Egyptian Journal of Medical Microbiology Volume 30 / No.3 / July 2021 111-117 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Evaluation of the Role of Immunotherapy in Allergic Fungal Rhinosinusitis

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Key words:
Allergic, Fungal, Rhinosinusitis, Immunotherapy

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ABSTRACT

Background: *Allergic fungal rhinosinusitis (AFRS) is a distinct form of chronic rhinosinusitis. Type I hypersensitivity to inhaled fungal allergens has been implicated as key pathogenesis. Immunotherapy as one of the therapeutic options is still controversial.* ***Objective:*** *to evaluate the role of immunotherapy in the management of AFRS patients not responding to medical treatment 3 months following endoscopic surgery.* ***Methodology:*** *A total of 35 patients diagnosed as resistant AFRS were included in this prospective study. Patients were diagnosed following clinical, radiological, and endoscopic examination of nose and paranasal sinuses. Specimens were collected during endoscopy and subjected to microscopic examination and fungal culture. Skin prick test and assessment of total IgE level were performed for all patients. Sublingual immunotherapy (SLIT) was initiated for all patients for 6 months. Clinical efficacy of SLIT was assessed using the 20-item sino-nasal outcome test (SNOT-20) score.* ***Results:*** *Aspergillus spp. was the most frequent fungus isolated (74.3%) from patients. All patients were sensitized to mixed fungi. Elevated total IgE (> 100 IU/mL) was found in all patients with 40% of them had peripheral eosinophilia. A significant improvement ($p < 0.001$) was recorded in the SNOT-20 score of examined patients recording a mean of 1.2 ± 0.3, 6 months after SLIT compared to 1.93 ± 0.44 before immunotherapy initiation.* ***Conclusion:*** *Immunotherapy appears to be a good adjunctive therapy for the management of resistant cases of AFRS.*

INTRODUCTION

Allergic fungal rhinosinusitis (AFRS) is a distinct form of chronic hypertrophic rhinosinusitis with a percentage of 6-9% among rhinosinusitis cases requiring surgery [1]. Higher percentages have been recorded particularly in countries with warm moist climate [2, 3].

Although the exact pathogenesis remains debated, it is believed that it results from hyperreactivity to inhaled environmental fungal allergens in atopic individuals [4]. This induces mucosal inflammation with eosinophilic mucin formation that further blocks the normal drainage pathways. The persistence of extramucosal fungal hyphae in sinuses eventually results in destructive effects, like bony erosions, due to activation of local immune mechanisms, though no fungal invasion occurs[5].

Aspergillus spp. was thought to be the main causative agent; however, other dematiaceous fungi like *Bipolaris*, *Curvularis*, and *Alternaria* spp. have been incriminated [6].

In 1994, Bent and Kuhn described five major in addition to six minor criteria to diagnose AFRS. They stated that all major criteria were necessary to define AFRS, while minor criteria were supporting features. Major criteria included; nasal polyposis, characteristic CT or MRI findings that confirm chronic rhinosinusitis, evidence of type I IgE-mediated hypersensitivity, presence of allergic eosinophilic mucous that contains fungal elements without tissue invasion, and positive fungal smear. Minor criteria included; asthma, unilateral predominance, radiographic bone erosion, fungal culture, Charcot-Leyden crystals, and serum eosinophilia [7].

In spite of the controversy regarding the diagnostic criteria [8], the presence of allergic mucin in sinuses that appears as thick yellow brown or dark green to black with the consistency of peanut-butter during endoscopy, the presence of CT or MRI evidence of sinusitis as expansion of paranasal sinuses, bony erosions and heterogeneous signal intensities, in addition to the presence of fungal elements within the mucin are considered essential for diagnosis by most authors [9, 10].

The disease typically affects young immunocompetent adults with a mean age between 21 and 33 years. High male to female ratio was recorded [11]. Cases are presented mainly with nasal congestion,

difficulty in breathing, sneezing, fatigue, difficulty in concentration, and poor performance [12].

The management of AFRS stays mainly on surgical debridement of sinuses that is followed by long term medications that suppress the inflammatory process e.g. steroids whether oral or nasal, antihistamines, and biological therapies that target type 2 inflammatory mediators like IgE, IL-4, IL-5 and IL-13 [13, 14]. In spite of this, high recurrence rate has been recorded [2]. The results of using antifungal therapy to prevent recurrences following surgery are inconclusive and controversial [15, 16].

