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Relation between Complicated Allergic Fungal Rhinosinusitis and the Mycological Profile of the Isolated Fungal Species

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ABSTRACT

Background: Allergic fungal sinusitis is a non-invasive pansinusitis that occurs in young immunocompetent individuals, with a strong history of atopy and elevated levels of total immunoglobulin E and peripheral eosinophilia.
Objective: The main target of the study was to know the relation between complicated allergic fungal sinusitis and the mycologic profile of the causative fungal species, as regards the genus and species of the isolated fungus, its antifungal susceptibility and its ability to produce destructive extracellular metabolic products and toxic agents.
Patients and Methods: Our cross sectional research included 50 individuals diagnosed with complicated allergic fungal rhinosinusitis who attended to the ENT outpatient clinic. All the studied patients were evaluated by full history, complete ENT examination, radiological evaluation, laboratory investigations and endoscopic sinus surgery.
Results: Aspergillus spp., particularly Aspergillus fumigatus and Aspergillus flavus, are the most often identified agents in allergic fungal rhinosinusitis. A multifaceted strategy to treat allergic fungal rhinosinusitis is necessary; surgery is the primary treatment for allergic fungal sinusitis. Corticosteroid treatment in its entirety is presently the gold standard of medical management, whereas alternative pharmacological treatments such as antifungals, antimicrobials, leukotriene modulators, and immunotherapy are reserved for those who are insufficiently responsive..
Conclusions: The most frequent kind of fungal rhinosinusitis is allergic fungal rhinosinusitis. It is found in immune-competent youth who have a history of allergic rhinitis and/or asthma. Allergic fungal rhinosinusitis has a relatively slow and indolent clinical course, but results in the growth of bone walls, resulting in their thinning or weakening and final erosion.
Keywords: Allergic sinusitis, Aspergillus, Fungal Species, Mycological Profile.

INTRODUCTION

Fungal sinusitis is a prevalent infection that has raised considerably in incidence over the last 30 years. This tendency might be related to the greater usage of immunosuppressive drugs in the modern era, as well as improved public awareness [(1)].

The clinical, radiologic, and histologic aspects of the host-pathogen interaction can be used to classify fungal illness of the nose and paranasal sinuses. The majority of widely recognized categorization methods categorize fungal rhinosinusitis as invasive or noninvasive based purely on histopathologic evidence of fungus penetration of host tissue. Acute fulminant invasive fungal sinusitis, granulomatous invasive fungal sinusitis (GIFS), and chronic invasive fungal sinusitis are all types of invasive fungal sinusitis (CIFS). Saprophytic fungal infection (SFI), sinus fungal ball, and allergic fungal rhinosinusitis are all examples of noninvasive fungal sinusitis [(2)].

Allergic fungal sinusitis is a non-invasive pansinusitis that often affects young immunocompetent people with a significantly strong history of atopy and increased total immunoglobulin E and peripheral eosinophilia [(1)]. The following characteristics constitute the diagnostic criteria for allergic fungal sinusitis: Sinusitis verified radiographically, with computed tomography (CT) indications of serpiginous regions of increased attention in afflicted sinuses, Atypical allergic mucin acquired surgically, the presence of fungal hyphae in allergic mucin or a positive fungal culture in correctly collected sinus material in an otherwise typical patient, no histopathologic evidence of mucosal fungal invasion, granulomata, mucosal necrosis, or giant cells must be eliminated, as well as other fungal rhinosinusitis disorders [(3)].

Species of Aspergillus especially *Aspergillus Niger, Flavus and Fumigatus* Diagnostic criteria for allergic fungal sinusitis include the following: Sinusitis is confirmed radiographically, with serpiginous areas of heightened attention in affected sinuses seen on computed tomography (CT). Atypical allergic mucin obtained surgically, the presence of fungal hyphae in allergic mucin, or the presence of a positive fungal culture in properly collected sinus material in an otherwise typical patient. No histopathologic evidence of fungal invasion, granulomata, mucosal necrosis, or large cells, as well as other fungal rhinosinusitis diseases, must be excluded [(4)].

