Middle-East Journal of Scientific Research 7 (3): 387-391, 2011
ISSN 1990-9233

Fungal Rhinosinusitis in Hospitalized Patients in Khorramabad, Iran

[1]Sabokbar Azar, [2]Bayat Mansour, [3]Kordbacheh Parivash and [1]Baradaran Babak

[1]Department of Microbiology, Islamic Azad University, Karaj, Iran
[2]Department of Medical and Veterinary Mycology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran
[3]Department of Parasitological Research and Mycology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Iran

Abstract: "Chronic Rhinosinusitis" (CRS) is characterized by mucosal inflammation of nasal cavity and Para nasal sinuses, accompanied by edema and obstruction. Various agents including bacteria, viruses and fungi have been introduced as etiological origins of the disease.Fungi with different abundances have been reported as a common cause of allergic Rhinosinusitis that among them *aspergillus* species were the usual. This study was associeted to explore the frequency of different fungi isolates by in vitro culture and histological assays from drainage and biopsy samples obtained from operated Rhinosinusitis patients and comparing with healthy controls. This prospective experimental study was undertaken on drainage or biopsy samples from 83 Rhinosinusitis patients who had been admitted thorough 18 months (from March 2006 until September 2007) in Khorram Abad's state hospital. Nasal from 34 age and sex matched healthy control subjects were also included. Direct microscopy with KOH, histopathology examinations on biopsies with gomori's methenamine silver; PAS and H&E staining and in vitro cultures of dispersed samples on SDA; SDA-C and BHI agar media were done.Samples from 12 out of 83 Patients (14.46%) had fungi elements approved by histopathiligical and cultural examinations. Frequencies of isolated fungi, *candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Mucor spp* were 6, 3, 2, 1; respectively. In conclusion, an overall frequency of fungal Rhinosinusitis in studied population was 14.46%. According to the findings, there is a major role for *Candida spp*. in rhinosinusitis generation that had not been noticed previously.

Key words: Rhinosinusitis · Fungi · *Candida albicans* · Khorramabad

INTRODUCTION

Sinusitis that usually coexists with rhinitis, named Rhinosinusitis, may be categorized to acute, sub-acute and chronic conditions based on severity and duration of the disease specific symptoms. Over 3 months history of symptoms, including nasal blockage, obstruction, congestion with or without facial pain, discharge, discolored postnasal discharge, anemia, may be the strong indicators for occurrence of chronic Rhinosinusitis (CRS) [1, 2]. The more common conditions that are associated with chronic form of the disease are perennial allergic and non-allergic rhinitis, nasal polyps and obstruction.

Fungal sinusitis may be invasive or noninvasive [3,4]. Granulomatous, acute fulminate and chronic invasive are 3 forms of invasive fungal sinusitis. Noninvasive involvement has two forms of allergic fungal sinusitis and fungus ball [3, 4]. Findings from histological examinations are used to defining invasive or noninvasveness of the disease.

Findings of the studies all around the world about etiologic agents of the disease are with controversies. Nonetheless, inflammation due to super antigens, bacterial infections with or without biofilm production, allergic reactions to fungal antigens and less commonly, gastro esophageal reflux, smoke and other environmental exposures, immune deficiencies (e.g. AIDS or neutrophil dysfunction), genetic susceptibility factors, diabetes and aspirin are the main etiological agents have been suggested [2].

Correspondence Author: Mansour Bayat, Department of Medical and Veterinary Mycology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran, E-mail:giau.iao@gmail.com.

Chronic rhinosinusitis (CRS) is a sinus disease related to hypersensitivity to fungal infection [5]. Fungal rhinosinusitis (FRS) is uncommon and account for 6-12% of culture or histological proven chronic sinusitis [6], but it suggested it might have a geographical variation [7, 8]. *Aspergillus* species have been reported as the most common cause of different types of fungal sinusitis [9-11]. Other reports insist *Mucor spp*. were more common in immune competent patients and *aspergillus spp.* have been isolated from immune compromised patients [12]. Fungal rhinosinusitis involves a wide spectrum of immune and pathological responses, including invasive, chronic, granulomatous and allergic disease [4, 6]. Many fungi exhibit rapid growth on mucous membranes of patient with chronic allergies and sinusitis [13]. Immunologic response to fungus is not necessarily associated with rhinosinusitis and immunologic response to fungal extracts appears to be heterogeneous and may differ based on geography, allergy status and/or other yet unknown factors [14, 15]. In addition, in allergic fungal rhinosinusitis, fungal growth in culture media is not always accompanied by an allergic reaction [16].

