean, SD & minimum & maximum).

C) Graphical presentation by (bar - charts).

2 – Inferential Statistics:

These were used to accept or reject the statistical hypotheses, they include the followings:

A) Chi-square $\chi 2$.

C) Repeated (t-test).

Note: The comparison of significant (P-value) in any test were:

S= Significant difference ($P<0.05$).

HS= Highly Significant difference ($P<0.01$).

NS= Non significant Difference ($P>0.05$) .

Results:

Table 1:: Age Distribution of Patients Included in the Study:

Age group	Rhinitis	Percentage %
5-14	27	27
15-24	30	30
25-24	21	21
35-44	13	13
45-54	5	5
545-64	6	6
>64	4	4
total	100	100%

0.00 Highly sig.(p>0.05)

Table 2: Gender Distribution of Patients Included in the Study.

Geneder	Rhinitis	%
Male	33	33
Femal	67	67
Total	100	100

0.00 Highly sig(P<0.01)

Table 3: Distribution of Patients According to the Types of Antigens used in the Skin Test:

Allergen		No. of (+ve) test		Rhinitis Vs control
		Control	Rhinitis	
D1+D2	N	13	57	P1= Highly Sig.
	%	26	57	.
HDM	N	6	44	.
	%	12	44	P1= Highly Sig.
M1	N	10	42	.
	%	20	42	P1= Highly Sig..
M2	N	1	27	
	%	2	27	P1= Highly Sig.
M3	N	0	25	
	%	0	25	P1= Highly Sig.
M4	N	2	31	.
	%	4	31	P1= Highly Sig.
Bermoda	N	4	56	
	%	8	56	P1= Highly Sig.
Plantain	N	3	52	
	%	6	52	P1= Highly Sig.
Grasses	N	2	49	
	%	4	49	P1= Highly Sig.
Mugwort	N	0	47	
	%	0	47	P1= Highly Sig.
Fagacae	N	0	30	
	%	0	30	P1= Highly Sig.
Betulacae	N	2	39	
	%	4	39	P1= Highly Sig.

Table 4: comparison of specific IgE levels according to the type of antigens used among two groups:

Specific IgE (+ve Anti- Allergen)		Studied groups		Rhinitis Vs Control
		Control	Rhinitis	
D1	Mean± SD	0.533 ± 0.147	1.346 ± 0.848	P1= Sig.
D2	Mean± SD	0.566 ± 0.133	1.468 ± 1.071	P1= Sig
D71	Mean± SD	0.536 ± 0.091	1.501 ± 1.345	P1= Sig.
M1	Mean± SD	0.506 ± 0.107	2.049 ± 1.079	P1= Highly Sig.
M2	Mean± SD	0.497 ± 0.098	1.922 ± 1.815	P1= Highly Sig.
M3	Mean± SD	0.497 ± 0.110	2.009 ± 1.198	. P1= Highly Sig.
M4	Mean± SD	0.45 ± 0.127	1.007 ± 0.418	P1= Sig.
M5	Mean± SD	0.53 ± 0.155	1.022 ± 0.337	P1= Sig.
M6	Mean± SD	0.487 ± 0.084	1.654± 1.143	P1= Sig.
G2	Mean± SD	0.63 ± 0.156	4.054 ± 3.699	P1= Highly Sig.
G5	Mean± SD	0.565 ± 0.148	4.077 ± 3.669	P1= Highly Sig.
W6	Mean± SD	0.53 ± 0.00	3.390 ± 2.463	P1= Highly Sig.
W10	Mean± SD	0.523 ± 0.047	2.914 ± 1.150	P1= Highly Sig.
T1	Mean± SD	-	3.040 ± 2.100	P1=Non Sig.
T3	Mean± SD	0.39 ± 0.00	2.534 ± 1.062	. P1= Highly Sig.

Table 5: comparison of levels of immunoglobulins among two groups:

Type of Ig	Groups	N	Mean	Std. Deviation	Mini.	Maxi.	P-value
T.IgE	Control	50	67.06	41.32	7	187	-
	Rhinitis	100	359.37	365.52	154	3466	0.00 HS
IgA	Control	50	211.392	89.244	94.7	352.2	-
	Rhinitis	100	174.749	65.596	90.9	351.5	0.003 HS
IgG	Control	50	882.080	180.705	649.5	1224.6	-
	Rhinitis	100	1241.217	467.891	692.9	2124.4	0.002 HS
IgM	Control	50	112.546	47.367	48.3	195.6	-
	Rhinitis	101	125.944	42.598	61.7	209.4	0.086 NS