Immunotherapy may otherwise alter the natural history of the disease as it is believed to drive TH1 response instead of TH2. This in turn, could down regulate the response to IL-4 and decrease the inflammatory process of the host. However, the clinical efficacy of immunotherapy in the management of AFRS is still debated [17].

This study aims to evaluate the role of immunotherapy in the management of AFRS patients not responding to medical treatment 3 months following endoscopic surgical debridement.

METHODOLOGY

A prospective study was conducted in Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University and Otorhinolaryngology Department, Faculty of Medicine, Zagazig University Hospitals over 3 months (January 2020 - March 2020) to collect eligible patients. Patients were then followed up for another 6 months to one year (June 2020 – December 2020).

This study was approved by the institutional review board (IRB) – Faculty of Medicine, Zagazig University. Written informed consent was obtained from all participants before enrolling in the study. We followed the ethical principles of the Declaration of Helsinki during the preparation of this study.

Participants:

A total of 35 adult patients (≥ 18 years old) diagnosed as having AFRS were included in this study. Along a period of three months, patients presented to the Otorhinolaryngology Out-patient Clinic, Zagazig University Hospitals and suffering from chronic sinusitis were evaluated and those fulfilling the criteria of AFRS were recruited.

Diagnosis of patients as AFRS was based upon a detailed history and thorough clinical examination, radiological findings in CT scan of nose and paranasal sinuses (axial and coronal view), nasal endoscopy to observe any shiny allergic mucin and nasal polyps and laboratory workup consisting of positive fungal culture and elevated total Ig-E (> 100 IU/mL). All recruited patients underwent surgical debridement of nasal polyps during endoscopy. Following surgical removal, patients with symptoms persisting for more than 3 months with no response to medication, and presenting with clinical symptoms of itchy nose, sneezing, rhinorrhea, and sleep disturbance, were included in the study. Patients with symptoms for less than 3 months or with immunosuppressive diseases were excluded from the study.

Collection of samples:

Allergic mucin and tissue biopsy from nasal polyps were collected during nasal endoscopy. Nasal biopsy specimens were cut into small pieces using sterile scissors and preserved in normal saline for microscopy and culture and formalin for histopathologic examination.

Venous blood samples were withdrawn from each participant to evaluate the absolute eosinophilic count and assess serum total Ig-E level. Eosinophilic count higher than 500 cells / mL was considered as serum eosinophilia. Ig-E levels > 100 IU/mL were considered raised [18].

Microscopy and fungal culture:

A portion of each nasal sample was examined using light microscopy after digestion with 10% potassium hydroxide (KOH). The remaining portions of the nasal samples were cultured on Sabouraud's dextrose agar (SDA) with chloramphenicol and gentamicin. Plates were incubated at 22°C and 37°C for 4 weeks. Fungal isolates were identified by the colonial morphology and microscopic examination of lactophenol cotton blue (LPCB) preparations as standard procedures [19].

Assessment of clinical symptoms (SNOT-20):

Once the diagnosis was confirmed, the clinical symptoms of AFRS patients were evaluated using the 20-item Sino-nasal outcome test (SNOT-20) score [20]. The score of each item ranged from 0 to 5, whereas 0=no problem, 1=very mild, 2=mild, 3=moderate, 4=sever, and 5=problem is bad as it can be. The total score was divided by 20, so that the possible range of SNOT-20 scores is 0 to 5, with a higher score indicating a greater manifestation.

Skin prick test:

Skin prick test was performed on the forearm of all recruited patients for allergens based on local climatic conditions. Drugs that affect the response of the skin prick test as antihistamines and antidepressants were discontinued for a maximum period of 3 weeks before performing the skin prick test. Different extracted allergens were used from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. The allergens tested were; house dust mites, date palm pollens, mixed fungi, nicotine smoke, cotton, and hay dust. A drop of each allergen extract was introduced via lancets into the skin on the volar side of the forearm. Histamine (10 mg/ml) and glycerinated saline were used as positive and negative controls. After 15 min, the mean of the largest diameter of the wheal and its

perpendicular diameter was recorded as the response. A response of at least 3 mm greater than the saline control was deemed positive [21]. All influential factors before the test were excluded and accurate record of each allergen and wheal diameters for each patient were recorded. Results of the skin prick were graded according to the diameter of the wheal (table 1).