Complications of allergic fungal sinusitis include orbital medial wall erosion seen on imaging, ophthalmoplegia, epiphora, diplopia, proptosis, orbital abscesses and also visual loss in rare cases. The proximity of important neurovascular structures including the internal carotid artery, cranial nerves, cavernous sinus, dura mater, and cerebral lobes puts any

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or all of these tissues at danger as a result of the disease process and surgical treatment [5].

The aim of study was to study the relation between complicated allergic fungal sinusitis and the mycologic profile of the causative fungal species, as regards the genus and species of the isolated fungus, its antifungal susceptibility and its ability to produce destructive extracellular metabolic products and toxic agents.

PATIENTS AND METHODS

Our cross sectional study was conducted on 50 patients with complicated allergic fungal rhinosinusitis who attended to the ENT outpatient clinic in the Otorhinolaryngology Department, Sohag University Hospital in collaboration with Medical Microbiology and Immunology Department.

Exclusion criteria were patients presented with complicated sinusitis due to any cause rather than allergic fungal sinusitis.

All the studied patients were evaluated by full history, complete ENT examination, radiological evaluation, laboratory investigations and endoscopic sinus surgery.

Microbiologic diagnosis: Specimens were obtained from the patients by endoscopic sinus surgery including tissue debris, wash fluid and fungal mud. Samples were transported to the laboratory on suitable transport media.

Direct microscopic examination:

Unstained wet mounts examination, examination of lactophenol cotton blue (LCB) stained wet mounts, culture, and identification of filamentous fungi.

Biochemical reactions:

1. **Urease Activity [6]:**

Locally prepared Christensen's urea broth containing tubes were inoculated with the isolated fungi and incubated at 25±2ºC for 3 days. After incubation, when a rich pink colour developed in the broth medium, the results were reported as positive.

2. **Lipase Production [7]:**

Detection of lipase production by isolated fungi was carried out on the medium of Ullman and Basins that was locally prepared. Test tubes were inoculated on the agar surface by 25µl of cell suspension and incubated at 25±2 ºC for filamentous fungi for 10 days. A fungus's lipolytic ability was apparent as a precipitate formed by crystals of the calcium salt of the oleic acid released by the enzyme. The depth of each visible precipitate was determined (in mm).

3. **Protease Production [8]:**

Locally prepared casein hydrolysis medium containing tubes were inoculated on the agar surface by 25µl of cell suspension of the tested fungal isolates and incubated at 25±2ºC for filamentous fungi for 7 days. Protease-producing fungus resulted in full breakdown of milk protein, which was seen as a distinct depth in the tube following incubation. The colony's clear depth was determined (in mm).

Ethical consent

An approval of the study was obtained from Sohag University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in this study.

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 16 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were represented as frequencies and relative percentages.

RESULTS

The study included 50 patients (27 females and 23 males) with complicated allergic fungal rhinosinusitis. The age of the patients ranged from 6 to 60 years. Twenty-six (52%) of the patients were presented with bilateral affection while twenty-four (48%) had unilateral affection. The majority of the cases were non-asthmatic (84%).

Inoculation of collected samples on SDA yielded 47 isolates of filamentous fungi that belong to four genera; Aspergillus fumigatus was the most common (Table (**1**).

Table (1): Distribution of filamentous fungi among cases with complicated allergic fungal sinusitis

Fungal isolate	No.	(%)
Aspergillus flavus	18	38.3
Aspergillus fumigatus	19	40.4
Aspergillus niger	6	12.8
Aspergillus terreus	4	8.5
Total	47	100.0

Antifungal susceptibility testing was performed on MHA using disc diffusion method. The highest percentage of sensitivity among isolates was to voriconazole **(**

Table (2).

