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Prevalence of Toxigenic fungi associated with Rhinosinusitis cases in AL-Diwanyah city

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Abstract

This aimed to investigate of toxigenic fungi associated with sinusitis therefore 75specimens were collected from patients suffers from Rhino sinusitis sinusitis after diagnosis by specialist physician and attended to Al-diwanyah teaching hospitals and investigate the ability of some fungi on mycotoxin production.

The prevalence of fungal sinusitis in our study was 60%. *Aspergillus* spp was the most commonly isolated species with percentage (44.44%)of fungus and this fungi had ability to produce Aflatoxin that appear as florescent blue spot on TLC plates when Checked under UV.

Key word: *toxigenic fungi. Mycotoxins, sinusitis.*

Introduction

The term Rhinosinusitis (RS) refers to the inflammation of nasal and paranasal sinus mucosa caused by either infectious (bacterial or fungal) or non-infectious (allergic or non-allergic or immunological causes. Fungal rhinosinusitis (FRS) is defined as the rhinosinusitis where fungi are responsible for causing the immunopathogenesis. The disease impairs the quality of life and creates socioeconomic loss. (Chatterjee and Chakrabarti,2009).

Rhino sinusitis affects about 20% of the population once in a lifetime, In the US, 4% of adults are affected annually [Schiller *etal*.,2012]. The prevalence of FRS is difficult to assess due to controversy about its definition. The prevalence would be very high if we agree with Ponikau et al.'s proposition that all Chronic Rhinosinusitis (CRS) cases are due to fungi (Ponikau *etal*.,1999). Overall, CRS affects 1%–1.5%, 11% and 12.5% of the population in North India (rural), the European Union and the US, respectively (Chakrabarti *etal*.,2015; Pleis *etal*.,2010] . Currently, AFRS is responsible for 7%–12% of CRS cases undergoing sinus surgery . Of the total cases of CRS, FRS is observed in 27.2% cases 1.1 persons per 1000 population) in India indicating high burden of FRS cases in rural northern India .Climate possibly plays an important role in the considerably high prevalence of FRS cases in India, Sudan, and Pakistan. (Bachert and Holtappels,2015; Rupa *etal*.,2002).

The allergic fungal rhinosinusitis (AFRS) is observed commonly in young adult males from rural areas attributed to their work in the fields in warm climates, thus predisposing them to nasal mucosal injury and fungal colonization . (Chakrabarti *etal*.,1992). Other predisposing factors include African-American origin, structural anomalies, and low socioeconomic status. Bony erosion is 15 times more common in African-Americans with higher rate of intra orbital and intracranial

extension of the lesion (White *etal.*,2015) While other researcher failed to observe any correlation between bony erosion and low socioeconomic status, other studies have found a significant correlation between the bony erosion and inhabitants of low-income countries with poor housing conditions [Wise *etal*.,2008]. However, the pathology of fungal rhinosinusitis in some patients could not be explained, as allergic inflammation was a predominant feature in those lesions. Safirstein first coined the term "allergic Aspergillus sinusitis" in a patient to explain simultaneous involvement of lung and sinuses with similar pathology . Subsequently in 1981, Millar described sinus symptoms with allergic pathology in five patients, though simultaneous history of allergic bronchopulmonary aspergillosis (ABPA) was seen in only one patient (Millar and Johnston,1981). He coined the term "allergic aspergillosis of paranasal sinuses" as the mucus from sinuses of these patients histologically simulated the mucus plugs expectorated by ABPA patients and patients demonstrating a type I hypersensitivity reaction to *A. fumigatus*. Aspergillus hyphae were detected in seven of those samples (mostly from young adult patients) simulating ABPA and leading to coinage of "Allergic Aspergillus Sinusitis (AAS)." This represented the fourth type of sinus aspergillosis described at that time following fulminant, indolent and localized non-invasive fungal ball (mycetoma)-like varieties (Katzenstein *etal*.,1983).

