A study on the prevalence of fungal isolates among Rhinosinusitis patients at CMCH, Coimbatore.

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In

MICROBIOLOGY– BRANCH IV

The Tamil Nadu

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MAY 2018 .

CERTIFICATE

This is to certify that the enclosed work **"A study on the prevalence of fungal isolates among Rhinosinusitis patients at CMCH, Coimbatore"** submitted by **Dr.N.Vandarkuzhali** to The Tamilnadu Dr.MGR Medical University is based on bonafide cases studied and analysed by the candidate in the Department of Microbiology, Coimbatore Medical College and Hospital, Coimbatore during the period from June 2016 to May 2017 under the guidance and supervision of **Dr.P.Shankar,MD.,** Associate Professor Department of Microbiology and the conclusion reached in this study are her own.

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I, **Dr. N. Vandarkuzhali ,** solemnly declare that the dissertation entitled **"A study on the prevalence of fungal isolates among Rhinosinusitis patients at CMCH, Coimbatore**" was done by me at Coimbatore Medical College Hospital, Coimbatore during the period from June 2016 to May 2017 under the guidance and supervision of **Dr. P.Shankar, M.D.,** Associate Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. MGR. Medical University towards the partial fulfilment of the requirement for the award of M.D. Degree (Branch – IV) in Microbiology.

I have not submitted this dissertation on my previous occasion to any University for the award of any degree.

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The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation Proposal is accepted / ~~Not accepted~~ and you are permitted / ~~Not permitted~~ to proceed with the above Study.

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ABBREVIATIONS

AFRS	:	Allergic Fungal Rhinosinusitis
BDM	:	Broth dilution method
CFRS	:	Chronic Fungal Rhinosinusitis
CGFRS	:	Chronic Granulomatous Fungal Rhinosinusitis
CIFRS	:	Chronic Invasive Fungal Rhinosinusitis
CLSI	:	Clinical Laboratory Standard Institute
DMSO	:	Dimethyl sulfoxide
E TEST	:	Epsilometer test
ELISA	:	Enzyme Linked Immunosorbent Assay
FB	:	Fungal ball
FRS	:	Fungal Rhinosinusitis
GMS	:	Gomori Methenamine silver
H&E	:	Haematoxylin and eosin
HPE	:	Histopathological examination
KOH	:	Potassium hydroxide
MIC	:	Minimum Inhibitory Concentration

MOPS	:	3N-Morpholino propane sulphonic acid
MRI	:	Magnetic resonance imaging
NFRS	:	Non fungal rhinosinusitis
PAS	:	Periodic acid schiff
PDA	:	Potato Dextrose agar
PNS	:	Paranasal sinus
RPMI	:	Rosewall Park Memorial Institute

INTRODUCTION

INTRODUCTION

Rhino sinusitis is a group of disorders characterized by mucosal inflammation of the nose and para nasal sinuses[9,21] and is a common disorder affecting 20% of the popuplation[6,21]. The Task force on Rhino sinusitis 2007-Formulated a Diagnostic criteria for classification of disease in to acute and chronic based on the duration of illness. A duration of less than 4 weeks as acute and more than 12 weeks without complete resolution is considered as Chronic Rhino sinusitis.

Acute Rhino sinusitis is well categorized. However controversies exist in the categorization of Chronic Rhinosinusitis[21] of which Several factors were implicated in the development. Ostia blockage by edema, inflammatory mucosa, and delayed recovery of muco ciliary function are some of the mechanisms that leads to a transition from acute to chronic inflammatory process[7].

Nasal polyp is considered as a part of the spectrum of Chronic Rhinosinusiti[7] and it is a multifactorial disease which is characterized by chronic inflammation. Virus, Bacteria, Fungi and genetic factors all can cause chronic inflammation which in turn leads to reactive mucosal hyperplasia and formation of nasal polyp[7].

The prevalence of Mycotic etiology was once thought as uncommon in cases of Rhinosinusitis. The first reported case was by Plaignaud in 1971[6]. However the frequency of fungal sinusitis has been increasing in the past two decades[18] especially in North India[9]. It is estimated that about 5-10% of patients with Chronic actually have fungus as an etiological agent[9]. This is because of improved techniques in Mycology, Histopathology, and Radiology which has led to improved detection rate, alteration in normal bacterial flora of nasal and para nasal sinuses due to increased use of broad spectrum antibiotics, and growing number of immunosuppressed indiviuals[3].

Fungal sinusitis is broadly defined as spectrum of pathological condition associated with sino nasal inflammation due to the presence of fungi[4]. Fungal sinusitis can occur in immunocompromised and as well as immunocompetent individuals[18]. The disease process is chronic and indolent in immunocompetent individual whereas it will be rapidly progressive and fulminant in immunocompromised individuals[18].