Table (2): Antifungal susceptibility pattern of isolates against various antifungal agents

Yeast Isolates	No	DS	flu	Itra	Vori	Clo	Amb	Nystatin	Terbinafin
			N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
A. flavus	18	**S**	0 (0%)	13 (72.2%)	12 (66.7%)	1 (5.5%)	4 (22.2%)	6 (33.3%)	0 (0%)
		I	4 (22.2%)	1 (5.6%)	5 (27.8%)	9 (50%)	2 (11.1%)	0 (0%)	2 (11.1%)
		R	14 (77.8%)	4 (22.2%)	1 (5.5%)	8 (44.5%)	12 (66.7%)	12 (66.7%)	16 (88.9%)
A. fumigatus	19	**S**	7 (36.8%)	11 (57.9%)	16 (84.2%)	2 (10.5%)	10 (52.6%)	7 (36.8%)	2 (10.5%)
		I	4 (21.1%)	5 (26.3%)	3 (15.8%)	2 (10.5%)	3 (15.8%)	3 (15.8%)	0 (0%)
		R	8 (42.1%)	3 (15.8%)	0 (0%)	15 (79%)	6 (31.6%)	9 (47.4%)	17 (89.5%)
A. niger	6	**S**	0 (0%)	0 (0%)	3 (50%)	0 (0%)	5 (83.3%)	1 (16.7%)	2 (33.3%)
		I	2 (33.3%)	2 (33.3%)	3 (50%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)
		R	4 (66.7%)	4 (66.7%)	0 (0%)	4 (66.7%)	1 (16.7%)	5 (83.3%)	4 (66.7%)
A terreus	4	**S**	2 (50%)	4 (100%)	4 (100%)	0 (0%)	1 (25%)	2 (50%)	0 (0%)
		I	1 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)	0 (0%)
		R	1 (25%)	0 (0%)	0 (0%)	4 (100%)	2 (50%)	1 (25%)	4 (100%)
Total	**47**	**S**	9 (19.1%)	28 (59.6%)	35 (74.5%)	3 (6.4%)	20 (42.5%)	16 (34%)	4 (8.5%)
		I	11 (23.4%)	8 (17%)	11 (23.4%)	13 (27.7%)	6 (12.8%)	4 (8.5%)	2 (4.2%)
		R	27 (57.5%)	11 (23.4%)	1 (2.1%)	31 (65.9%)	21 (44.7%)	27 (57.5%)	41 (87.3%)

S = sensitive, I = Intermediate and R = Resistant

80.8 % of isolates were positive for urease enzyme production that contributes to their virulence (Table (**3**).

Table (3): Screening of filamentous fungi isolates for urease enzyme production

Mould isolates	No	Urease activity	
		Positive	Negative
		No. (%)	No. (%)
Aspergillus flavus	18	15 (83.3)	3 (16.7)
Aspergillus fumigatus	19	13 (68.4)	6 (31.6)
Aspergillus niger	6	6 (100)	0 (0)
Aspergillus terreus	4	4 (100)	0 (0)
Total	47	38 (80.8)	9 (19.2)

68% of isolates possessed high proteolytic activity (Table (**4**).

Table (4): Screening of filamentous fungi isolates for proteolytic activity

Mould isolates	No	Proteolytic activity			
		H	I	L	N
		No. (%)	No. (%)	No. (%)	No. (%)
Aspergillus flavus	18	11 (61.1)	5 (27.8)	2 (11.1)	0
Aspergillus fumigatus	19	17 (89.4)	1 (5.3)	1 (5.3)	0
Aspergillus niger	6	1 (16.7)	4 (66.6)	1 (16.7)	0
Aspergillus terreus	4	3 (75)	1 (25)	0	0
Total	47	32 (68.1)	11 (23.4)	4 (8.5)	0

H (high enzyme producer): the clear depth is ≥ 18 mm.
I (intermediate enzyme producer): the depth falls in the range 15-17 mm.
L (low enzyme producer): the clear depth is ≤14 mm.
N (non enzyme producer): there is no clear depth below the fungal colony.

75.4% of isolates possessed high lipolytic activity (Table (**4**).

Table (5): Screening of filamentous fungi isolates for lipolytic activity

Mould isolates	No	Lipolytic activity			
		H	I	L	N
		No. (%)	No. (%)	No. (%)	No. (%)
Aspergillus flavus	18	10 (55.6)	5 (27.8)	0 (0)	3 (16.6)
Aspergillus fumigatus	19	12 (63.2)	6 (31.5)	1 (5.2)	0 (0)
Aspergillus niger	6	3 (50)	1 (16.7)	0 (0)	2 (33.3)
Aspergillus terreus	4	2 (50)	2 (50)	0 (0)	0 (0)
Total	47	27 (57.4)	14 (29.8)	1 (2.1)	5 (10.6)

H (high enzyme producer): the depth of visible precipitate is ≥ 15 mm.
I (intermediate enzyme producer): the depth of visible precipitate range is 6-14 mm.
L (low enzyme producer): the depth of visible precipitate is ≤5 mm.
N (non enzyme producer): there is no visible precipitate below the fungal colony.

