Assessment of Nasal Immunoglobulin E Level in Atopic and Non-atopic Rhinitis Patients: A Tool for Diagnosis of Local Allergic Rhinitis

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Many cases of AR can be miss-diagnosed due to deficiency in the conventional laboratory tools. Detection of local Ig E immune response and allergy associated genes may aid in diagnosis of these cases. The local immune response and the allergy associated genes of these suspected cases must be evaluated as they may help in their characterization. This study was conducted on 129 patients with chronic rhinitis to determine the frequency of LAR, and analyze the association of IgE receptor (FcεR1β) gene polymorphism with LAR. All participants were subjected to clinical questionnaire, skin prick test, specific IgE measurement in serum and nasal secretions and analysis of FcεR1β gene polymorphism. LAR constituted 24.8 % of total rhinitis cases and 44.4% of non-allergic cases. Cockroach was the main sensitizing agent in local allergic rhinitis in comparison with allergic cases (OR =0.11; 95% CI= 0.04-0.34; *P*<0.001). In LAR, nasal specific Ig E was significantly lower than that in AR patients (*P* < 0.001). FcεR1β genotype TT was more frequently expressed in LAR and AR than non-allergic rhinitis (NAR) (*P*< 0.001). It is concluded that LAR is an emerging allergic condition that could be diagnosed by nasal specific IgE, and that FcεR1β polymorphism is one of the genetic factors associated with AR and LAR.

Rhinitis is an upper respiratory disease that affects 20- 40% of the population (Cho, 2013). In general, Rhinitis as inflammatory condition is classified into AR and NAR based on the clinical history, allergic skin test, and serum-specific Ig E to the expected aeroallergens (Rondon *et al*., 2010). NAR is mainly idiopathic rhinitis and is usually precipitated by different causes as hormones, medication with nonsteroidal anti-inflammatory drug and dysfunction of autonomous nervous system (Toppila-Salmi *et al*., 2015).

Many idiopathic rhinitis (IR) patients who were previously diagnosed as non-allergic proved to have LAR, or entopy (Rondon *et al*., 2010). The concept of local allergy has been proposed as it is now generally accepted that mucosal local Ig E play an important role in the expression of atopic allergic disease. In these cases, specific Ig E may be presents in serum at levels below the threshold of detection or the production of specific Ig E may occurs only in the nasal mucosa (Sakaida *et al*., 2014).

LAR is characterized by suspected clinical history, negative skin prick test, absent serum specific Ig E, positive response to nasal provocation test, and elevated nasal specific IgE and inflammatory mediators such as tryptase and eosinophilic cationic peptide in the nasal fluids (Cho, 2013).

AR is an IgE-mediated airway disease where allergen exposure triggers the immune response (Melchiotti *et al.*, 2014) with a prevalence of 10–20 % in the worldwide population (Toppila-Salmi *et al*., 2015). Diagnosis of AR is based on conventional methods as clinical history questionnaire, skin prick test (SPT), and measurement of serum specific immunoglobulin E (sIgE) to environmental aeroallergens (Sakaida *et al*., 2014).

Nasal specific Ig E plays an important role in respiratory mucosa during allergic airway inflammation (Joyce, 2013). Local production

of specific Ig E has been demonstrated by detection of Ig E-positive mast cells, plasma cells, and B cell in inferior turbinate tissue from patients with AR. In addition, specific IgE was measured by enzyme allergosorbent test (Schryver *et al*., 2015).

The immune-pathogenesis of both LAR and AR depends on Immunoglobulin E-mediated activation of mast cells and basophils through the high-affinity IgE receptor (Fc ε RI) (Shi & Pamer, 2011; Melchiotti *et al.*, 2014). In addition, previous studies reported increase in the expression level of mRNA for the heavy chain of Ig E in nasal and bronchial mucosa (Cameron *et al,* 2003; Coker *et al.,* 2003).