Based on several studies in literature, the prevalence of fungal sinusitis varies widely in geographical distribution[7]. This is because of difference in the technique of specimen collection, fungal detection techniques and geographical condition. Geographical condition affects both the prevalence rate and the type of organism isolated[7]. Environmental factors like agriculture,

economy, warm moist climate and host factors also play a significant role in the causation of Fungal Rhinosinusitis[6,7].

Fungal sinusitis can be broadly classified in to Invasive and non-invasive forms[3]. Allergic fungal rhino sinusitis(AFRS) and fungal ball (Mycetoma) comes under Non-invasive forms whereas Invasive form includes Acute fulminant and chronic invasive sinusitis[2].

It is evident from most of the studies that, AFRS is the most commonest form in India[10] . Aspergillus flavus being most frequently isolated followed by Aspergillus fumigatus[3]. Dematiaceous fungi like Curvularia, Bipolaris, Alternaria are common isolates in western world[3].

Diagnosis of invasive fungal sinusitis needs high index of clinical suspicion in immunocompromised individuals who present with fever, nasal congestion, discharge and facial pain[2]. Based on Direct microscopy, Fungal culture and Histopathological examination[5] diagnosis should be made. Though Histopathology is important to distinguish invasive from non-invasive form based on mucosal integrity and classifying the disease, Direct microscopy and fungal culture clinches the diagnosis of type of fungal isolates[5].

As fungal sinusitis can cause severe symptoms which can impede the normal day to day activities, it can result in huge economical burden to the individual. Invasive form can cause lot of morbidity and Acute fulminant

form sometimes causes life threatening situation. So early accurate diagnosis and appropriate treatment can reduce the morbidity and mortality.

The recommended treatment is surgical debridement of necrotic tissue combined with intravenous antifungal agent. Amphotericin-B is the treatment of choice because it is effective against Zygomycetes and Aspergillus[2].

The purpose of this study is to isolate the Fungi in the nasal and sinus secretions, sinonasal polyps and their antifungal susceptibility pattern by CLSI micro broth dilution and E-strip method, then a comparative analysis is to be done between the two methods. As compared to other forms of FRS, the clinical presentation of most of the acute fulminant forms are subtle, and a high index of clinical suspicion is needed. Early isolation of fungi, histopathological evidence of invasion and study of their antifungal susceptibility pattern is of paramount importance. In addition, because of growing number of resistant fungal strains the study of Antifungal susceptibility pattern will helps in early appropriate administration of Antifungal therapy combined with surgical debridement of all the involved tissues and preserving the natural barrier will prevents the morbidity and mortality.

AIM & OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY

1. To isolate and identify the fungi among the chronic rhinosinusitis patients.

2. To categorise the disease by its clinical presentation and histopathological examination.

3. To assess the risk factors causing fungal rhinosinusitis

4. To do antifungal susceptibility by CLSI reference microbroth dilution method and E-strip method .

5. To compare CLSI microbroth dilution method and E-strip method of antifungal susceptibility.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Sinusitis- Inflammation of Paranasal sinuses. Rhinitis- Inflammation within the nasal cavity. In 1996, the Task force on Rhinosinusitis[27], sponsored by American Academy of Otolaryngology- Head and Neck surgery, The American Rhinology Society and The American Academy of Otolaryngologic Allergy recommended the replacement of the term Sinusitis with Rhinosinusitis. This is because Rhinitis commonly precedes sinusitis and also occurrence of one without the other is extremely rare.

The Task force on Rhinosinusitis 2007- Formulated a Diagnostic criteria for Rhinosinusitis[27]

Acute Rhinosinusitis	Upto 4 weeks of purulent nasal discharge(anterior, posterior, or both) accompanied by nasal obstruction, facial pain-pressure-fullness or both
Sub Acute Rhinosinusitis	Symptoms persisting for 4-12 weeks
Chronic Rhinosinusitis	12 weeks or longer of two or more of the following signs and symptoms. • Muco purulent drainage(anterior, posterior, or both) • Nasal obstruction

	• Facial pain-pressure-fullness • Decreased sense of smell AND inflammation is documented by one or more of the following findings: • Purulent mucus or edema in the middle meatus or Ethmoid region. • polyps in nasal cavity or middle meatus • Radiographic imaging showing inflammation of paranasal sinuses.
Recurrent Acute Rhino sinusitis	>4 acute episodes in 1 year with resolution between episodes

Predisposing factors:

There are a lot of host and environmental factors that play a role in the pathogenesis of Rhino sinusitis like allergy, infectious agents like Bacteria, Virus, Fungi , Ciliary dysfunction in case of cystic fibrosis and Mechanical obstruction to sinus ostia like concha bullosa.