Table 1: Grading of skin prick test response [22].

Skin prick test wheal	Result	Grading
0 mm	Negative	0
Up to 3 mm	Positive	+1
3 – 5 mm	Positive	+2
5 -7 mm	Positive	+3
7 mm and above	Positive	+4

Sublingual Immunotherapy (SLIT):

After documentation of skin prick test responses for each patient, dilutions of allergens extracts were prepared using glycerin 50% in 20 ml simple bottles with a glassy dropper. Extracts were given as sublingual drops which were kept under the tongue for two minutes and then swallowed. The sublingual drops were administered in the morning on an empty stomach [23]. SLIT was divided into two phases; the build-up and the maintenance phases [24]. During the build-up phase, three increasing concentrations of the allergens were administrated (1/200 W/V, 1/100 W/V, 1/50 W/V) for 3 months, followed by the maintenance phase which started from the 4th month (1/50 W/V) till the end of the 6th month. Patients were instructed to carefully notice and document any change in symptoms severity or any adverse reactions like gastrointestinal disturbances, angioedema, difficulty in breathing, pruritis or skin rash development.

Reassessment of clinical symptoms:

Patients were followed up after the completion of immunotherapy within at least 6 months follow up. The improvement of the clinical symptoms was evaluated by SNOT-20. The impact of treatment is measured by calculating the difference between SNOT-20 scores before and after treatment.

Statistical analysis:

Collected data were statistically analyzed using SPSS software (Statistical Package for the Social Sciences software version 25). Quantitative variables were represented as the mean value ± standard deviation (SD), and categorical variables were represented as absolute numbers and percentages. The independent sample t-test was used to compare the mean of the two groups. Results were considered statistically significant when *p* (probability) values were equal to or less than 0.05.

RESULTS

This study enrolled 35 adult patients (≥ 18 years old) with allergic fungal rhinosinusitis; 16 (45.7%) males and 19 (54.3%) females, with a mean age of 34.14 ± 10.31 years. 85% of cases were from urban areas. Peripheral eosinophilia (> 500/mL) was found in 14 (40%) cases. Serum total Ig-E levels were raised (> 100 IU/mL) in 100% of AFRS cases.

Direct microscopic examination of KOH preparation was positive in 27 (77.1%) cases and septate hyphae were seen in these positive cases. All recruited patients were positive for fungal culture. The results of the fungal culture are demonstrated in table (2). *Aspergillus* spp. was found to be the most frequent fungus detected being isolated from 74.3% of cases (n=25) with *Aspergillus fumigatus* (54.3%) being the commonest.

Table 2: Frequency of fungal species isolated from AFRS patients (n=35).

Isolated fungus	Number (percentage), (n=35)
Aspergillus fumigatus	19 (54.3%)
Aspergillus flavus	7 (20%)
Zygomycetes species	8 (22.8%)
Scopularis species	1 (2.9%)

Table (3) demonstrates the results of the skin prick test. All patients were either monosensitized or polysensitized to the used allergens. All patients (100%) were sensitized to mixed fungi.

Table 3: Skin prick test results in AFRS patients.

Skin prick test	Positive, n (%) (n=35)	Negative, n (%) (n=35)
Date palm pollens	28 (80%)	7 (20%)
House dust mites	32 (91.4%)	3 (8.6%)
Mixed fungi	35 (100%)	0 (0%)
Smoke	15 (30%)	20 (70%)
Cotton	4 (11.4%)	31 (88.5%)
Hay dust	2 (5.7%)	33 (94.3%)

The baseline mean SNOT-20 score and that after completion of immunotherapy (6-months later) are summarized in table (4). The highest 5 mean item scores at the initial visit were runny nose, postnasal discharge, need to blow nose, thick nasal discharge, and sneezing. A significant improvement (p<0.001) in the SNOT-20 score after immunotherapy is demonstrated recording a mean of 1.93 ± 0.44 at the initial visit, compared to 1.2 ± 0.3, 6-months later after completing immunotherapy. No side effects were recorded along the follow up period in all treated patients.

Table 4: The SNOT-20 score at the initial visit, and after the completion of immunotherapy 6-month later.