The development of AR and LAR disease may be the result of the interaction of environmental factors and different genetic polymorphism of allergy associated genes on chromosome 11q13 (Misra *et al*., 2015). As a result, a positive significant association of genes polymorphism of leukotriene and IL-4, IL-13, polymorphisms and allergic rhinitis was previously described (Korzycka-Zaborowska *et al*., 2014). One of the important genes involved in the development of AR is FcεR1, also known as high-affinity IgE receptor for the Fc region of IgE and consisting of one α (FcεR1α-antibody binding site), one β (FcεR1β-which magnify the signal), and two disulfide bridge connected γ chains (FcεRIγ-the site where the intracelluar signal initiates) (Liao *et al.,* 2015). Mutation in the promoter region of FcεR1 β was previously identified and proved to be associated with other allergic condition as atopic bronchial asthma and chronic spontaneous urticarial (Korzycka-Zaborowska *et al*., 2014, Rasool *et al*., 2015)

This study was designed to evaluate the role of nasal specific Ig E measurement in diagnosis of LAR patients, so affecting the management strategy of these cases. Also, to analyse the association of FcεR1β gene polymorphism with LAR as one of the important allergy associated genes.

Patients and Methods

Study Design

This study is a cohort study that was conducted during the period from August 2014 to August 2015. The study was approved by the ethics committee of the Faculty of Medicine, Zagazig University.

Study Patients

For a population of 4253 adult rhinitis patients, with an estimated prevalence of 20% and 5% precision, the sample size required was 129 patients. They were selected using simple random sampling from a population evaluated for the first time for rhinitis in Allergy and Immunology unit and otorhinolaryngology department, Faculty of medicine; Zagazig University, Egypt. Written informed consents were obtained from all patients enrolled in the study.

The inclusion criteria; All patients included in the study had suspected clinical diagnosis of chronic rhinitis that was made by the history of paroxysms of sneezing, watery rhinorrhea, and nasal blockage with or without itching of the eyes, nose, and palate, postnasal drip, cough, irritability, and/or fatigue for more than 6 months (Wallace *et al,* 2008).

Exclusion criteria; Patients were excluded from the study if they had infective rhino-sinusitis, generalized skin allergy, severe dermatographism, severe asthma, or Patients with autoimmune diseases. Also, pregnant and lactating female were excluded. The patients were instructed to avoid systemic corticosteroids (5 days), antihistamines (first generation 3 days, second generation 5 days) and mast cell stabilizers (2 days)

Allergic Rhinitis Clinical Scoring

All participants were subjected to a clinical questionnaire developed by Ciprandi *et al*. (2004) covering the detailed symptoms. Each nasal symptoms including nasal congestion, sneezing, rhinorrhea and itching were scored subjectively as follows: 0, no symptoms; 1, symptom positive but did not interferes with daily activities or night time sleep; 2, symptoms were troubling but did not affect daily activities or night sleep; 3, symptoms disturbed daily activities or sleep. Scores for each symptoms was recorded and sum of the scores were calculated (0–12) (mild:1-4; moderate: 5-8; sever: 9-12) (Ciprandi *et al*., 2004).

Skin prick test (SPT)

A routine skin prick test was performed by aeroallergen panel using kits containing 17 common inhalant allergens (*D. pteronyssinus, D. farinae,* grass mix, Birch pollen, sunflower seeds, *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Alternaria species,* cat epithelium, dog epithelium, feather mix, cockroach, latex, wasp venom, bee venom) supplied in 5 ml vials (Omega Laboratory) (Montreal, Canada), negative control (saline 0.9%) and positive control (histamine 1 mg/ml).

A drop of solution of each test allergen, and controls was placed on the flexor surface of the forearm, and skin was pricked at drop midpoint. The results of SPT were read at 15 min and the reaction was considered positive if the wheal area with a diameter of ≥ 3 mm (Rondo *et al.,* 2012).