Diagnostic criteria for CRS, after specific symptoms of the disease confirmed by primary examinations are nasal endoscopy, X-ray radiography, MRI and computed tomography (CT) [17-20]. Definitive diagnosis is necessary for making proper treatment procedures [2, 7], that can be done using different methods, such as direct microscopically observation of dispersed samples in KOH, histo-pathological studies of dissected polyps or mucosal tissues by Hematoxylin and Eosin, Gomori's methylamine silver and periodic acid Schiff stains. In addition to histological findings, in vitro culture assays are used for identification of the fungus and further confirmation of the observations.

The current study was designed to explore the frequency of different fungi isolates by in vitro culture assays or by histo-pathological observations of drainage and biopsy samples obtained from operated rhinosinusitis patients and healthy controls in Khorramabad; capital of Lorestan province of Iran. Fungi with different abundances have been reported as a common cause of allergic rhinosinusitis and *aspergillus* species were frequently isolated from patients, but differential abundances of fungi isolates from Iranian patients were unknown.

MATERIALS AND METHODS:

Eighty-three patients with clear symptoms of chronic rhinosinusitis that their diagnosis had been confirmed by CT-scan were included in the study. The patients admitted thorough 18 months (from March 2006 until September 2007) in Khorramabad's state hospital. All patients completed preoperatively questionnaires about their other interfering variables such as history of operations and treatments, age, sex, location etc,. Biopsy samples from their polyps and sinusoidal mucosa dissected under surgery operations and aseptically transferred to sterile phosphate buffered saline to make in vitro cultures or to assemble paraffin-embedded tissues latter. Nasal lavages from 34 age and sex matched healthy control subjects were also included.

Direct observations of samples, prepared in 10% KOH and dimethyl sulphoxide, were done using light microscopy. Gram stain was used to observe the yeasts. Histopathological examinations of smears, prepared from parafin embeded tissues, reviewed after staining with gomori's methenamine silver; Periodic Acid Schiff and Hematoxylin & Eosin stains. In vitro cultures of dispersed biopsy samples or nasal lavages on Sabouraud dextrose agar (SDA); SDA with Chloramphenicol and Brain Heart Infusion agar media were done. All cultures done in parallels, one in 25°C and another incubated in 35°C. All cultures were kept in proper condition up to 3 weeks and identification of growing fungi was established using standard methods. Slide cultures were also provided to explore microscopic features of the isolates.

Any specimens that had fungi in direct examinations and had two successive culture growths considered as positive.

RESULTS

The 83 patients were included in the current study. Mean age was 41.5 years old (range: 9-74), with 34 to 49 men and women rate respectively. Thirty-four sex and age matched healthy controls also were included that their mean age was 43 years old (range: 14-70) with 13 to 21 men and women rate respectively.

Most of the patients had a history of chronic rhinosinusitis or Asthma and a history of previous sinus or polyp surgery. A few of them had long time usage of nasal drops or spray.

Table 1: Respective and absolute abundance distribution of taken samples from patients who suffered from polyp and fungal sinusitis based on Microorganism and pathology result (1385-1386)

	Positive		Negative		Total	
Type of Fungi	Number	Percentage	Number	Percentage	Number	Percentage
Mucor	1	1.20	82	98.80	83	100
Aspergillus fumigatus	3	3.61	80	96.39	83	100
Aspergillus flavus	2	3.41	81	97.59	83	100
Candida albicans	6	7.23	77	92.77	83	100

On microscopic examination, septated fungal hyphens (5 cases), unrepeated hyphae (1 case) and yeast like budding cells (6 cases) were identified and influx of leukocytes (neutrophils or eosinophils) with evidence of chronic inflammation was seen.