Item	Initial visit (n=35) Mean ± SD	Six month (n=35) Mean ± SD	t (dt)	*p*-value
Need to blow nose	2.7 ± 1.08	1.7 ± 0.9	4.15	<0.001*
Sneezing	2.3 ± 1.3	1.3 ± 0.8	3.55	<0.001*
Runny nose	3.08 ± 1.19	2.1 ± 1.19	3.301	<0.001*
Cough	1.9 ± 1.3	1.14 ± 0.9	2.78	0.003*
Postnasal discharge	3.02 ± 1.2	1.9 ± 1.17	3.8	<0.001*
Thick nasal discharge	2.9 ± 1.3	1.9 ± 1.17	3.34	<0.001*
Ear fullness	1.9 ± 1.3	1.14 ± 1.08	2.67	0.004*
Dizziness	1.3 ± 1.1	0.7 ± 0.7	2.57	0.006*
Ear pain	1.5 ± 1.3	0.9 ± 1.13	2.1	0.01*
Facial pain/pressure	1.2 ± 1.1	0.6 ± 0.9	2.25	0.013*
Difficulty falling asleep	1.6 ± 1.2	1.3 ± 1.07	1.006	0.15
Wake up at night	2.05 ± 1.3	1.6 ± 1.19	1.5	0.06
Lack of a good night's sleep	2.05 ± 1.3	1.7 ± 1.19	1.05	0.14
Wake up tired	2.6 ± 1.2	1.7 ± 1.17	3	0.0018*
Fatigue	2.02 ± 1.17	1.4 ± 1.09	2.21	0.015*
Reduced productivity	1.6 ± 1.16	1.02 ± 0.9	2.21	0.015*
Reduced concentration	1.8 ± 1.2	1.3 ± 1.08	1.5	0.06
Frustrated/restless/irritable	1.2 ± 1.01	0.9 ± 0.8	1.6	0.051
Sad	0.9 ± 0.8	0.6 ± 0.8	1.29	0.09
Embarrassed	0.7 ± 0.8	0.4 ± 0.6	1.5	0.06
SNOT-20 score	1.93 ± 0.44	1.2 ± 0.3	6.5	<0.001*

t-test of significance, *$p \leq 0.05$ is statistically significant

DISCUSSION

In the current study, 35 cases were diagnosed as having AFRS. The mean age of these cases was 34.14 years with a range of 18 – 52 years. A slightly higher incidence was observed in the female with a male to female ratio of 1: 1.18. Most of cases (85%) of cases were from urban areas.

Most of the studies on AFRS come from countries with humid climate. Some authors consider it exclusively a disease of areas with high humidity where high mold counts exist [25]. In India, Kaur and his coworkers [26] reported that AFRS was more common among adolescents and young adults and more common in rural areas. They reported that the mean age of AFRS cases was 28.4 years with a range of 18 -48 years, and the male: female ratio was 1.1: 1, slightly higher in males. However, in another Indian study [27], 58% of fungal sinusitis cases were females compared to 42% males, which comes in agreement with the current study.

All AFRS cases in the current study had elevated serum total IgE level > 100 IU/mL, while peripheral eosinophilia (> 500/mL) being detected in 14 (40%) cases. Uri and his colleague [28] reported that 90% of AFRS cases have increased blood levels of IgE. Kaur et al.[26], found that 80% of AFRS patients had raised serum total IgE, however, elevated peripheral eosinophilia was detected in fewer AFRS cases (25.7%), which comes in agreement with the current study.

All patients in the current study were subjected to nasal endoscopy. Obtained samples were sent for microscopic examination and fungal culture. Evidence of fungal hyphae by microscopic examination of KOH preparation was positive in 77.1% of cases. Shetty et al. [27] recorded a ratio of 80.2%. Fewer cases were recorded by Kaur and his coworkers [26] who found that only 20% of cases were positive for fungal hyphae by direct microscopic examination. The failure of detection of fungal elements under the microscope in some cases could be attributed to the uneven distribution of fungal hyphae in the obtained specimen.