DISCUSSION

Katzenstein *et al.* [9] first identified allergic fungal rhinosinusitis (AFRS) in 1983. They showed a subclass of fungal rhino sinusitis that accounts for up to 10 % of CRS cases in the United States. It most commonly affects young, immunocompetent people with fungal atopy, it is unilateral, and has a geographic predilection to humid and even arid environments, like those seen in the southern United States, Middle East, Australia, and Africa[2]. Nasal obstruction is characteristic of various kinds of chronic polypoidal sinusitis, as thick black mucus rhinorrhea or postnasal discharge, hyposmia, face discomfort and pressure, and, in advanced illness, orbital or facial deformation. The disease was initially described in a patient with allergic bronchopulmonary aspergillosis (ABPA)-like mucus plugs within the paranasal sinuses[1].

Diagnostic criteria for AFRS vary by author, but the criteria that were described by **Bent and Kuhn**[10] in 1994 are the most widely accepted one. Their five criteria have (a) nasal polyposis, (b) allergic mucin (eosinophilic mucin) with no signs of any invasion, (c) hyper attenuating signal density seen by CT scan (double density sign), (d) positive fungal stain or culture and (e) type I hypersensitivity to fungi diagnosed by history, skin examining or serology [11]. Serum IgE levels are frequently elevated, and these levels fluctuate in response to disease activity. Low IgE levels are conceivable, particularly in individuals with quiescent illness. **Kuhn and Javer**[12] discovered that total IgE was both more specific and sensitive for predicting AFS persistence or recurrence than fungus specific IgE.

The most frequent non-invasive form is allergic fungal rhinosinusitis (AFRS). Historically it is a disease with a sluggish and indolent clinical history. AFRS causes bone wall growth, resulting in weakening and eventual erosion [13]. Ophthalmic symptoms or consequences of allergic fungal sinusitis vary from orbital medial wall erosion seen on imaging to proptosis and loss of vision in severe instances. The closeness of important neurovascular systems such as the internal carotid artery, cranial nerves, cavernous sinus, dura mater, and cerebral lobes puts any or all of these tissues at danger, both throughout the disease process and after surgical treatment [5].

In our study, 52% of our cases were presented with proptosis, and visual loss was recorded in only 2% of studded cases. According to **Alzarei and Assiri** [14] orbital complications were encountered: (26.7%) eye proptosis, (11.7%) diplopia and (6.7%) unilateral complete blindness. Proptosis of the eye responded well to surgical and postoperative treatment, but diplopia improved more slowly following surgical and medicinal treatment. Proptosis and a strong index of suspicion on the part of the ophthalmologist are critical components of the first diagnosis of allergic fungal sinusitis.

A large-scale retrospective study was conducted in 2008, where Aspergillus flavus was found to be the most common etiological agent in allergic FRS, followed by Aspergillus fumigatus [15]. A research was done in Gujarat to evaluate 100 instances of FRS from various areas in the States. Aspergillus species was the most often isolated fungus from AFRS, with Aspergillus flavus being the most frequently isolated species in 82.85% of cases [16]. Worldwide, the prevalence of causal fungus species varies. Aspergillus flavus is the most frequently isolated fungus in India and Sudan, particularly in north India. However, in the United States of America, Aspergillus fumigatus and Rhizopus arrhizus are the most prevalent. Bipolaris is a newly recognized disease, particularly in hot regions such as the southern United States of America, Australia, Pakistan, and India [11]. Retrospective study was done on 2015 where Aspergillus species were most entirely isolated (61.84 %) [17].

In our study aspergillus fumigatus is the most commonly isolated fungal species representing 40.4% followed by Aspergillus flavus representing 38.8%.

In a study, antifungal susceptibility test of 200 clinical isolates of A. fumigatus was done and voriconazole was the most active drug [18]. According to **Jain *et al.*** [17], voriconazole, anidulafungin and micafungin were most effective against Aspergillus species.