In vitro cultures on mycotic mediums of the patients' samples, showed the pure growth of the fungus (see Table 1). The isolated mold fungi were *Aspergillus fumigatus* (3 cases), *Aspergillus flavous* (2 cases), *Mucor* (one case) and the yeasts were only *Candida albicans* (6 cases).

DISCUSSION

Fungi are ubiquitous organisms and their spores find easily their ways to sinusoidal cavities through respiration. The human immune system potentially eliminates these invaders; however, in 10% of cases, fungal spores remain active and lead to manifestation of the disease. Fungal involvement of the nasal and par nasal sinuses varies from the benign to malignant and fatal forms. Fungal rhinisinusitis can also be categorized as invasive and noninvasive forms [3, 4].

Although Brook has reported that fungal sinusitis is most common in the immune compromised and diabetic populations [21], but the disease can also occur in certain immune competent people [5, 12].

The primary factors that influence colonization and localized infection of sinusoidal mucosa by various fungi are not clearly understood. Studies have showed that mast cells express MMPs and interact with extracellular matrix proteins and ASM and may play a role in nasal and bronchial hyper pensiveness as well as tissue remodeling to forming nasal polyps [22].

A nasal polyp is a benign tumor that grows from the lining of the nose or sinuses and is found in 20% of chronic rhinosinusitis patients [23-28]. Studies by Marfani indicate that all Allergic rhinosinusitis originates from allergic reactions to fungi and polyps are present in all of the allergic fungal rhinosinusitis (AFR) (100% of AFR cases) [5]. In another study conducted by Bassiouny 92% of CRS patients with allergic fungal sinusitis have had polyposis [29]. The relevant mechanisms proposed to polyp formation are localized immune responses to fungal antigens, including various cytokines and polyconal IgE and recruitment of eosinophils and mast cells to altering mucosal tissues [30]. On the other hand, a study by Rozanska-Kudelska suggests that allergy for fungi don't play a significant role in etiology and pathogenesis of the chronic rhinosinusitis with or without polyps [31].

While polyps are located in the nasal cavities, they are able to cause various problems such as nasal congestion, vertigo and even internal involvements in the cranium [2, 5].

Studies by Newman (1994) indicated that 39% of patients with chronic rhinosinusitis had asthma as well as eosinophilia and elevated IgE [32, 33]. In deed, allergic inflammation, polyclonal IgE and cytokines production and eosinophil recruitments are the major factors leading to polyp formation [30]. Thus eosinophilic mucin seen in histologic examinations is one of the five major criteria for disease dignosis [10, 34-37].

The defensive mechanisms provided by healthy immune system, located in the sinusoidal mucosa, eliminates as quickly as all microorganisms that diffuse through respiration. Thus, in vitro cultures of samples from respiratory channels of the people with healthy immune system rarely show fungal elements.

In a study performed at Mayo Clinic on sampled patients with CRS and healthy volunteers for fungus, approximately 96% of patients with CRS demonstrated positive fungal cultures, while 100% of healthy volunteers demonstrated fungal colonization [38]. This is the only example with surprising results, but in all other studies the frequencies of fungi in CRS patients range from 5% to 12% and rare reports of fungi isolation from healthy people have been presented. In the current study none of healthy control subjects had positive cultures according to the established criterion.

Among the studied population, 14 out of 83 patients (16.87%) had fingi in histologic exams and in vitro cultures. The invasion of fungus to involved tissues was seen in 14.46% of the patients, confirmed by histopathological tests. Eosinophilic musin, eosinophil and neutrophil infiltrations were also seen.

Most of the patients had history of chronic cold and sinusitis and the others had used drugs like aspirin, nasal drop or spray.

Similar studies has shown different percentages (5-57.4%) in patients who suffered from chronic rhinosinusitis by standard cultures of the fungus in nasal mucosa [39]. The reasons for the significant difference in determining the percentage are unusual methods of study and gathering the samples which are done, mostly by washing nose. Thus, illogical statistics can be related to contaminations, not fungus factors.