However, the term AAS was changed to "allergic fungal sinusitis" when etiologic agents other than *Aspergillus* spp. (dematiaceous group including *Bipolaris* spp., *Alternaria* spp., *Curvularia* spp.) were identified (Allphin *etal.*,1991;, Slavin ,2006).Some of these fungi had ability to produce the mycotoxins which are toxic compounds that are naturally produced by certain types of moulds (fungi). several hundred different mycotoxins have been identified, but the most commonly observed mycotoxins that present a concern to human health and livestock include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone and nivalenol/deoxynivalenol. Mycotoxins can cause a variety of adverse health effects and pose a serious health threat to both humans and livestock.(Winnie and Sabran,2018).

In this study we amid to investigation ability of some fungi isolated from rhino sinusitis on producing of mycotoxins.

Material and Methods

After having the Ethical agreement of the patients, we started nasal specimens' collection form patients suffers from Rhino sinusitis whom attended to AL-Diwaniyah teaching hospitals with help specialist doctor, throughout the period from October 2018 till February 2019. There were 75 patients were screened during the study period.

Specimens collection

Specimens for fungal culture were obtained After checking patient do not use the antibiotics with the help of the specialist doctor, by diagnostic nasal endoscopy. sinus aspirate was subjected to potassium hydroxide mount, and, if fungal elements were identified, then fungal culture was done on Sabouraud dextrose agar that contain chloramphenicol (0.25 l mg / ml) to prevent the growth of bacteria and incubated at

25°C and 37°C for 4 weeks, after growing of fungi were purified by re culture it on the media to get pure colonies. (Ferguson ,2005).

The developing colonies were diagnosed depending on the phenotypic characteristics of the colonies, which included color, odor, growth speed, height, tissue and pigment production, as well as microscopic characteristics by using15% potassium hydroxide solution and blue cotton dye to detect shape of hyphae, spores of fungi depending on ((Baron *etal*., 1994) .

Toxins Extraction

The isolates of Aspergillus spp. were investigated for the production of the AFs using Coconut liquid medium. About 1ml of the mold spore suspension was inoculated in 25ml of Coconut liquid medium in sterile cups and incubated at 28 ± 2ºC for 10 days in triplicates, for AFs detection (Pane *etal.*,2012). After incubation, the contents of each cup were filtered through whatman filter paper No.1 under vacuum. The filtrate was extracted with 25ml of chloroform in a separating funnel with gentle shaking for 1min then let until two layers separated. The lower layer (organic) was taken and evaporated to dryness in oven at 40-50ºC. The residue was re-dissolved in 1ml of chloroform and the containers were stored at -20ºC until uses.(AL-Jassani and AL-Meamar ,2017)

TLC Test

TLC was carried out on a silica gel 60 plate 20 × 20cm (Merck, Germany) and with modification using toluene: chloroform: acetone (15:75:10 v/v) as mobile phases [Yazdani *etal.*,2011]. AFs mix standard (20µl) (Sigma, USA) and50µl of test samples were spotted on TLC plates and was run for 60min at room temperature. Plates were air-dried and checked over a UV Transilluminator (360nm). The AF spots were marked and the retention factor (R*f*) value was calculated for each spot in comparison with the standard.(AOAC,1984).

Results and Discussion

Results of this study showed that 45/75 (60%) from patients had positive culture for fungi (Figure 1). this results disagree with results of (Suresh *etal*.,2016) whom showed that (30%) from patients infected with fungi . Fungal infections can occur in any age group, but symptoms differ based on the immunity status of the individual. A high index of suspicion is needed for the diagnosis of fungal rhinosinusitis when patients present with symptoms similar to chronic sinus infection resistant to conventional antibiotic therapy. (Milosevic *etal*.1969).