Measurement of serum specific IgE

Serum was separated from the blood samples (5 mL) at the time of diagnosis. Immune blot assay was used for the quantitative determination of specific IgE in human serum against circulating aeroallergen (same allergens used in the skin prick tests and anti-goat Ig G as positive control) with Allergy Screen Panel 2A EGY (MEDIWISS analytic GmbH, Underinger, Germany) according to the manufacturer's instructions. Briefly, serum was pipetted into a trough of nitrocellulose membrane coated with specific allergens, followed by addition of biotin coupled anti-human IgE antibody, streptavidin conjugated with alkaline phosphatase and substrate; in order. The colour reaction of each precipitates line on the test trough indicated serum specific antibody content. Serum specific IgE was analyzed by Rapid Reader (Improvio, Germany) using the densitometer curve of the membrane and concentration data for each intensity. The result was expressed in IU/ml reflecting allergen specific Ig E content of serum according to the manufacture instructions (table 1). The test was valid if positive control Ig E> 3.5 IU/ml

Table 1. Relationship between the classes found and allergen specific Ig E content of serum

Ig E level (IU/ml)	Allergen specific Ig E content
<0.35	Non or hardly found
0.35-0.69	Low
0.7-3.4	Increased
3.5-17.4	Significantly increased
17.5-49.9	High
50-100	Very high
>100	Extremely high

Measurement of Nasal Specific IgE

Nasal secretions were collected by cotton-wool sticks then placed in a 2 mL Eppendorf tube containing 1 mL of transfer medium (phosphate- buffered saline with gentamycin 50 µg/mL, penicillin G 340 U/mL, and fungizone 500 µg/mL) for 30 min to allow for mediators to diffuse into the medium, and were then stored at 4 °C for a maximum of 2 h. Nasal secretion samples were centrifuged at 1000x*g* for 10 minutes to separate cellular components (Perić *et al*., 2011). Supernatants were stored at -70 °C, until specific IgE level was measured as described in the serum Ig E Assay.

PCR-Restriction Fragment length polymorphism Analysis of FcεR1 B gene

PCR amplification of FcεR1 B promoter at 109: Genomic DNA from the blood was extracted using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Waltham, USA) according to manufacture instruction. The FcεR1 B promoter at 109 bp region was first amplified then subjected to restriction digestion and analysis. PCR amplification was carried out using Forward primers of 5 -GTG GGG ACA ATT CCA GAA GA-3 (sense) and reverse primer 5 -CCG AGC TGT CCA GGA ATA AA-3 (antisense) (Rasool *et al*., 2015).

Amplification of β-actin gene was performed in each PCR reaction as an internal positive control using Forward primers of GCA CCA CAC CTT CTA CAA TG and reverse primer TGC TTG CTG ATC CAC ATC TG (Glare *et al*., 2002). Non template negative control (water instead of DNA extract) was also included.

For each reaction all primers were added to PCR Master Mix beads (Bioron, Germany) containing 1x PCR buffer (10mM Tris-HCL, 50 mM KCL,1.5 mMMgCl2, 0.2 mM of each dNTP, 5% glycerol, Xylene Cyanol FF, 50 units/ml Taq DNA polymerase) and nuclease-free water was added to final volume (20 µl). Amplification was performed in Biometra thermocycler (T-Gradient Biometra, Germany) and Cycling conditions were 1 cycle at 95°C for 5 min, 40 cycles at 95°C for 30s, annealing at 60 °C for 45s, extension at 72°C for 1 min, and a final extension at 72 C for 10 min (Rasool *et al.,* 2015).

PCR amplified products were detected by 2% agarose gel electrophoresis. Ten µl of each amplified DNA & 100 bp ladder; molecular weight marker (GeneOn, Germany) were loaded in agarose gel containing 0.3 mg/ml of ethidium bromide. The bands were visualized using UV transilluminator (312 nm).

DNA bands were analyzed after confirming the presence of positive control band (838 bp), and absence of bands in negative control sample as compared to the DNA ladder. PCR product of 382 bp length indicating positive amplification of FcεR1B promoter at 109 bp region.

Restriction digestion of FcεR1 B promoter amplicon: The PCR product which was 382 base pairs was digested by Tru9I restriction endonuclease enzyme (New England Biolabs, UK). Briefly, 10µl PCR products were incubated with 1µl of Tru91 containing 5 units of the enzyme at 67 °C for 12 hours. Digested products were run on 2.5% agarose in 1× TAE buffer (promega, USA) with 100 bp ladder; molecular weight marker (GeneOn, Germany). The electrophoresis was carried out at 70 V and 230 mA for 45 minutes.