Signs and Symptoms associated with Diagnosis of Rhinosinusitis(1996 Rhinosinusitis Task Force) [27]

Major Factor	Minor Factor
• Facial pain/pressure • Nasal obstruction • Nasal discharge/discoloured postnasal discharge. • Hyposmia/Anosmia • Purulence on examination • Fever(acute only)	• Head ache • Fever(all non acute) • Halitosis • Dental pain • Fatigue • Cough • Ear pain/pressure/fullness

Fungal Sinusitis:

The incidence of Fungi as an etiological agent in the diseases of Nose and Para nasal sinuses has been increasing over the past 30 years. As fungi are ubiquitous, inhalation of fungal spores gain access into the Nose and Para nasal sinuses and further pathogenesis depends not only on the inherent nature of the fungus but also on the host immune response and complex fungus-host interaction.

Historical Perspective:[21]

- Plaignaud was the first who described Fungus Tumor in Maxillary sinus in a 22 Year old soldier in 1791.
- In 1897 Oppe described Aspergillus species causing sinusitis in Sphenoid sinus.
- The two categories of fungal sinusitis i.e non-invasive and invasive was first recognized by Hora in 1965.
- Fulminant form of sinusitis in immunocompromised patients was recognized by McGill et al, in 1980.
- Miller et al, in 1981 and Katzenstein in 1983 independently discovered the pathophysiologic resemblance between Chronic rhino sinusitis with mucosal plug and patient with Allergic broncho pulmonary aspergillosis. Thus leading to the description of fourth form of the disease i.e Allergic Aspergillus sinusitis. But later Dematiaceous fungi was found to be the commonest cause of Allergic form of sinusitis which results in the replacement of the above term into Allergic Fungal Rhino sinusitis(AFRS).
- Ponikau et al. had given a new name to allergic type of disease as Eosinophilic fungal Rhino sinusitis.

Classification of Fungal sinusitis[2,3,10,21]

Classification is based on clinical, radiological and histopathological manifestations of host-pathogen relationship. Broadly it can be classified into Invasive and Noninvasive disease based on the sinus mucosal invasion by the fungus.

Invasive Fungal Rhino sinusitis (IFS)	• Acute invasive fungal Rhino sinusitis(AIFR) • Granulomatous invasive fungal Rhino sinusitis(GIFR) • Chronic invasive fungal Rhino sinusitis(CIFR)
Non invasive Fungal Rhino sinusitis(Non IFS)	• Localised colonization of the Nasal and Para nasal sinus mucosa by fungi. • Sinus Fungal Ball(FB). • Allergic Fungal Rhino sinusitis(AFRS)

i) Invasive Fungal Rhinosinusitis:

It is defined as the invasion of fungal elements into the sinus mucosa, sub mucosa, blood vessels and bone on histopathological examination

Diagnostic criteria by deShazo for diagnosing Invasive FRS[2,3].

i) Mucosal thickenings or air fluid levels within the sinus cavity on radiological imaging. Histopathological evidence of hyphal forms within the sinus mucosa, sub mucosa, blood vessel or bone.

ii) In Granulomatous invasive sinusitis, histopathological evidence of fungal hyphae within the sinus mucosa, sub mucosa, blood vessel or bone in association with granuloma consisting of giant cells.

a) Acute Invasive Fungal Rhinosinusitis[2,3,27]

It can also be termed as Acute fulminant Invasive or Acute Necrotizing Fungal sinusitis. The term acute denotes very protracted course of < 4 weeks. Some form of immunosuppression is always found in the patient presenting with this kind of disease. Members of Zygomycetes like Mucor, Rhizopus and Absidia, or Aspergillus spp are the commonest fungi causing invasive form of disease. Bipolaris, Candida, Fusarium etc are rarely reported. Invasion of fungal hyphae into the blood vessels like carotid arteries and cavernous sinus leading to necrosis and neutrophilic infiltration are the histopathological findings. Dense fungal hyphae are usually seen in the necrotic tissues. The term fulminant denotes rapid progression with fatal outcome. So it necessitates early intervention which will cut down the disease progression and prevents the fatal outcome. A high index of clinical suspicion is needed in the diagnosis of AIFRS in immunocompromised patients, presenting with

symptoms of sinusitis as most of the times the clinical findings are subtle. Patients will have headache, rhinorrhea, nasal congestion. Fever is the most important finding in 50% to 90% of the patients.