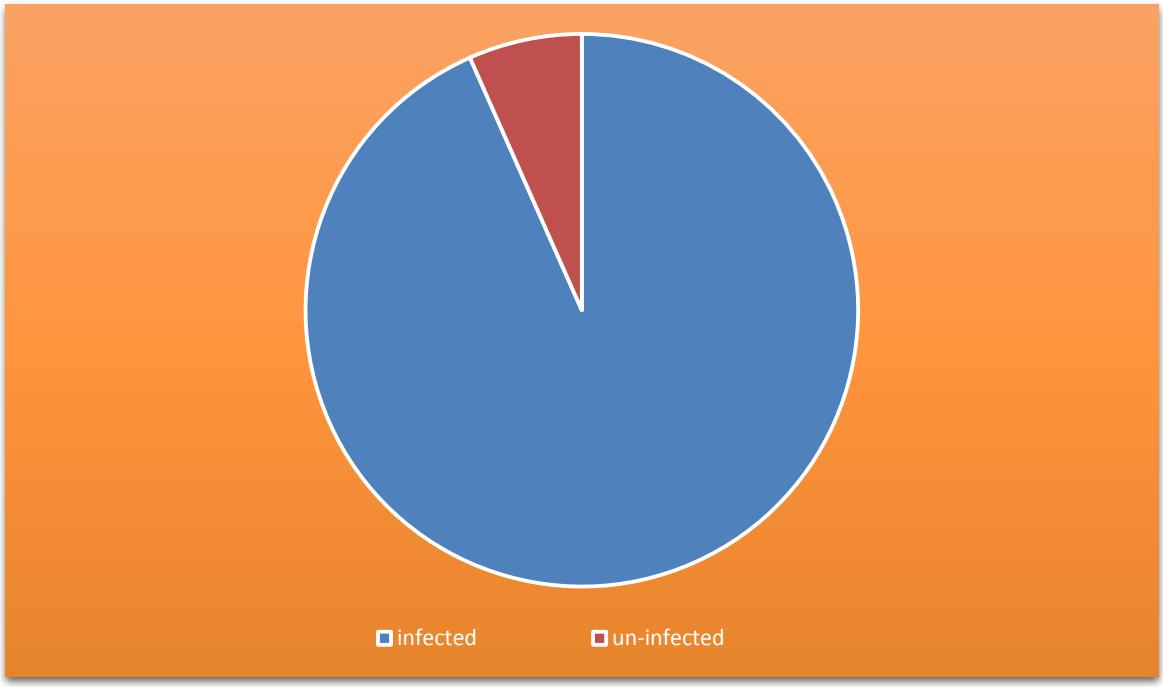

Figure (1) percentage of infected and un –infected patients with fungi

Aspergillus spp is the most common isolates in fungal rhinosinusitis. Where number of its isolates were 20(44.44%) table (1) this results Similar to results of AL-shibly *etal*.,2019 who record that *Aspergillus* spp was most common fungi isolated from sinusitis while Sandeep *etal*.(2016) referred that *Candida* was the most common organism isolated (26.6%) in rhino sinusitis . *Alternaria* spp and *Candida albicans* account 20% from isolates while *Mucor* spp and *Penicillium spp* formed 11.11% and 4.44% respectively.

Aspergillus, a common mold that lives indoors and outdoors Most people breathe in *Aspergillus* spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to *Aspergillus*. It is had large numbers of spores which is the main source of infection and spread in the weather and It is known for its strong resistance to antibiotics.(CDC,2019).

Chronic fungal sinusitis in apparently normal hosts is probably more common in the Recent years , and a variety of saprophytic fungi have been isolated. Infection may take the form of a fungus ball in the sinus, allergic fungal sinusitis or, rarely, locally invasive infection which may be confused with Wegener's granulomatosis or squamous cell carcinoma. Community-acquired chronic fungal sinusitis is a relatively common problem in some tropical and subtropical countries, eg in Africa and India, and imported cases may be encountered. The commonest cause overall is *A. flavus*. In

some instances invasive disease will develop, other fungi should be considered in relation to the country of origin and travel history of the patient.(Cheng *etal*.,2014).

Fungal Rhino sinusitis RS with complex immune modulation in its pathogenesis Geographical variation, it is reported in areas with warm, dry and humid climate (Aeumjaturapa *etal*.,2003). The high prevalence of the disease is noted in India, North Africa, the Middle East and southeastern and southwestern parts of the US (especially Mississippi basin) (Saravanan, *etal*.,2006; Lanza *etal*.,2006). Northern states of the US have a lower frequency of 0.4%, while Southern states reported 10% (Ferguson *etal*.,2000). AFRS constitutes the highest number of cases of CRS in India accounting for 56%–79% of cases (Das *etal*.,2009; Michael *etal*.,2008). FRS cases are also reported from Australia, Malaysia, and Thailand [Aeumjaturapat *etal*.,2003;, Goh *etal*.,2005).