The digestion fragments of 221 and 161 bp were corresponding to the C allele. Whereas fragments of 182, 161, and 39 bp indicated T allele (Rasool *et al.,* 2015). So, CC homozygous wild genotype was expressed by DNA bands of 221, 161 bp, TT homozygous variant genotype by DNA bands of 182, 161, 39 bp & CT as heterozygous variant by 221, 182, 161, 39 bp.

Statistical Analysis

Qualitative variables were expressed as percentages and the association between the categorical variables was carried out by Chi-square, and Fisher exact test. Quantitative variables were summarized as mean and standard deviation (SD). Statistical analysis of quantitative variables was performed using the *t* test for continuous variable. Odds ratios (ORs) and 95% confidence intervals (CIs) were used for probability calculations. Values of $P<0.05$ were considered significant. All statistical analysis was carried out using SPSS 13 (USA).

Results

Patients grouping

129 patients with clinical manifestations of chronic rhinitis were studied. A total of 57 (44%) patients showed positive results for SPT and serum specific IgE and therefore classified as AR. In addition, 72 (56%) patients were not positive by both tests. Within those 72 patients, 32 (44.4%) were positive for nasal specific Ig E only and grouped as LAR. The remaining 40 patients out of 72 were classified NAR.

Demographic data of the patient and triggering factors

It was found that patients with LAR were younger than allergic and NAR patients ($P<0.001$). A higher significant association with female gender was found in LAR (62.5%) compared to that in NAR (20%) (OR = 0.15; 95% CI 0.04-0.48; $P=0.001$) and also between female gender in LAR compared with AR (50.9%) (OR= 0.24; 95% CI 0.08-0.67, $P = 0.002$).

Irritant odours and dust were the main triggering factors reported in LAR (59, 53% respectively). However, in the AR patients, smoking was the most frequently triggering agent (49%). Family history of atopy (FHA) was significantly associated with local allergic rhinitis and allergic rhinitis compared to NAR (OR= 12.3; 95% CI 2.79-62.53; $P<0.001$) and (OR =26.72; 95% CI, 6.61-125.88, $P<0.001$), respectively.

There was significant association between bronchial asthma and both local allergic rhinitis and allergic rhinitis compared to asthma association with NAR (OR 5.4; 95% CI 1.36-23.2; $P=0.005$) and (OR 4.5; 95% CI 1.27-17.42; $P= 0.007$). Data were presented in table 2.

Table 2. Demographic data of the allergic rhinitis, local allergic rhinitis and non allergic rhinitis patients

Variable		Allergic No.=57		Local allergic No.=32		Non-allergic No.=40		*P value
		Mean ±SD		Mean ±SD		Mean ±SD		
Age		25.2±2.7		19±6.5		32.9±2		< 0.001
		No.	%	No.	%	No.	%	*P* value
Sex	Male	28	49.1	12	37.5	32	80	< 0.001
	Female	29	50.9	20	62.5	8	20	
Family history		39	68.5	16	50	3	7	< 0.001
Smoke		28	49	6	18.7	2	5	< 0.001
Dust trigger		10	17.5	17	53	1	2.5	< 0.001
Irritant odour		8	14	19	59.3	0	0	< 0.001
Aspirin		0	0	0	0	9	22.4	< 0.001
Bronchial asthma		19	33.3	12	37.5	4	10	0.012
Conjunctivitis		3	5.2	5	15.6	0	0	0.022

**P* value<0.05 is significant

Clinical characterization of the patients

Patients with LAR mainly presented with perennial symptoms (OR 2.1; 95% CI 0.76-5.3; *P*>0.1) more frequently than AR. The majority of LAR and AR patients had moderate to severe nasal symptoms (85% and 89%, respectively). In LAR, sneezing was the most frequently reported symptom (81% of LAR cases). However, rhinorrhea was the most frequent in patients with AR (63%). Although, nasal symptoms in LAR and AR presented with different frequency, there is no significant difference in their clinical manifestations (*P*>0.1). However, significant differences were detected between LAR and NAR (*P*<0.001).