In this study, the highest number of isolated fungus of the polyp and sinus belonged to *Candida albicans* (6 cases) and *Aspergillus* (3cases) patients and there was a Mucor case too. The isolation, particularly *Aspergillus* isolation has been mentioned in the other studies. Meanwhile, statistics did not prove any relation among job, sexuality, age and suffering.

Endoscopic sinus surgery was performed in all cases and recurrent or residual disease was observed in 19.14 percent.

ACKNOWLEDGMENTS

Special thanks to pathology and operation room of the social security hospitals and Shohadaye Ashayer hospital in Khoramabad and mycology group of Lorestan Medical Science University, particularly, Dr. Ahmad Golshan, Dr. Asghar Sepah vand, Dr. Rooh Allah Sobhani, Dr. Babak Maleki, Dr. Madhani and Ms. Kazem Zadeh, Ms. Ravan Bakhsh who helped us in this research.

The relation of fungal sinusitis and polyps and its ethologic factors:

From 83 samples, totally 12 cases (14.46%) have been positive. *Aspergillus flavus* showed 2.41%, *Aspergillus fumigatus* 3.61%, *Mucor* 1.2% and *Candida albicans* showed 7.23% respectively.

REFERENCES

1. Ng, M.L., R.S. Warlow, N. Chrishanthan, C. Ellis and R. Walls, 2000. Preliminary criteria for the definition of allergic rhinitis: a systematic evaluation of clinical parameters in a disease cohort (I). Clin Exp Allergy, 30(9): 1314-31.
2. Marple, B.F., 2009. Diagnosis and management of chronic rhinosinusitis in adults. Postgrad Med., 121(6): 121-39.
3. DeShazo, R.D., K. Chapin and R.E. Swain, 1997. Fungal Sinusitis. N Engl. J. Med., 337(4): 254-9.
4. DeShazo, R.D., 1997. A new classification and diagnostic criteria for invasive fungal sinusitis. Arch Otolaryngol Head Neck Surg, 123(11): 1181-8.
5. Marfani, M.S., 2004. Allergic fungal rhinosinusitis with skull base and orbital erosion. J. Laryngol. Otol., 124(2): 161-5.
6. Challa, S., 1998. Fungal rhinosinusitis: a clinicopathological study from South India. Eur. Arch Otorhinolaryngol., 267(8): 1239-45.
7. Torres Macedo, N.L., E. Segovia Forero and H. Aguirre Mariscal, 2001. Mycotic allergic sinusitis. Monographic review of the literature. Rev Alerg Mex., 48(1): 28-32.
8. Wise, S.K., 2008. Socioeconomic factors in the diagnosis of allergic fungal rhinosinusitis. Otolaryngol Head Neck Surg., 138(1): 38-42.
9. Chakrabarti, A., S.C. Sharma and J. Chandler, 1992. Epidemiology and pathogenesis of paranasal sinus mycoses. Otolaryngol Head Neck Surg., 107(6 Pt 1): 745-50.
10. Saravanan, K., 2006. Allergic fungal rhinosinusitis: an attempt to resolve the diagnostic dilemma. Arch Otolaryngol Head Neck Surg., 132(2): 173-8.
11. Washburn, R.G., 1998. Chronic fungal sinusitis in apparently normal hosts. Medicine (Baltimore), 67(4): 231-47.
12. Robey, A.B., 2009. The changing face of paranasal sinus fungus balls. Ann Otol Rhinol Laryngol., 118(7): 500-5.
13. Tichenor, W.S., 2006. Fungal sinusitis. NewYork Publication, pp: 1-4.
14. Orlandi, R.R., 2009. Immunologic response to fungus is not universally associated with rhinosinusitis. Otolaryngol Head Neck Surg., 141(6): 750-6 e1-2.
15. Wise, S.K., 2008. Antigen-specific IgE in sinus mucosa of allergic fungal rhinosinusitis patients. Am. J. Rhinol., 22(5): 451-6.
16. El-Morsy, S.M., 2008. Allergic fungal rhinosinusitis: detection of fungal DNA in sinus aspirate using polymerase chain reaction. J. Laryngol. Otol., 124(2): 152-60.
17. Baumann, I., A. Koitschev and F. Dammann, 2004. Preoperative imaging of chronic sinusitis by multislice computed tomography. Eur Arch Otorhinolaryngol., 261(9): 497-501.