Table (1)Number and percentage of fungal isolates of patients with Rhino sinusitis

Fungal species	No. of isolates	%
Aspergillus spp	20	44.44
Alternaria spp	9	20
Candida spp	9	20
Penicillium spp	5	11.11
Mucor spp	2	4.44
Total	45	100

In this study we examined the ability of most common fungi in this study (*Aspergillus* spp) on Aflatoxin production that appear as Blue spot under UV on TLC Plate with R*f*= (73-80) results showed that 11/20 (55%) from isolate had ability of AF production as show in figure (2,3).

Aflatoxins are poisonous carcinogens that are produced by certain molds (Aspergillus flavus and Aspergillus parasiticus) Aflatoxins have been reported to affect the various body organs like the liver, kidneys, lungs, brain, testes and many endocrine and exocrine organs, the heart, skeletal muscles and the different body systems After entering the body, the aflatoxins are absorbed across the cell membranes where they reach the blood circulation. They are distributed in blood to different tissues and to the liver, the main organ of metabolism of xenobiotics. Aflatoxins are mainly metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1 (Usha *etal*.,2017). In humans and susceptible animal species, aflatoxins especially Aflatoxins B1 are metabolized by cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8,9-epoxide, a reactive form that binds to DNA and to albumin in the blood serum, forming adducts and hence causing DNA damage (Godfrey *etal*.,2013). Orbital cellulitis was the most common complication of toxigenic fungal rhinosinusitis.

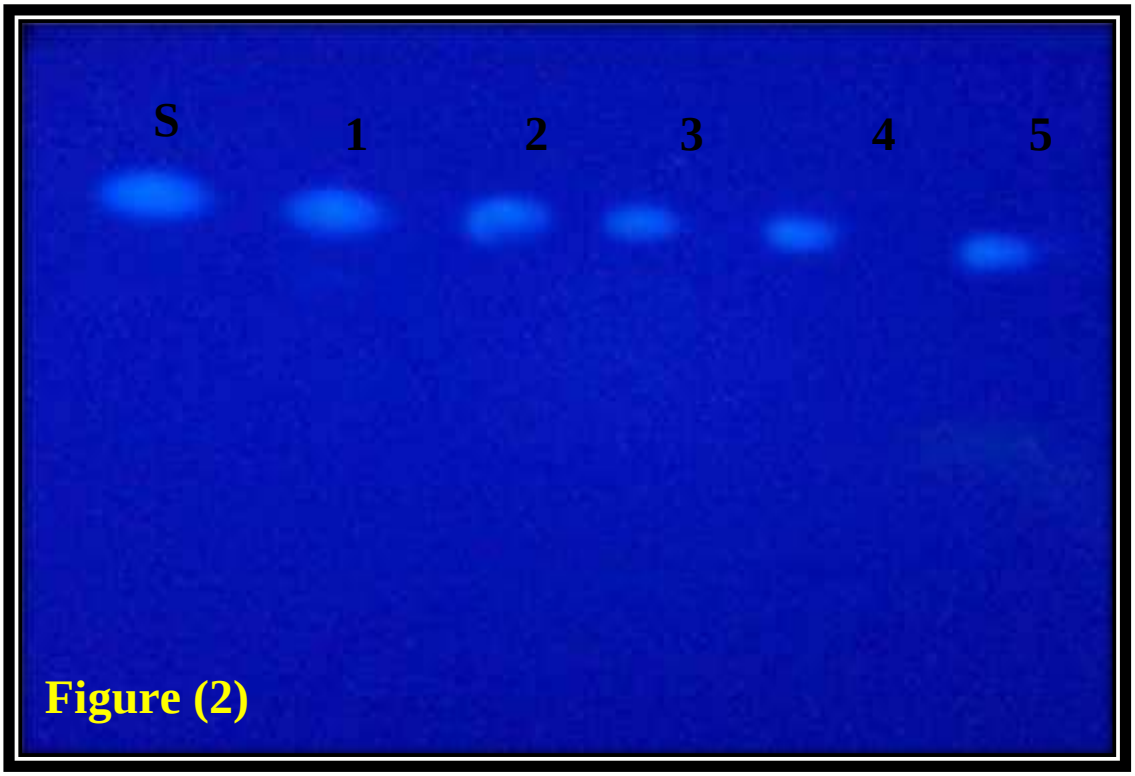

Figure (2)