The aeroallergens sensitization by Skin pricks test (SPT)

With a panel of 17 aeroallergens, only nine allergens gave positive skin prick test. Out of 57 patients who showed positive results of SPT, 15 (26.3 %) patients showed positive result to only one allergen, the remaining cases (73.7%) were polysensitized and distributed as the following: 29 (50.8%) patients showed positive result to two allergens , 7 (12.2%) patients showed positive result to three allergens, and 6 (10.5%) patients showed positive result to four allergens.

The level and pattern of Serum and Nasal specific Ig E

In AR patients, serum and nasal specific Ig E showed positive results of the same nine aeroallergens and same aeroallergens distribution of the skin prick test results (Table 3). There was a strong significant correlation between serum specific Ig E and specific Ig E in nasal secretions of AR patients (r; 0.8, $P < 0.001$).

According to results of nasal specific Ig E measurement in patients with LAR, and by comparing its result with AR patients, (55%) patients with LAR rhinitis were polysensitized in contrast to AR patient who expressed mostly polysensitization pattern to the tested aeroallergens. Cockroach was the main sensitizing aeroallergen in LAR (OR =0.11;

95% CI 0.04-0.34; *P*<0.001), and in AR patients, *Dermatophagoides fabrinae* was the most frequently sensitizing aeroallergen (OR =8.11; 95% CI 2.02-37.8; *P*<0.001). Also, sensitization to *Dermatophagoides pteronyssinus* was detected more frequently in LAR than AR patients (OR =0.28; 95% CI0.1-0.76; *P*=<0.005) (Table 3).

Table 3. Aero-Allergen reactivity distribution among allergic rhinitis and local allergic rhinitis patients as determined by measuring nasal specific Ig E

Allergen	Allergic rhinitis No.=57		Local allergic No.=32		*P* value
	No.	%	No.	%	
Candida albicans	6	10.5	0	0	NS
Alternaria	13	22.8	5	15.6	0.418
Aspergillus fumigatus	18	31.5	4	12.5	0.045
Cat epithelium	11	19.2	0	0	0.007
Cockroach	9	15.7	20	62.5	< 0.001
Feather mix	2	3.5	3	9.3	NS
Mixed grasses	12	21.05	5	15.6	NS
Derm. Fabrinae	26	45.6	3	9.3	< 0.001
Derm. Pteronyssinus	15	26.3	18	56.2	0.005

**P* value>0.05 is not significant (NS)

In LAR, the mean nasal specific Ig E for Cockroach was the highest (12.04 IU/ml) among the tested nasal specific Ig E. In general, the mean nasal specific Ig E level for individual allergens in local AR was significantly lower than those in allergic rhinitis group (*P*<0.001). The results are presented in table 4.

Table 4. Comparison between nasal specific IgE level among allergic rhinitis and local allergic rhinitis patients

Allergen	Allergic Ig E* Mean ±SD (IU/ml)	Local Allergic Ig E* Mean ±SD (IU/ml)	*P* value
Candida	15.02±3.01	-	-
Alternaria	16.1±3.2	4.7±3	< 0.001
Aspergillus fumigatus	29.1±5.4	9.3±5.5	< 0.001
Cat epithelium	10±2.6	-	-
Cockroach	33.7±4.9	12.04±5	< 0.001
Feather mix	15.8±1.8	3.2±1.1	< 0.001
Mixed grasses	23.6±3.3	8.1±2.8	< 0.001
Derm. Fabrinae	61.418.7	5.3±2.2	< 0.001
Derm. Pteronyssinus	21.1±3.5	10.9±1.7	< 0.001

*Nasal Ig E Assay by ELISA with cut off≥ 0.35 IU/ml. *P* value<0.05 is significant.