b) Granulomatous Invasive Fungal Rhinosinusitis[2,3,27]

This form of disease is usually seen in immunocompetent individuals who presents with an enlarging mass in the cheek, nose, orbit, para nasal sinuses with proptosis. On histopathologic examination non-caseating granulomas with multinucleated giant cells and scanty fungal hyphae are seen. This form of disease has been primarily seen in Sudan, India, Pakistan, and Saudi Arabia.

c) Chronic Invasive Fungal Rhinosinusits[2,3,27]

It is characterized by dense accumulation of fungal hyphae, vascular invasion and sparse inflammatory reaction. Aspergillus fumigatus is the commonest agent isolated from this type of disease. It usually affects immunocompromised individuals. Some times patients may present with orbital apex syndrome.

ii)Non-Invasive Fungal Sinusitis:[2,3,27]

a) Localized fungal colonization of Nasal or Paranasal sinus mucosa:

This condition was previously termed as Saprophytic Fungal Infection. The condition usually occurs in patients who had undergone previous sinus surgery, in whom alteration in the function of muco ciliary apparatus leads to the formation of mucus crusts over which the fungus may grow. The condition is diagnosed not only by fungal growth on culture but also on visible fungal material in nasal and paranasal sinus cavity on diagnostic nasal endoscopy.

b) Fungal Ball:[2,3,27]

Literatures have shown that various terms are used to name this condition which includes Mycetoma, Aspergilloma, Chronic non-invasive Granuloma but it was found that Fungal Ball was the correct term to be used. Aspergillus spp is the commonest organism encountered. It is characterized by the presence of mucopurulent cheesy clay like material in the sinus cavity, radiological evidence of sinus opacification with or without calcification, dense conglomeration of fungal hyphae separate from the sinus mucosa, nonspecific chronic inflammation without eosinophilic predominance.

c) Allergic Fungal Rhinosinusitis[2,3,27](AFRS)

Though lot of controversies exist in the definition of AFRS, Bent and Kuhn proposed a diagnostic criteria which includes,

i) Type-I hypersensitivity

ii) Nasal polyps

iii) Characteristic CT findings(central areas of hyperattenuation within the sinus cavity correspond to areas of hypointensity on T1 weighed MR images and signal void on T2 weighed MR images)

iv) Positive fungal stain or culture

v) Allergic mucin with fungal elements and no tissue invasion.

Of all these criteria, presence of fungi in allergic mucin is essential for diagnosis. Millar et.al proposed this condition as an upper airway version of Allergic Broncho pulmonary Aspergillosis.

AFRS patients will present with typical symptoms of CRS like nasal polyp, progressive nasal congestion, airway obstruction and periods of exacerbated symptoms. Aspergillus flavus is most commonly isolated organism followed by Aspergillus fumigatus in our country. However studies have shown that Black fungi are being commonly isolated in western countries.

Epidemiology:

i) Prevalence of the Disease:

The prevalence of Fungal rhino sinusitis has increased over the past decade especially in the tropical countries like India, Pakistan and Sudan[1,2]

When compared to US. various studies have shown that AFRS is the most commonest form of Fungal Rhinosinusitis.[1,2,10]. This increasing prevalence can significantly affect the physical quality of life which in turn causes huge economic burden. Environmental factors like Agriculture, Economy and Warm moist climate also plays an important role in the epidemiology of Fungal Rhinosinusitis[6].

ii) Geographical Distribution:

Geographical area is an important determinant in the occurrence of FRS particularly AFRS and Granulomatous invasive FRS [10]. AFRS occurs more commonly in India, North Africa, middle-east and parts of USA like Mississipi basin and south-east and south-west parts of United states[10]. The presence of warm dry climate in North India, Sudan, Saudi Arabia and Arizona had recorded a high number of cases[10]. Granulomatous invasive FRS are exclusively seen in India, Pakistan and Sudan but very rare in US[10]. Fungal ball cases are found to be more common in Taiwan, France and Italy[10].

iii) Host Factors:

For unknown reasons in most of the studies AFRS was found commonly in males[10] especially in middle aged males in rural areas because they frequently go to fields and acquire the fungal spores upon injury to the nasal mucosa. Some studies have shown that malnourishment, undiagnosed DM, etc., due to poverty plays a major role in occurrence of AFRS[10]. Most frequently Atopic individuals are more prone to get AFRS, because of allergic response to the etiological fungi being harboured in the sinus cavity resulting in the formation of sinonasal polyposis and Pansinusitis[1]. Fungal ball is more common in middle aged or elderly females [10].Previous sinus surgery, Endodontic treatment on maxillary teeth are important risk factors for the development of Fungal ball[10].