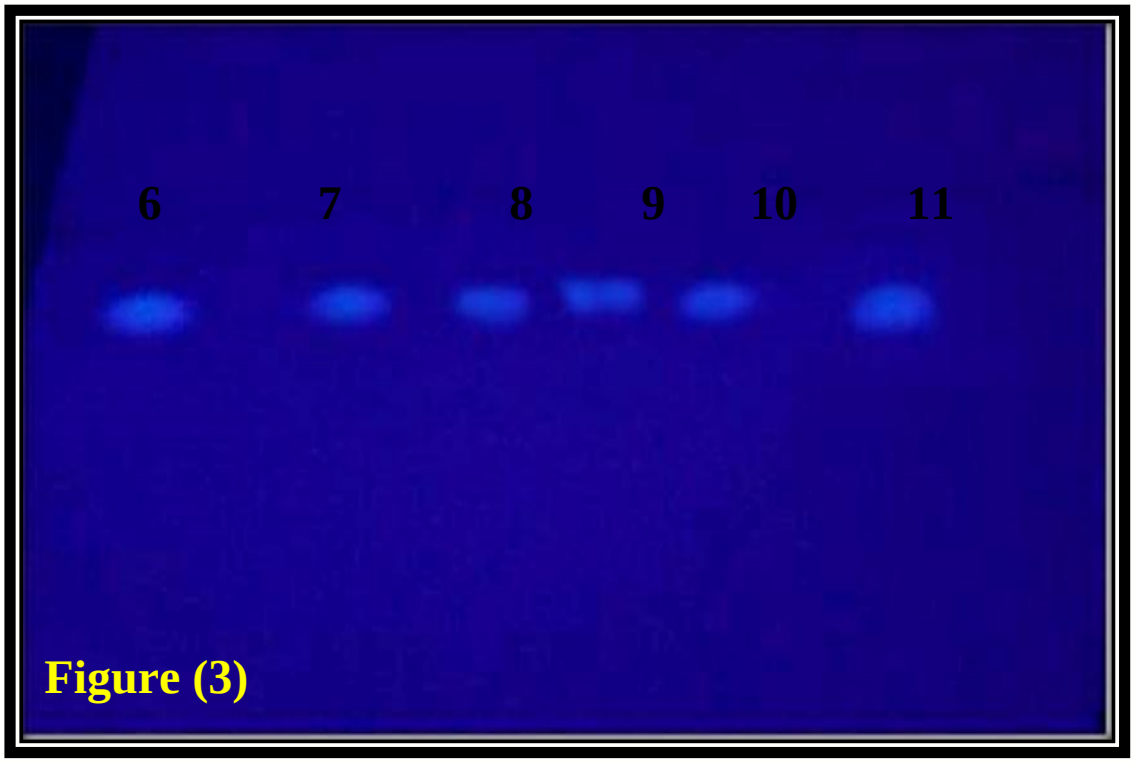

Figure (3)

Figure (2) & Figure (3) Ability of (1-11) Aspergillus isolates to produce Aflatoxin S =Standard Aflatoxin

Aflatoxins cause acute and long term (chronic) carcinogenicity and toxicity ,However, there are some microsomal systems present in the body which are quite helpful in converting harmful B1 Aflatoxins into non-toxic metabolites. These

tiny systematic structures, microsomal systems, are not quite sufficient in converting all the aflatoxins into the mild form. Thus, we are still left with potential vulnerability towards the toxicity caused by the aflatoxins (Patterson and Alicroft, 1970). It has been reported from Uganda that a 14 years old boy died because high levels of aflatoxins in his diet caused acute necrosis in liver (Serck-Hanssen, 1970).