Genotypes characterization of FcεR1 B in LAR, AR, and NAR groups

Patients with allergic and LAR exhibited T allele more frequently than NAR cases at frequency of 0.79 and 0.78; respectively. However, NAR patient were mainly associated with C allele (0.75). TT genotypes of FcεR1 B was the predominant type in both local allergic and allergic rhinitis compared to NAR (OR=6.5, 95% CI=2.08-21.57; *P*<0.001; and 5.9(2.17-16.5); *P* <0.001 respectively).

In NAR, wild genotype (CC) was more frequently reported than that in LAR and AR patients (OR=0.04; 95% CI=0.01-0.18, *P* <0.001, and 0.01 (0.0-0.07), *P*<0.001; respectively) (Figure 1). (Tables 5, 6).

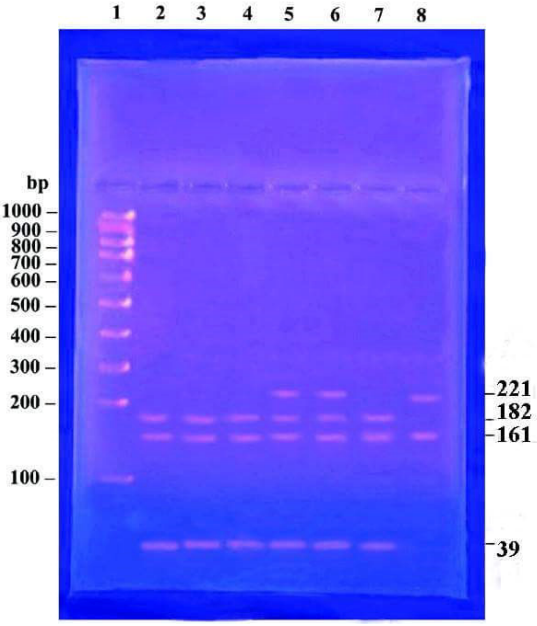

Figure 1. UV transilluminated agarose gel of FcεR1β gene promotor by RFLP in patients with local allergic rhinitis. Lane 1 represents 100 bp DNA Step Ladder which consists of 10 DNA fragments ranging from 100bp to 1000 bp in exactly 100 bp increments. Lanes No. 2 , 3, 4, and 7 show three DNA bands at 39, 161, and182 bp represent TT allele. Lanes No. 5 and 6 show four DNA bands at 39, 161, 182, and 221bp represent CT allele. Lanes No. 8 shows two DNA bands at 161, and 221bp represent CC allele

Table 5. Genotypes distribution of FcεR1β promoter region among allergic, and NAR patients

Allelic Genotypes	AR(57)	NAR (40)	OR	*P* value
	No. (%)	No. (%)	(95% CI)	
TT Allele	36 (63%)	9(22.5%)	5.9(2.17-16.5)	<0.001
CT Allele	19 (33.3%)	2(5%)	9.5(1.92-63.57)	<0.001
CC Allele	2(3.5%)	29(72%)	0.01(0.0-0.07)	<0.001

P value<0.05 is significant.

Table 6. Genotypes distribution of FcεR1β promoter region among local allergic, and NAR patients

Allelic Genotypes	LAR (32)	NAR (40)	OR (95% CI)	*P*-value
	No. (%)	No. (%)		
TT Allele	21 (65.6%)	9 (22.5%)	6.58(2.08-21.57)	<0.001
CT Allele	8 (25%)	2 (5%)	6.33(1.09-47.51)	0.014
CC Allele	3(9.3%)	29 (72.5%)	0.04(0.01-0.18)	<0.001

P value<0.05 is significant.

Discussion

Atopy is a term used to describe IgE-mediated allergic disease in individuals with positive serum specific Ig E. It has become evident that IgE-mediated disease can occur in non-atopic patients due to mucosal local Ig E (entopy) (Powe *et al*, 2010).

In the present study, similar to the studies of Carney *et al*. (2002), and Rondo *et al*. (2012), the LAR cases constituted 24.8% of rhinitis and 44.4% of the cases that gave negative results for skin prick test and serum specific IgE. Similarly Lo Pez *et al* (2010) demonstrated that 62.5% of patients previously diagnosed as NAR or idiopathic rhinitis actually has LAR or entopy.