18. Cyr, C., 2001. Do symptoms and initial clinical probability predict the radiological diagnosis of acute sinusitis in children? Paediatr Child Health, 6(8): 536-9.
19. Engels, E.A., 2000. Meta-analysis of diagnostic tests for acute sinusitis. J Clin Epidemiol., 53(8): 852-62.
20. Zinreich, S.J., 1998. Fungal sinusitis: diagnosis with CT and MR imaging. Radiol., 169(2): 439-44.
21. Brook, I., 2005. Microbiology and antimicrobial management of sinusitis. J. Laryngol Otol., 119(4): 251-8.
22. Pawankar, R., 2005. Mast cells in allergic airway disease and chronic rhinosinusitis. Chem Immunol Allergy, 87: 111-29.
23. Settipane, G.A., 1996. Epidemiology of nasal polyps. Allergy Asthma Proc., 17(5): 231-6.
24. Settipane, G.A., 2000. Asthma, aspirin intolerance and nasal polyps. N Engl. Reg. Allergy Proc., 7(1): 32-7.
25. Settipane, G.A. and F.H. Chafee, 1977. Nasal polyps in asthma and rhinitis. A review of 6,037 patients. J. Allergy Clin Immunol., 59(1): 17-21.
26. Fokkens, W., V. Lund and J. Mullol, 2008. European position paper on rhinosinusitis and nasal polyps group. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi., 43(4): 317-20.
27. Fokkens, W., V. Lund and J. Mullol, 2007. European position paper on rhinosinusitis and nasal polyps Rhinol Suppl, 20: 1-136.
28. Huizing, E.H., 2005. European Position Paper on Rhinosinusitis and Nasal Polyps. Rhinol., 43(3): 238-238.
29. Bassiouny, A., 2000. Prevalence of extramucosal fungal elements in sinonasal polyposis: a mycological and pathologic study in an Egyptian population. Am J. Otolaryngol., 20: 30-36.
30. Li, J.Y. and S.Y. Fang, 2008. Allergic profiles in unilateral nasal polyps of bilateral chronic rhinosinusitis. Am. J. Rhinol., 22(2): 111-4.
31. Rozanska-Kudelska, M., 2009. Mold fungi and the role of allergy on fungi in chronic rhinosinusitis. Otolaryngol. Pol., 63(3): 245-8.
32. Szucs, E., 2002. Eosinophilia in the ethmoid mucosa and its relationship to the severity of inflammation in chronic rhinosinusitis. Am. J. Rhinol., 16(3): 131-4.
33. Newman, L.J., 1994. Chronic sinusitis. Relationship of computed tomographic findings to allergy, asthma and eosinophilia. Jama, 271(5): 363-7.
34. Bent, J.P. and F.A. Kuhn, 1994. Diagnosis of allergic fungal sinusitis. Otolaryngol Head Neck Surg., 111(5): 580-8.
35. Hidir, Y., 2008. Rate of allergic fungal etiology of chronic rhinosinusitis in Turkish population. Eur Arch Otorhinolaryngol., 265(4): 415-9.
36. Orlandi, R.R., S.L. Thibeault and B.J. Ferguson, 2007. Microarray analysis of allergic fungal sinusitis and eosinophilic mucin rhinosinusitis. Otolaryngol Head Neck Surg., 136(5): 707-13.
37. Ryan, M.W. and B.F. Marple, 2007. Allergic fungal rhinosinusitis: diagnosis and management. Curr Opin Otolaryngol Head Neck Surg., 15(1): 18-22.
38. Ponikau, J.U., 1999. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc., 74(9): 877-84.
39. Elmorsy, S., 2001. Sinus aspirates in chronic rhinosinusitis: fungal colonization of paranasal sinuses, evaluation of ICAM-1 and IL-8 and studying of immunological effect of long-term macrolide therapy. Rhinol., 48(3): 312-7.