Conclusion

The role of Fungi is clearly important to ENT surgeons and has been very necessary subject to much discussion in the in many studies. The impact of fungi in the upper airways is wider than just its role in fungal sinusitis and it has ability to produce the mycotoxins that had very dangerous effects.

References

- Aeumjaturapat, S.; Saengpanich, S.; Isipradit, P.; Keelawat, S. (2003). Eosinophilic mucin rhinosinusitis: Terminology and clinicopathological presentation. J. Med. Assoc. Thail. 86:420–424.
- AL-Jassani, M. J and AL-Meamar, T.S. (2017) Aflatoxina and Aflatoxigenic fungi contaminated of dried fruit in Iraqi market. Journal of gloubal pharma technology 9(10):299-308.
- Allphin ,A.L.; Strauss, M.; Abdul-Karim, F.W.(1991) Allergic fungal sinusitis: Problems in diagnosis and treatment. Laryngoscope. 101:815–820.
- Alshibly1, M. K. ; Al Zamily, I.A. and Haider,R.(2019) Antibiotic Sensitivity of Fungi Isolated from Patients with Sinusitis. Sci. J. Med. Res. 3 (10): 64-69
- AOAC (1984). Official methods of analysis (Gentennial Edition) ,Association of official analytical chemists,Washington,DC,USA.
- Bachert, C. and Holtappels, G. (2015). Pathophysiology of chronic rhinosinusitis, pharmaceutical therapy options. GMS Curr. Top. Otorhinolaryngol. Head Neck Surg. 94:S32–S63.
- Baron, E.J.; Pererson, L.R.; Finegold, S.M .(eds)(1994) Bailey and Scott's Diagnostic Microbiology. 9th ed. CV Mosby, St. Louis.
- Centers for Disease Control and Prevention(CDC) (2019).Fungal diseases .Rewiew .CDC24/7:saving lives,protecting peoples ™ .
- Chakrabarti, A.; Rudramurthy, S.M.; Panda, N.; Das, A.; Singh, A. (2015).Epidemiology of chronic fungal rhinosinusitis in rural India. Mycoses. 58:294–302.
- Chakrabarti ,A.;Sharma, S.C.; Chandler, J. (1999).Epidemiology and pathogenesis of paranasal sinus mycoses. Otolaryngol. Head Neck Surg. 107:745–750.
- Chang ,C.C.; Incaudo, G.A.; Gershwin, M.E.(2014). Diseases of the Sinuses: A Comprehensive Textbook of Diagnosis and Treatment. Springer; Berlin, Germany.
- Chatterjee, S.S.and Chakrabarti, A. (2009). Epidemiology and medical mycology of fungal rhinosinusitis. Otorhinolaryngol. Clin. An. Int. J.;1:1–13.
- Ferguson, B.J.; Barnes, L.;Bernstein ,J.M.; Brown, D.; Clark, C.E.; Cook, P.R.; DeWitt, W.S.; Graham, S.M.;Gordon, B.; Javer, A.R., et al.

(2000).Geographic variation in allergic fungal rhinosinusitis. Otolaryngol. Clin. N. Am. 33:441–449.