Analysis of the result of current work revealed that 44% of the clinically suggested cases diagnosed by skin prick test as AR. However, in the studies done by Rondo *et al* (2012), and Ashour *et al* (2014), higher percentages of the patients were proven to have AR by SPT (63.1% and 73.2%, respectively). This difference may be due to different numbers and types of aeroallergens used in SPT panel.

In consistence with results obtained by Rondo *et al* (2012), LAR mainly presented in patients significantly younger than patients with allergic and non-allergic rhinitis ($P< 0.001$). However, Rodrigues *et al* (2015) found that the mean age of LAR was 35.1 years old. Female gender was found to be more frequently associated with LAR compared with NAR (OR = 0.15; 95% CI 0.04-0.48, $P= 0.001$). This result was different from that obtained by Moitra *et al* (2014) who found that M/F ratio was 62/40 = 1/ 1.6. This study result indicates that LAR may initiate in childhood of female mainly, although this analysis requires further prospective evaluation.

In this study, family history of atopy (FHA) was significantly associated with AR (68%) and LAR (50%) compared to NAR (7%). This may indicated the role of the genetic factors in development of LAR in addition to their role in AR (Fokkens *et al*, 2005).

The triggering factors presented more frequently in the LAR patients than AR or NAR ($P= 0.001$). Irritant odours and dust were the main triggering factors reported in LAR (59%, 53% respectively). However, in the AR patients, smoking was the most frequently triggering agent (49%). In contrast, Rondo *et al* (2012) revealed that House dust was the main triggering factor reported in LAR (47.3%) and AR (38.1%). This contrast could be due to environmental exposure to different triggering agents.

The present study revealed that 59% of patients with LAR expressed perennial symptoms which was more frequently than AR patients (42%). However, Wedback *et al* (2005) and Rondon *et al* (2008) demonstrated that the majority of patients with LAR have seasonal symptoms depending on the multiple host demographic data.

In association with the study of Molgaard *et al* (2007) and Cho, (2013), the current study, reported that many patients with LAR have clinical criteria indicative of AR, as moderate to severe symptoms of sneezing, congestion, rhinorrhea, and a high rate of comorbidity. The same finding was obtained by Rondo *et al* (2012) who demonstrated that the majority of those patients suffered from rhinorrhea and nasal obstruction, and commonly associated with conjunctivitis. All these results indicate the allergic nature and pathogenesis of LAR.

In this study, all patients were subjected to measurement of nasal specific IgE level as the current routine diagnostic tools in rhinitis may not be sufficient to differentiate between non-

allergic and LAR, as local IgE normally is not measured.

The result obtained by skin prick test regarding the number of allergens to which the patients were sensitized, 15 (26.3 %) patients were monosensitized, and 74% were polysensitized. These results were the same as those obtained by Ashour *et al*. (2014) who reported that 14.6% of these patients showed positive result to only one allergen (monosensitized), whereas the remaining patients were polysensitized.

In association with the result obtained by Rodrigues *et al.* (2015), nasal specific IgE gave positive results for same allergens as serum-specific IgE in AR. The result of this study and in association with the study of Sakaida *et al.* (2014), demonstrated that nasal specific Ig E presented in 100% of AR. However, Rodrigues *et al.* (2015) reported that nasal specific IgE presented in 77% of AR.

Analysis of this study results revealed significant strong correlation between the level of serum specific IgE and nasal specific IgE in AR patients (r= 0.8; *P*< 0.01). Also, this finding was demonstrated by Sakaida *et al.* (2014) (*r*= 0.79, *P*< 0.001). In addition, Fuiano *et al.* (2010) revealed a significant association between the results of the 2 tests but only for house dust mite, grass pollen, and olive pollen and such association was not observed for *Alternaria.* However, Yoshida *et al* (2005) found that levels of specific IgE measured in nasal secretions exceed levels measured in serum of patients diagnosed with AR to cedar pollen. On the other hand, Rodrigues *et al*. (2015) revealed that the mean serum specific IgE for *Derm. ptyrossinus* and *Derm. flarinaea* was higher than those in nasal lavage. This difference in nasal and serum Ig E levels measured in different studies was expected due to the variations of clinical manifestation of AR in individuals sensitized to aeroallergen.