Table 6: comparison of complement levels among two groups:

complement	groups	N	Mean	Std. Deviation	Mini.	Maxi.	P-value
C3	Control	50	106.348	20.689	84.3	191.9	-
	Rhinitis	100	97.390	18.013	68.7	148.1	0.008 HS
C4	Control	50	34.574	7.819	24.8	49.5	-
	Rhinitis	100	33.367	13.498	14.1	78.3	0.581 NS

Table 7: comparison of interleukin levels among the groups:

Type of IL	groups	N	Mean	Std. Deviation	Mini.	Maxi.	P-value
IL-4	Control	50	1.948	1.673	0.25	10.22	-
	Rhinitis	100	41.336	19.991	2.12	82.42	0.00 HS
IL-10	Control	50	2.546	1.072	0.82	4.88	-
	Rhinitis	100	5.738	2.336	3.05	22.36	0.00 HS

Table 8: comparison of Eosinophil count (%) among two groups:

groups	N	Mean	Std. Deviation	Mini.	Maxi.	P-value
Control	50	2.46	1.25	1	5	-
Rhinitis	100	6.93	2.75	3	14	0.00 HS

Discussion:

Allergic diseases are in general an important world wide health problem.

Among the ten leading pathologic conditions in United States, it ranks second in order after cardiac problems, and is one of the most coasty health problems [22].

Relationship between Age, Sex and Diseases:

The results of this study, demonstrated AR is still affecting all age groups and the majority of cases occurred between the ages 5-45 years, and tend to diminish gradually with aging.The data showed, in AR the age groups 15-24 years and 5-14 years were higher than other age groups with (HS $p < 0.01$).

These observations are in agreement with the findings of other investigators as Bjorksten et al in [23], Couwnberge et al in [24], Al-Shartslify in [25] and Al-Taee in [26], who stated that the

signs and symptoms AR generally appear before the fourth decade of life and decrease with old age.However, other studies conducted by Yawn et al in [27] and Kasuba in [28], showed that AR can begin at any age group and the onset is usually during childhood and adolescence.The decreased incidence of allergic diseases among older subjects is related to an absolute decrease in the number of cells capable for releasing mediators in response to specific stimulation and also to decrease in the level of serum total and specific IgE [25].

Concerning the Gender distribution there was female predominance in AR patients. These results agreed with Norman's study in [29], who found a female predominance in AR by 60-80% for all age groups, but this disagreed with Osslon et al study in [30], who observed an equal gender distribution in AR patients in all age groups.

Comparison of Immunoglobulin Level among All Groups:

Total IgE level:

The data demonstrated significant differences in the mean concentration of total serum IgE level between the two studied groups. IgE is produced by plasma cell, predominantly in lymphoid tissue adjacent to the respiratory and gastrointestinal tracts. Its concentration at birth is about 0.22 IU/ml. Adult levels are reached by the age of 10-15 years and decline after the age of 70 years. Healthy non allergic adults have an expected IgE of up to 120 IU/ml [31]. In fact, increased IgE is observed in 30% of patients with AR .Conversely, IgE may also be increased in conditions like allergic bronchopulmonary aspergillosis, immunodeficiency, lymphoma, parasitic disease, HIV, alcoholism, smoking and sever burns (IgE is present in extremely small amounts in serum, its concentration may increases in response to specific stimuli. thus in the years immediately following its identification by Ischizaka and colleagues in 1966, many authors documented extremely wide range of serum total IgE value present in different study groups.

In this study the mean of total serum IgE level in all allergic patients was significantly higher than the healthy control. These findings were also reported by Burrws et al in [32] and Bentley et al in [33] and AL-Diwan et al in [34]..

Immunoglobulin IgA, IgG, IgM Levels:

Antibodies are gamma globulin proteins (immunoglobulin) that react specifically with the antigen that stimulate their production. They makeup about 20% of the protein in the plasma [35]. Evaluation of the humeral immunity consists primarily of measuring the amount of each of the three important immunoglobulin in the serum [36]. IgA is the main immunoglobulin in secretions, it comprises 7-15% of total serum immunoglobulin, and it prevents attachment of microorganisms like bacteria and viruses to mucous membranes. IgA has a role in mucosal homeostasis and host defense. Selective IgA deficiency is associated with increase prevalence of atopy [37]. In this study, the mean concentration of serum IgA in control is significantly higher than in the AR group. IgG, is the predominant Abs in the secondary response were large amount of IgG are produced and constitutes an important defense against bacteria and viruses, it comprises 75-85% of total serum Abs. It is the only Abs that crosses the placenta and can opsonize and can activate complement [38]. In the present study, the mean concentration of serum IgG is significantly higher in AR groups, when compared to control, but IgM is the main immunoglobulin produced early in the primary response and it is the most efficient Abs in agglutination, complement fixation and in defense against bacteria and viruses [39]. A significant difference was found between the AR group and the control group. These results disagree with the findings of other authors like AL-Tawil et al [38], and Al – Taee [40], who reported that there were no significant deviations from normal controls, regarding the serum concentration of IgA, IgG and IgM in Iraqi asthmatic

and AR patients. While there is agreement to some extent with the findings of AL-Naimi [41], who reported a significant deviation from normal controls regarding IgG serum level in the AR groups. The results of this study concerning the serum concentration of IgA, IgG and IgM in the two studied groups agree with the finding of AL-Haidari [42], who reported the same results.