While a successful treatment strategy for AFRS is multifaceted, surgery is the primary therapy for allergic fungal sinusitis. The objectives of surgery are to remove the polyps, to widen the affected sinuses, to drain any eosinophilic mucus, and to establish access for topical intranasal medicine [19]. Over the course of a seven-year follow-up period, one longitudinal research discovered that patients required an average of two surgical operations and three doses of systemic steroids per year, as well as chronic polypoidal mucosal edema and increased total IgE [20].

Antifungal medication with itraconazole is now widely regarded as the major management strategy for ABPA. Antifungal therapy aims to decrease the fungal antigenic stimulation for inflammation, therefore lowering corticosteroid needs [21]. Given the apparent similarities of AFRS, antifungal treatment may be considered to attain the same aims. Randomized controlled trials support the efficacy of itraconazole in ABPA. Regrettably, the majority of research on AFRS to date have been retrospective in nature or have significant methodological problems, limiting the conclusions that may be drawn. **Rains and Mineck** [22] conducted a retrospective study of 139 individuals with AFRS. In addition to long-term topical corticosteroids, patients were treated with short-course systemic steroids or systemic steroids and oral itraconazole (200–400 mg/day for 3 months or longer, depending on response). The recurrence rate was 20% in the itraconazole-treated group, compared to 50% in the steroids-only group. In this 12-year retrospective cohort, the reason for assigning certain patients to itraconazole treatment is unclear, and recurrence was defined as the requirement for further medical treatment.

Chan *et al.* [23] reported results on 32 patients with allergic fungal rhinosinusitis who did not respond adequately to sinus surgery, short course oral corticosteroids, and nasal amphotericin B therapy (6 mL three times a day of 0.1 mg/mL amphotericin B deoxycholate). All patients were treated with oral itraconazole (200–300 mg/day) for 3 months. Twelve patients demonstrated endoscopic scoring improvement, while 15 demonstrated no difference, and five demonstrated worsening. **Seiberling and Wormald** [24] made the review charts of 23 patients with AFRS and non-allergic eosinophilic fungal rhinosinusitis who had previously failed medicinal treatment and sinus surgery. For a minimum of six months, patients received 100 mg itraconazole BID. Three patients needed to discontinue therapy because of hepatic adverse effects, four patients did not respond, and sixteen patients experienced subjective symptom relief in addition to a decrease in oral steroid use and a better endoscopic appearance (decreased eosinophilic mucin and polyps). **Khalil *et al.*** [25] studied 50 adult patients who had sinus surgery after being diagnosed with AFRS. Following surgery, patients received systemic and topical steroids and were randomly allocated to one of five treatment arms: (1) oral itraconazole, (b) fluconazole nasal spray, (c) combination of oral itraconazole and fluconazole nasal spray, (d) fluconazole nasal irrigation, or (e) placebo. The authors found a reduced recurrence rate in groups treated with topical fluconazole, however itraconazole did not appear to offer any advantage.

CONCLUSIONS

Allergic fungal rhinosinusitis is the most frequent kind of fungal rhinosinusitis. It is found in immune-competent young adults who have a history of allergic rhinitis and/or asthma. Allergic fungal rhinosinusitis has a sluggish and indolent clinical course but results in bone wall growth, weakening, and final erosion. Complications of allergic fungal rhinosinusitis develop as a result of fungi's strong lipolytic, proteolytic, and urease enzyme synthesis, all of which contribute to their pathogenicity.