Fungal culture was positive in all AFRS patients in this study. *Aspergillus* spp. was isolated from 74.3% of AFRS cases with *Aspergillus fumigatus* and *Aspergillus flavus* representing 54.3% and 20%, respectively. This was followed by *Zygomycetes* representing 22.8% then *Scapularis* spp. 2.9%. Dematiaceous fungi were not isolated in the current study. Shetty et al. [27] in India found that *Aspergillus* species was the most common fungus isolated from AFRS patients with a frequency of 79%. They reported *Aspergillus flavus* (64.2%5) to be the most common followed by *Aspergillus fumigatus* (13.6%), *Mucormycosis* (11.1%), *Rhizopus* (7.4%), and *Scopularis* (2.5%). *Aspergillus flavus* was reported to be the most common in other studies [26, 29]. However, *Aspergillus fumigatus* and dematiaceous fungi were

reported to be the most common in another study [6]. The type of isolated fungi depends greatly on the dominant fungal species in the environment which in turn is influenced much by climate and geographic conditions.

During the initial visit, the baseline clinical assessment of AFRS patients was done using the SNOT-20 score. The highest 5 mean item scores at the initial visit were runny nose, postnasal discharge, need to blow nose, thick nasal discharge, and sneezing. Piccirillo et al. [20] found that the 5 items with the highest mean item scores for rhinosinusitis cases at the first visit were postnasal discharge, facial pain/pressure, need to blow nose, wake up tired, and fatigue.

Sublingual immunotherapy was initiated for 6 months in patients who did not respond to adequate medical treatment 3 months following endoscopic surgery. Before initiating immunotherapy, all patients in the current study underwent skin prick test where 80% were sensitized to date palm pollen, 91.4% to house dust mites, 11.4% to cotton, and 5.7% to hay dust. All patients were sensitized to mixed fungi. Similar results were reported by Chaitanya and Kalavathi[12], who reported allergen sensitization in 72.4% for pollen, 82.7% for dust, and 65.55 for mites and fungi.

Evaluation of the response to immunotherapy in AFRS patients was done by assessing the improvement in their clinical symptoms. A significant improvement (p<0.001) in the SNOT-20 score after immunotherapy was detected. The mean ± SD SNOT-20 score at the initial visit was 1.93 ± 0.44, while the mean ± SD SNOT-20 score 6-months later was 1.2 ± 0.3. Furthermore, there was a highly significant improvement in the five symptoms most frequently reported at the first visit after completion of immunotherapy. No side effects were detected in all treated patients.

The exact pathogenesis of AFRS remains to be revealed. It seems that the initiation of the inflammatory process is a multifactorial event. Atopy to inhalant fungal allergens and IgE production by itself may not be sufficient to induce AFRS. However, it plays a central role in the pathogenesis [4]. A specific expression of fungal antigens to T cells and aberration of local mucosal defense mechanisms may also have their roles[30]. The development of specific IgG to etiologic molds has been suggested to have a significant role in immunopathogenesis[4]. Probably for this reason, immunotherapy was debated for years for the concerns that it may induce allergen-specific IgG that theoretically could induce complex-mediated hypersensitivity reactions [31].

However, several trials have proved immunotherapy to be safe with contradicting results regarding its clinical efficacy [32]. In their 4-year retrospective study, Marby et al., in one of the most distinguished studies, found a significant reduction in allergic mucin, fungal debris and crusts with reduction in the use of intranasal steroids after one year of immunotherapy in 11 AFRS patients. At the end of four years, they reported that even after stopping immunotherapy for up to 7 to 17 months, there was no recurrence of the disease. However, on longer term follow-up (4-10 years), no additional benefit was added to the immunotherapy group compared to the non-immunotherapy group [33]. Other studies have demonstrated improved patient outcomes with immunotherapy with reduction in the need for both oral and nasal steroids [34]. In their study, Piccirillo et al. [20] reported that the mean ± SD SNOT-20 score at the initial visit was 1.9 ± 0.9, while the mean ± SD SNOT-20 score 6-months was 1.3 ± 1.0, indicating a significant improvement following immunotherapy.

Currently, most of the literature supports a role for immunotherapy particularly in recalcitrant AFRS. In their evidence-based review, Gan et al. [35] declared an aggregate quality of evidence grade C regarding the use of immunotherapy in AFRS.

CONCLUSION

The current study demonstrates immunotherapy to be a good adjunctive therapy for the management of AFRS patients not responding to adequate medical treatment.

The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

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