- Ferguson ,B.J. (2005).The diagnosis of allergic fungal sinusitis. In: Leviene HL, editor. In sinus surgery, endoscopic and microscopic approaches. Thiem Medical;. pp. 290–299.
- Godfrey, S. ;Bbosa, D. K.; Lubega,A.; Ogwal-Okeng,J.; William, W. Anokbonggo and Kyegombe,D.B(2013). Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems. Open access peer-reviewed
- Goh, B.S.; Singh, G. B., Mohamed, R. I.;, Pit S.; Abdul Samad, S. (2005).Prevalence of allergic fungal sinusitis in refractory chronic rhinosinusitis in adult Malaysians. Otolaryngol. Head Neck Surg. 133:27–31.
- Katzenstein ,A.L.A.; Sale, S.R.; Greenberger ,P.A. (1983).Allergic Aspergillus sinusitis: A newly recognized form of sinusitis. J. Allergy Clin. Immunol. 72:89–93.
- Lanza, D.C.; Dhong, H.J.; Tantilipikorn, P.; Tanabodee, J.; Nadel, D.M.; Kennedy D.W. (2006).Fungus and chronic rhinosinusitis: From bench to clinical understanding. Ann. Otol. Rhinol. Laryngol. 115:27–34.
- Michael, R.C.; Michael ,J.S.; Ashbee, R.H.; Mathews, M.S. (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. 51:493–496.
- Millar J.W.and Johnston A.L.D. (1981).Allergic aspergillosis of the maxillary sinuses. Thorax. 36:710.
- Milosevic, B. El-Mahgoub, S.; Aal, O.A. El-Hassan, A.M. (1969) Primary aspergilloma of the paranasal sinuses in Sudan. A review of seventeen cases. Br J Surg 56:132–137.
- Pane ,B. ;Ouattara – Sourabie; Philippe, A. ;Nikiema ;Nicolas, B. Aly ,S. (2012).Aflatoxigenic potential of Aspergillus spp. isolated from groundnut seeds, in Burkina Faso, West Africa. African journal of microbiology research 6(11):2603-2609
- Patterson, D. S. P. and Alicroft. R. (1970). Metabolism of aflatoxin in susceptible and resistant animal species. Food Cosmet. Toxicol,8:43
- Pleis ,J.R.; Ward, B.W.; Lucas, J.W. (2010).Summary health statistics for U.S. adults: National Health Interview Survey. Vital Health Stat. 10:1–207.
- Ponikau, J.U.; Sherris, D.A.; Kern, E.B.; Homburger, H.A.; Frigas, E.; Gaffey T.A.; Roberts, G.D. (1999).The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin. Proc. 74:877–884.
- Rupa, V.; Jacob, M.; Mathews, M.S.; Job, A.; Kurien, M.; Chandi, S.M. (2002).Clinicopathological and mycological spectrum of allergic fungal sinusitis in South India. Mycoses. 45:364–367.
- Sandeep ,S. M.; Dayanand ,A.; D; George, Z. M.; Sengottaiah ,P.; M., Ravisankar , M.S., and Vaidyanathan, V.S.(2016). Prevalence and clinical profile of fungal rhino sinusitis Allergy Rhinol (Providence). 7(2): e115–e120.
- Saravanan, K.; Panda, N.K.; Chakrabarti, A.; Das, A.; Bapuraj; R.J. (2006).Allergic fungal rhinosinusitis: An attempt to resolve the diagnostic dilemma. Arch. Otolaryngol. Head Neck Surg.132:173–178.

- Schiller, J.S.; Lucas, J.W.; Ward, B.W.; Peregoy, J.A. (2012).Summary health statistics for U.S. Adults: National health interview survey. Vital Heal. Stat. 2012;10:1–171.
- Serck-Hanssen, A. (1970). Aflatoxin-induced fatal hepatitis? A report from Uganda. A. Arch. Environ. Health, 20:729.
- Slavin ,R.G. (2006). Sinusitis: Viral, bacterial, or fungal and what is the role of staph? Allergy Asthma Proc. 27:447–450.
- Usha, P. Sarma, Preetida, J. Bhetaria; Prameela D.i; and Anupam V.(2017). Aflatoxins: Implications on Health. Indian J. Clin .Biochem. 32(2): 124–133.
- White, L.C.; Jang, D.W.; Yelvertan, J.C.; Kountakis, S.E. (2015).Bony erosion patterns in patients with allergic fungal sinusitis. Am. J. Rhinol. Allergy. 29:243–245. 22.
- Winnie-Pui,P.L. and Sabran M. R. (2018) Mycotoxin: Its Impact on Gut Health and Microbiota .Front. Cell. Infect. Microbiol.
- Wise, S.K.; Ghegan, M.D.; Gorham, E.; Schlosser, R.J. (2008). Socioeconomic factors in the diagnosis of allergic fungal rhinosinusitis. Otolaryngol. Head Neck Surg. 138:38–42.
- Yazdani .D.;Zain al Abidina ,M.A.;Tana,Y.H.;Kamaruzamana.(2011). Molecular identification of Aspergillus and Eurotium species isolated from rice and their toxins producing ability .Microbiolgy ,80(5):720-727.