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REFERENCES

1. **Chakrabarti A, Rudramurthy S, Panda N *et al.* (2015):** Epidemiology of chronic fungal rhinosinusitis in rural India. Mycoses, 58(5):294-302.
2. **Chakrabarti A, Denning D, Ferguson B *et al.* (2009):** Fungal rhinosinusitis: a categorization and definitional schema addressing current controversies. Laryngoscope, 119(9):1809-18.
3. **Fokkens W, Lund V, Mullol J *et al.* (2012):** European position paper on rhinosinusitis and nasal polyps 2012. Rhinol Suppl., 23: 3-6.
4. **Montone K, Livolsi V, Feldman M *et al.* (2012):** Fungal rhinosinusitis: a retrospective microbiologic and pathologic review of 400 patients at a single university medical center. Int J Otolaryngol., 12:684-89.
5. **Vashishth A (2015):** Extensive allergic fungal rhinosinusitis: Ophthalmic and skull base complications. Indian J Otolaryngol Head Neck Surg., 67(3):227-33.
6. **Seeliger H (1956):** Use of a urease test for the screening and identification of cryptococci. J Bacteriol., 72(2):127-31.
7. **Sierra G (1957):** A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie Van Leeuwenhoek, 23(1):15-22.
8. **Paterson R, Bridge P (1994):** Biochemical techniques for filamentous fungi. Wallingford, Oxon, UK: CAB International. Pp. 125. https://lib.ugent.be/en/catalog/rug01:000340141
9. **Katzenstein A, Sale S, Greenberger P (1983):** Allergic Aspergillus sinusitis: a newly recognized form of sinusitis. J Allergy Clin Immunol., 72(1):89-93.
10. **Bent J, Kuhn F (1994):** Diagnosis of allergic fungal sinusitis. Otolaryngol Head Neck Surg., 111(5):580–588.
11. **Aribandi M, Bazan C (2007):** CT and MRI features in Bipolaris fungal sinusitis. Australas Radiol., 51(2):127-32.

12. **Kuhn F, Javer A (1999):** Utilizing fungal specific IgE levels as a serological marker for allergic fungal sinusitis activity. Am Rhinologic Soc Spring Meet., 111: 580-588.
13. **Bozeman S, deShazo R, Stringer S *et al.* (2011):** Complications of allergic fungal sinusitis. Am J Med., 124(4):359-68.
14. **Alzarei A, Assiri S (2016):** Orbital complication of allergic fungal rhino-sinusitis. Bahrain Medical Bulletin, 38(3): 139-141.
15. **Michael R, Michael J, Ashbee R *et al.* (2008):** Mycological profile of fungal sinusitis: An audit of specimens over a 7-year period in a tertiary care hospital in Tamil Nadu. Indian J Pathol Microbiol., 51(4):493-6.
16. **Waghray J (2018):** Clinical study of fungal sinusitis. International Journal of Otorhinolaryngology and Head and Neck Surgery, 4: 1307-1311.
17. **Jain R, Singhal S, Singla N *et al.* (2015):** Mycological profile and antifungal susceptibility of fungal isolates from clinically suspected cases of fungal rhinosinusitis in a tertiary care hospital in North India. Mycopathologia, 180(1):51-9.
18. **Dannaoui E, Meletiadis J, Tortorano A *et al.* (2004):** Susceptibility testing of sequential isolates of Aspergillus fumigatus recovered from treated patients. Journal of Medical Microbiology, 53(2):129-34.
19. **Marple B (2001):** Allergic fungal rhinosinusitis: current theories and management strategies. Laryngoscope, 111(6):1006-19.
20. **Marple B, Newcomer M, Schwade N *et al.* (2002):** Natural history of allergic fungal rhinosinusitis: a 4- to 10-year follow-up. Otolaryngol Head Neck Surg., 127(5):361-6.
21. **Walsh T, Anaissie E, Denning D *et al.* (2008):** Treatment of aspergillosis: Clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis., 46(3):327-60.
22. **Rains B, Mineck C (2003):** Treatment of allergic fungal sinusitis with high-dose itraconazole. Am J Rhinol., 17(1):1-8.
23. **Chan K, Genoway K, Javer A (2008):** Effectiveness of itraconazole in the management of refractory allergic fungal rhinosinusitis. J Otolaryngol Head Neck Surg., 37(6):870-4.
24. **Seiberling K, Wormald P (2009):** The role of itraconazole in recalcitrant fungal sinusitis. Am J Rhinol Allergy, 23(3):303-6.
25. **Khalil Y, Tharwat A, Abdou A *et al.* (2011):** The role of antifungal therapy in the prevention of recurrent allergic fungal rhinosinusitis after functional endoscopic sinus surgery: a randomized, controlled study. Ear Nose Throat J., 90(8):1-7.