Complement Component Levels:

The complement system is an important soluble component of the innate Immune system; it is a series of plasma enzymes and regulatory proteins resulting in cell lyses. C3, is a key component required to switch on three different types of effector molecules during complement activation.C4, is an important molecule in the classical and Mannan binding Lectin Pathways of complement activation [43]. C3 and C4 facilitate microbe and damaged cell clearance. C3 and C4 deficiency are linked to autoimmune diseases like SLE and lead to an increase in bacterial infection particularly with encapsulated type like streptococcal pneumonia [35]..

Eosinophil Percentage:

The main function of Eosinophils is to defend against parasitic diseases and to mitigate the effects of immediate hypersensitivity reactions. For routine clinical usage, most labs give the normal peripheral blood Eosinophil percentage as 0-6%, while the normal absolute Eosinophil count is considered as 40-440 cell/cu.mm [44]. Table and figure 9, demonstrated HS differences in the mean Percentage of Eosinophil count in the peripheral blood of AR group, when compared with the control group.. These results are in agreement with the findings of Bosquet in [22],

IL4 and IL10 Levels:

Cytokines are low molecular weight soluble proteins, produced by a wide variety of hematopoitic and non hematopoitic cell types. They are critical for both normal innate and adaptive immune response. They are involved in the regulation of growth, development and activation of immune system cells, also in the mediation of inflammatory responses, many

cytokines are pleiotropic. Th2 and mast cells secret IL4 which target Bcell, leading to its proliferation, growth and isotype switching to IgG1, IgG3, IgG4 and IgE. Also it inhibits maturation along Th1 pathway. In this study there was HS difference in its mean concentration between the atopic groups and the healthy control group. These results agree with the findings reported by Hussain [45], and Alhaidari [42]. While our results disagree with Sheikh et al [46], Boccangi et al [47],

IL10 is secreted by many cells (Th2, macrophage, B cells). It targets B cell causing isotype switch to IgG1 and IgG3, it promotes maturation along Th 2 pathway and inhibits Th1 pathway maturation. So IL10 is considered an immunoregulatory cytokine [41]. In this study, there was a highly significant difference in the mean concentration of Serum IL10 between the two studied groups. The same result reported by Wong et al [48], Sanchez et al [49] and AL-haidari [42]. The possibility that large number of patients included in AR group were on immunotherapy explain the high concentration of IL10 in their sera when compared to other group. The repeated exposure to antigens as with immunotherapy will stimulate CD4 and CD25 T cells in the lymph nodes to produce IL10 [50].

Skin Test:

It is an in vivo method which identifies IgE sensitivity to common allergens. About 57% of patients with AR show a response to D1+D2 allergen followed by pollens (Bermoda 30.7%, Plantain 52%, Grasses 49%, Mugwort weed 47%), then M1 allergen 24%. These results are inconsistent with the results declared by Dakhlalla [51]. The D1+D2 are the most common indoor allergens triggering atopy and the First allergens faced in our indoor environment. They are small in size easily transported and inhaled [52].

The possibility of the presence of patients suffering from seasonal AR in the present study group makes the outdoor pollens the second offending allergens. Some of the control also shows a positive skin test to D1+D2 and M1 and this result was also

reported by AL –Taee [40] and AL –Niami[41]

Specific IgE Levels:. The mean serum concentration of IgE Abs in AR group against the pollen allergens (Grasses, Weeds, Trees) were (3.699, 2.163, 2.100 IU/ml respectively), these were higher than that for M2 and D71. These results agree with Rodrigues Et al [53], who found a high specific IgE Abs concentration in AR patient against the pollen allergens, followed by dust mite and molds allergens. While the results disagree with the finding of Dakhlalla [51] and AL-Haidari [42], who found the highest specific serum IgE Abs, were against dust mite and mold allergens.

All these results show that the outdoor allergens are causing mainly AR. This is the same result reported by Yawn et al [54] .