Acute invasive sinusitis most commonly occurs in elderly who are possibly diabetic, or on chemotherapy[10]. Patient with neutropenia or impaired neutrophil function like diabetic ketoacidosis, malignancies, aplastic anaemia, solid organ transplantation are at risk[10]. As fungus are ubiquitous , inhalation of fungi is an unavoidable one which can breech the normal immunological barrier in an immunocompromised host. Infection spreads along blood vessels and nerves infecting the sinus tissue, creating an acidotic environment which further favours the growth of the fungi[10].

iv) Agent Factors:

Members of Zygomycetes are more common in causing Acute invasive FRS, especially Rhizopus spp. mainly in tropical countries[10]. The commonest fungi causing Granulomatous invasive and Chronic invasive FRS are Aspergillus flavus and Aspergillus fumigatus respectively [10]. It has been evident from most of the studies that Aspergillus flavus followed by Aspergillus fumigatus are commonly responsible for AFRS in both North and South India but Phaeohyphomycotic fungi like Bipolaris, Curvularia, Alternaria are more common in causing AFRS in North America[10]. In case of Fungal ball mostly the cultures are sterile except when Aspergillus species are isolated[10].

Investigations:

i) routine investigations for diagnosing the underlying risk factors which include,

- **Total count, differential count, absolute eosinophil count, ESR**

  In case of allergic fungal sinusitis where absolute eosinophil count will be raised. In case of Acute invasive FRS neutrophil may be low which implies an underlying immunosuppression.

- **Blood sugar levels**

  Poor glycemic status implies an immunosuppresssive state

- **HIV testing**
- **Liver function**- Chronic liver disease- Immunosuppression
- **Total serum IgE**
- **Anergy panel for cellular and humoral immunity.**
- **Renal function test**

  Chronic renal failure-Immunosuppression.
- **CHEST X-RAY**

  Patients with allergic fungal sinusitis can have co-existence of Allergic Broncho pulmonary Aspergillosis which can be detected by chest X-ray PA view.

ii) Investigations to detect the type and extent of the disease, which includes

- **PLAIN RADIOGRAPHY OF PARANASAL SINUSES:**

  Radiologic evidence of sinusitis of one or more paranasal sinuses with or without flocculent calcifications is supportive of allergic FRS.
- **COMPUTED TOMOGRAPHY:**

  CT Paranasal sinuses documents the changes within the sinus cavity and baseline information of anatomy which is essential both for surgery and detection of disease progression.[21] Bony erosion, facial soft tissue thickening and extrasinus involvement are the classical features of

AIFRS[21]. Though unilateral involvement with homogenous opacification of the sinuses are common [21] Complete or partial sinus opacification of a single sinus i.e., maxillary followed by sphenoid sinus is more in favour of diagnosis of Fungal ball[21].

MAGNETIC RESONANCE IMAGING:

Though the CT PNS detects the bony erosion impending intracranial spread are detected only by MRI[27].

DIAGNOSTIC NASAL ENDOSCOPY:[27]

Findings may include

- Fungal tufts –growing on retained secretions
- Polypoidal swellings /polyps
- Allergic mucin , in cases of allergic fungal sinusitis.(golden yellow peanut butter like), purulent discharge.
- Soft cheese like material(white to brown/black)
- Granulomatous mass
- Pale White necrotic debris ,Black coloured eschar

HISTOPATHOLOGY:

Histopathological appearance of lesions is important and are an adjunct in establishing the diagnosis of the disease its prognosis and for deciding treatment protocols

HPE:

i) **ALLERGIC FUNGAL SINUSITIS:** The features are Scattered fungal hyphae in mucinous material with abundant eosinophils and Charcot Leyden crystals. Allergic mucin is characterized by clumps of eosinophil and other cellular debris, within a background of pale eosinophilic - basophilic, amorphous mucin. The fungal elements tend to be sparse and are without subepithelial tissue invasion or fungal ball formation[27].

ii) **FUNGAL BALL:** It is characterized by non specific inflammatory reaction with no eosinophilic or neutrophilic predominance, no evidence of vascular invasion or granuloma but with formation of dense conglomeration of fungal hyphae separate from the sinus [27]

iii) **GRANULOMATOUS INVASIVE FRS:** Non-caseating granuloma with foreign body type of giant cells, vasculitis, vascular proliferation and perivascular fibrosis with scanty fungal hyphae may be seen[27].

iv) **CHRONIC INVASIVE FRS:** Sparse inflammatory reaction, presence of vascular invasion, and dense accumulation of hyphae are the characteristic features of CIFRS[27].