In this study, 55% of patients with local allergic rhinitis were poly-sensitized. However Rondo *et al. (*2012) found that mono-sensitization was the predominant pattern in LAR (62.7%). This different pattern may be influenced by geographical area of the study and presence of different triggers.

In the current study, Dermatophagoides fabrinae was the main sensitizing aeroallergen in allergic rhinitis (45.6%) (*P*<0.001). However, Ashour *et al* (2014) study demonstrated that House dust was the most common allergens in allergic rhinitis (46.3%).

The assay of nasal Ig E in LAR patients in this study, and in comparison with AR, revealed that Cockroach was the main sensitizing aeroallergen in LAR (62.5%). In contrast, Fuiano *et al* (2012) suggests that sensitization to *Alternaria* was frequently expressed by exclusive production of specific IgE in the nasal mucosa. Also, Rondo *et al* (2012) results was different as it demonstrated that Dermatophagoides pteronyssinus was the main sensitizing aeroallergen, in both LAR (60%) and AR (54%), with no significant differences. In addition, Rondon *et al.* (2009) demonstrated that house dust mites was the main sensitizing aeroallergen both in LAR and AR (60 and 54%, respectively).

Concerning sensitization to fungal allergens, this study revealed that *Aspergillus fumigates* was the most prevalent in 31.5% of allergic rhinitis. In agreement with Rondo *et al.* (2012) *Alternaria spp.* was significantly more frequent in LAR. Also, Ashour *et al* (2014) demonstrated that the most common fungal allergens were *Aspergillus fumigates* (19.5%), and *Rhizopus spp.* (19.5%).

Sensitization frequencies to different aero-allergens vary depending on the geographic region as measured in population-based manner (Ashour *et al*., 2014). Difference in exposure rates to various environmental allergens, genetic differences or alterations in the allergenicity of these allergens that

possibly occur over time can explain some of these variations (Bryce *et al*., 2010).

In this study, the level of nasal specific Ig E for different allergens tested in local allergic rhinitis patients were significantly lower than those quantified in allergic rhinitis patients. Also, Schryver *et al.* (2015) reported that the patients who were only positive to nasal specific IgE, the mean nasal specific IgE for all tested allergens was significantly higher in AR than local AR. The highest among the tested specific Ig E was for *Derm. Fabrinae*.

In agreement with Hizawa *et al.* (2000), the polymorphic variant FcεR1 B was observed more frequently in AR than non-allergic patients. Genotype TT was strongly associated with AR and LAR compared to that genotype in nonallergic rhinitis patients Also, Young *et al.* (1992) and Korzycka-Zaborowska *et al.* (2014) reported the association of FcεR1 B polymorphism with allergic rhinitis. These findings may be in association with the environmental factors precipitate to the development and progress the atopic diseases. The effect of this polymorphism may be explained by increasing the signal transmission of this chain of FcεR1. In addition, these SNPs may be used as a disease marker for IgE-mediated allergic rhinitis even in the absence of serum specific Ig E.

However, Coker *et al.* (2003) stated the link between variations in FcεR1α gene and allergic diseases such as atopic dermatitis and asthma. In addition, Liao *et al*. (2015), results demonstrated that SNPs in the FcεR1α promoter region were significantly higher in allergic patients than in non-allergic controls. This polymorphism may be associated with increased Ig E production, so expression of allergic manifestations (Sallmann *et al*., 2011).

This study concluded that local allergic rhinitis constitutes 44% of rhinitis patients who are initially diagnosed as non-allergic rhinitis. Also, nasal specific IgE is an important marker of AR and it could be an alternative tool to skin prick test and serum specific Ig E. In addition, polymorphism of FcεR1β gene is strongly associated with IgE based AR and LAR even in the absence of serum specific Ig E. However, we recommend further studies for standardization of nasal sample collection, measurement of nasal specific Ig E and the genetic factors specially FcεR1β gene expression determining the local allergic rhinitis.

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