Conclusions:

Allergic rhinitis is the most common allergic conditions which have increased since past decades and posing a heavy burden on health care system.

The current data implies the following conclusions:

1- The majority of cases occurred between the age of 5 and 35 years, and tends to diminish gradually with age.

2- The female predominance was reported in allergic rhinitis group.

3- Significantly high levels of serum total IgE antibodies values were demonstrated in patients with AR as compared with control group.

4- Although higher levels of serum IgG and IgM were recorded in allergic rhinitis, they did not attain the significance levels.

5-the data had clearly demonstrated a significance difference in the serum concentration of complement in term of C3 and C4 components between the AR group and control group.

6. High Eosinophil percentages encountered in AR group when compared to control group with statistically significant levels.

7- Tthe outdoor allergens, mainly pollens, remain the most familiar offenders for allergic rhinitis sufferers.

8- IL4 levels in the serum of AR groups documented an elevate value. In

contrast to a highly significant elevation in IL10 levels in allergic rhinitis group versus the Control group.

Acknowledgment

1- Profound novel immunological studies are essential to clarify the role of different cytokines, such as IL5, IL6, IL8, IL13 in allergic diseases and the possibility of their role in diagnosis and treatment purposes.

2- To determine the role of allergen in allergic diseases, an aero-allergic map is needed to establish the annual prevalence of allergens in the atmosphere and their concentrations in different parts of our country.

Furthermore, it will enable the allergist to speculate appropriate panel of tests for their patients. And to set up a perfect preventive strategy to abort the drama of atopy, which is worth to be studied ever so.

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APPENDIX : 1

Questionnaire form :

Name **SEX** **Age** **Address**

Occupation

Chief complain **Duration**

History of other diseases **smoking**

Family history

Triggers

Exercise **URTI** **Pets** **Emotion**

Food **weather** **Fumes** **Others**

1.Clinical evaluation

Asthma **allergic rhinitis**

Cough **sneezing**

Dyspnoea **Rhinorrhoea**

Wheeze **Obstruction**

Others **Others**

2- Examination

Wt **Bp** **RR** **PR**

Chest exam and breathing sounds

3- Spirometry:

Actual **predicted**

FEV1

FVC

Ratio

PEFR

MMEFR

4- Skin test:

HDM **MOLD** **GRASSES** **WEEDS** **TREEES**

5- Investigations:

Total serum IgE level:

Specific serum IgE level:

Serum IL4 level:

Serum IL10 level:

Serum complement C3 level:

Serum complement C4 level:

Serum immunoglobulin levels:

IgA **IgG** **IgM**

Eosinophil %

Instruments and Tools used in the investigations:

1- ELISA system: TECAN/ MSP 9500, CARVO, SCINETFIC INSTRUMENT –USA

2- Light Microscope – Olympus – Japan

3- Ordinary Centrifuge – Nova, NF815 –Turkey.

4- Incubator – Memmert-Germany.

5- Oven – Memmert- Germany.

6- Spirometry – Erich Jaejer Jmbh- Germany.

APPENDIX : 2

Kits and Reagents used in the investigations:

Kits and reagents	company	country
Total IgE ELISA Kits	BIOMAGHREB	TUNISIA
S.IgE EIA kits	BIOMAGHREB	TUNISIA
Radial Immunodiffusion Test kits	BIOMAGHREB	TUNISIA
IL4 ELISA kits	BIOSOURCE	BELGIUM
IL10 ELISA kits	BIOSOURCE	BELGIUM
WBC SOLUTION	VACSIN AND SERA INSTETUTE	IRAQ
LISHMAN SOLUTION STAIN	BDH CHEMICALS	ENGELAND

APPENDIX 3 : ALLERGEN EXTRACTS USED IN SKIN TEST :

Code Name	Scientific Name	Common Name
D1+D2 Mite	Dermatophagoids Pteronyssiens D1 + Der. Farinae D2	Housdust
M1	ALTERNARIA	MOULD 1
M2 2	CLADOSPORIUM	MOULD
M3 3	PENCILLIUM MIXTUER (P.DIGITATUM,P.EXPANUM P.NOTATUM).	MOULD
M4 4	ASPRGILLUS NIGER	MOULD
G2 BERMODA GRASS	CYNODOM DACTYLON	
G5 MIX ernal Grass Grass Grass	ANTHEXANTHUN ODORATUN DACTYLIS GLOMERATA PHLEUM PRATENS POA PRATENS LOLIUM PERENNE	Sweet V Orchard Timothy Meadow Rye
W6 MUGWORT	ARTEMISIA VULGARY	
W10 PLANTAIN	LAMBS QUARTERS	
T1	FAGACAE TREE 1	
T3		BETULACAE