SPECIMEN COLLECTION AND PROCESSING [25,26]

The collection, transport and processing of clinical specimens encompass one of the most important considerations in determining the etiology of fungal disease.

IDEAL SAMPLE [25,26]

Surgical samples like polypoidal tissues, cheesy material or necrotic material in sinonasal cavity should be transported in a saline filled sterile container in an order to maintain fungal viability. Ideally processing should be done immediately without any delay. Another container containing 10% formalin is used for histopathological examination of the specimen . The most beneficial ideal sample would be, collection before the initiation of antimicrobial therapy.

DIRECT MICROSCOPIC EXAMINATION [25,26]

Direct microscopy should be done using 10% KOH .Fluorescent calcofluor white stain with or without KOH is superior to the use of KOH alone.

i) It will provide a rapid information which aids the clinician to initiate the treatment.

ii) It is useful in preliminary identification of the organism which may be detected later in culture.

iii) Identification of unique fungal elements may help in deciding the need for additional specimen from other sites, special media or serological tests.

Zygomycetes on Direct microscopy [26]

Broad upto 25 µm in diameter , aseptate , non dichotomously branching hyaline hyphae are seen. Some times branching may occur at rght angles. Thick walled chlamydospores of 15-30 µm in diameter may form. The special stain like GMS, PAS, and Gridley fungus do not colour as deeply as they stains other fungi.

Aspergillus species on Direct microscopy [26]

Septate hyphae of 3-12µm in diameter with dichotomous branching at 45º angles. Hyphae are nearly parallel to one another. They often tend to grow radially.

CULTURE:

A minimum of 0.5 ml of homogenized tissue should be inoculated into two slants of Sabouraud Dextrose Agar with antibiotic Gentamicin . Inoculated tubes were incubated at 25ºC and 37 ^{O} C. Cultures were examined for expected growth, daily in the first week and twice a week for the subsequent period. Cultures were incubated for a minimum of 4 weeks before being considered as culture negative.[26]

Macroscopic appearance of the colony [26]:

After the appearance of mature growth, texture and surface colour of the colony on obverse and colour reverse along with any pigment which diffuses in to the media should be noted.

Microscopic examination of growth [26]

It can be done by;

i) Tease mount using Lactophenol cotton blue

ii) Cellophane tape mount.

iii) Slide culture technique.

Non-Cultural methods of identification of Fungi: [25]

It includes, -detection of circulating Antigens and Antibody.

-Fungal constitutive macromolecules

-Fungus-specific metabolities

-Fungus-specific nucleic acid sequences.

i) Detection of Fungal Antigens [25] :

Detection of Fungal Antigens is very useful in case of invasive disease. Galactomannan polysaccharide antigen detection in serum and urine of the patients with invasive Aspergillosis is very useful for early diagnosis. Several tests for detection of soluble antigens of Aspergillus spp in serum, urine or other body fluids have been developed. Radio immunoassay, Enzyme linked immune sorbent assay, Biotin avidin linked immunosorbent assay, Latex agglutination and I mmunoblotting have been the most commonly used method. [25]

ii) Detection of fungal antibody [25]

Exposure of the immune system to cell wall specific, cytoplasmic and extracellular fungal antigen may elicits an Antibody response, which can be detected by Immunodiffusion(ID) in agarose and Counter-immunoelectrophoresis detects fungal precipitins. ELISA, RIA, Complement fixation test and Indirect fluorescent antibody test can also detect fungal antibody with high sensitivity rate. Thus serology is helpful in correlating the clinical significance of positive fungal culture.

iii) Detection of Fungal constitutive macromolecules:

G-test-This test detects the (1,3)-beta-D-glucan by using modification of Limulus assay which is used for the detection of endotoxin and has a

specificity of ˷20pg/ml. It is used to confirm invasive mycosis. This assay is manufactured by removing bacterial endotoxin sensitive factor C from limulus lysate making this reagent specific for beta glucan. This modified lysate is formulated with a synthetic chromogenic substrate and salts.[25]

iv) Detection of Fungus-specific metabolites:

This is useful in case of disseminated fungal infection like Aspergillosis and Candidiasis by using Gas liquid chromatography(GLC). [25]

v) Detection of Fungus specific Nucleic acid sequence:

PCR-Polymerase chain reaction is a primer-mediated enzymatic amplification of specific DNA sequence.

PCR for fungal identification uses **Internal Transcribed Spacer region (ITS) ,** which is a non-coding region present in-between the genes encoding ribosomal RNA of the fungus. These genes encoding r-RNA and spacer are present in tandem repeats and the ITS region is the most widely sequenced region, which is the universal fungal barcode sequence [15,22,23,24]. Primers are designed targeting these ITS regions.

Panfungal PCR- Here, after amplifying the ITS region, using specific probes different fungi can be identified by the size of amplification product on an automated sequencer [15.]

ANTIFUNGAL SUSCEPTIBILITY TESTING

As the incidence of invasive fungal infection is increasing, especially during the last few decades, development of resistance to some of these antifungal drugs has made Antifungal susceptibility testing inevitable **.**

i) Reference method for Broth dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard(M-38A)

CLSI [30] document M38A second edition is the reference standard and it is used for testing filamentous fungi causing invasive fungal infections. Aspergillus species, Fusarium species, Rhizopus species, Pseudallescheria boydii and mycelial form of Sporothrix schenckii.

Inoculum	$0.4x10^4$-$5x10^4$ CFU/ml
Inoculum Standardization	Spectrophotometrically
Test medium	RPMI 1640
Format	Microdilution
Temperature	35°C
Duration of incubation	24 h/48h
Endpoint	No visible growth

Minimum inhibitory concentration is the lowest concentration of an antifungal drug that causes specified reduction in the visible growth of the microorganism in agar or broth dilution susceptibility testing.

ii) AGAR DILUTION:

Agar dilution method has been done in yeast nitrogen base agar with good reproducibility [34,35]

iii) E TEST:

E-test is a commercially available agar diffusion-dilution [31], which quantitatively determines the antimicrobial MICs [32]. It is designed in such a manner, where a calibrated plastic strips impregnated with antifungal drug with continuous concentration gradient [32] is used. An inhibition ellipse is formed centered along the strip. MICs is the concentration where the edge of the ellipse intersects the strip.

iv) DISK DIFFUSION:

Disk diffusion interpretive criteria are available by the latest CLSI document. Espinel –Ingroff et al in a multicenteric evaluation, have studied the disk diffusion assay for filamentous fungi [33] and concluded that the optimal conditions were (i) plain Mueller Hinton agar,(ii) incubation times varying from 16-24 hours for zygomycetes, 24 hours for Aspergillus fumigatus, A.flavus,

A.niger and 48 hours depending on the species and (iii) Itraconazole, Amphotericin10µg, Posaconazole 5µg, Voriconazole 1 µg, Caspofungin 5 µg disks are used.

v) Sensititre calorimetric method: .

This is a commercially available calorimetric microdilution method based on the CLSI M27-A2 standard for yeast. Each test consists of a disposable microtitre plate, which contains dried serial dilutions of six antifungal agents, Amphotericin B (range 0.008-16 µg/ml), Fluconazole (range 0.125-256 µg/ml), Itraconazole (range 0.008-16µ g/ml), Ketoconazole (range 0.008-16 µg/ml) and 5-Flucytosine (range 0.03-64 µg/ml), Voriconazole (range 0.008-16 µg/ml) in individual wells . The wells also contain Alamar Blue as a colorimetric indicator, which greatly improves the end point readability by a colour change from blue to pink. Results are expressed as an MIC and comparative studies against the NCCLS method have shown favorable results [31] . Excellent shelf life and the test also works with moulds, especially those that sporulate freely like Aspergillus.[31]

TREATMENT:

NON INVASIVE FUNGAL SINUSITIS:

1. SUPERFICIAL MYCOSIS/FUNGAL BALL:

Treatment includes complete removal of the Fungal Ball [27] and post operative Antifungal agents are not recommended [27] unless the patient is severely immunocompromised. Culture directed antibiotics to combat co existent bacterial infection may be used.

2. ALLERGIC FUNGAL SINUSITIS:

Treatment includes Functional Endoscopic Sinus Surgery(FESS), which in turn improves the sinus ventilation and mucociliary clearance [27]. Since AFRS is an immunologically mediated hypersensitivity to the fungal antigens, immunomodulators like topical and systemic corticosteroids both during preoperative and early post operative period will prevent the recurrence and improve the systems.[27] Till date there is no sufficient data which shows the use of topical antifungal agents to improve the systems.

INVASIVE FUNGAL SINUSITIS:

1. CHRONIC INVASIVE FUNGAL SINUSITIS:

Surgical debridement which should remove all the involved tissues with the maintenance of the natural barriers to infection like orbit combined with the use of newer less toxic Antifungal agent is the recommended

treatment [27].The duration of Antifungal therapy is individualized and long term follow-up is recommended.

2. ACUTE INVASIVE FUNGAL SINUSITIS:

Debridement of all grossly infected and devitalized tissue is mandatory. Orbital exentration in patients with known cerebral involvement and very poor vision may help reduce the burden of infected tissue. Wound packing that is impregnated with Amphotericin can be used. Following surgery, irrigation of nasal cavity with Amphotericin B (50 mg /L of water) irrigations (20 ml 4 times a day) may be performed. Granulocyte-colony stimulating factor infusion along with white blood cell transfusion will improve the Absolute Neutrophil count above 1000 cells/cmm. Concurrent initiation of systemic Antifungal agents ,especially second generation extended spectrum Azole [27] derivatives along with the restoration of neutropenia or treatment of Diabetic ketoacidosis will improve the overall prognosis [27]. Despite aggressive therapy and surgical debridement, the mortality rate is very high.

ANTIFUNGAL THERAPY [37].

Antifungals used for moulds:

- Polyenes: Amphotericin B, Amphotericin B lipid formulation
- Azoles: Triazoles- Itraconazole, Voriconazole, Posaconazole
- Echinocandins: Caspofungin, Micafungin, Anidulafungin

AMPHOTERICIN B [37]

It is obtained from Streptomyces nodosus [37]

Mechanism of action:

It has a macrocyclic ring with hydrophobic conjugated double bonds on one side and hydrophilic OH group on other side. AMB through its hydrophobic end gets binds to ergosterol in fungal cell membrane. Several molecules of it form a Micropore. The hydrophilic side forms the inner side of the micropore through which ions are transported thus increasing permeability and causing leakage of intracellular components. Membrane channel activity is increased at lower doses and pores are formed at higher doses [37].

Antifungal Spectrum:

It has a wide range of action against yeast and moulds, -Candida albicans, Cryptococcus neoformans, Blastomyces dermatiditis, Rhodotorula, Aspergillus, etc [37]

NEWER FORMULATIONS OF AMPHOTERICIN B [37]

i) Amphotericin B colloidal dispersion
ii) Amphotericin B lipid complex
iii) Liposomal amphotericin B

Dosage: It is available in a 50mg vial. First it should be suspended in 10 ml of distilled water and then mixed with 500ml of glucose solution. Initially 1mg test dose is given as slow i.v over 20min, if no reaction occurs it should be given in a dose of 0.3mg/kg/dose of slow infusion over 4-8 hours(37). Daily dose may be gradually increased upto 0.7mg/kg depending on the tolerance of the patient.

AZOLES [37]

Mechanism of action:

Inhibition of cytochrome P-450- dependent lanosterol 14-demethylase, an enzyme required for the synthesis of ergosterol, the main component of fungal cell membranes. This results in the accumulation of methylated sterols , depletion of ergosterol and inhibition of cell growth.

Dosage:

Itraconazole: 200 mg b.i.d

Voriconazole 6 mg/kg q12 h IV OR 200 mg q12 h

Posaconazole 100 mg b.i.d

Indications:

Itraconazole: Invasive aspergillosis refractory to amphotericin.

Voriconazole: Approved as primary therapy in invasive aspergillosis.

Posaconazole: Prophylaxis of invasive fungal infections. Shown to have good activity against zygomycetes.

SUSCEPTIBILITY OF DIFFERENT MOULDS TO AZOLE GROUP OF ANTI FUNGAL AGENTS

ORGANISM	**ITRACONAZOLE**	**VORICONAZOLE**	**POSACONAZOLE**
A.fumigatus	+	++	++
A.flavus	++	++	++
A.terreus	++	+	++
Fusarium	-	-/+	-/+
Rhizopus spp	-/+	-	+
Mucor spp	-/+	-	-
Scedosporium apiospermum	+	+/++	+/++
S.prolificans	-	-/+	-

ECHINOCANDINS [37]**-**Caspofungin, Micafungin and Anidulafungin**.**

MECHANISM OF ACTION:

Mechanism of action is noncompetitive inhibition of enzyme glucan synthase which produces (1,3)β d glucan. The destruction of cell wall structure leads to osmotic instability and ultimately lysis of the fungal cell

Caspofungin : 70 mg iv loading dose followed by a daily 50 mg IV dose.

INDICATIONS:

It is indicated in the treatment of invasive aspergillosis in patients who are refractory to or intolerant of other antifungals.It is also approved as empirical therapy for presumed fungal infections in neutropenic patients.

MATERIALS & METHODS

MATERIALS AND METHODS

PLACE OF STUDY:

This prospective study was conducted in the Department of Microbiology, in association with Department of Pathology, Coimbatore Medical College, Department of Otorhinolaryngology, Coimbatore Medical College Hospital, Coimbatore.

STUDY PARTICIPANTS:

All patients undergoing functional endoscopic sinus surgery (FESS) and/or diagnostic nasal endoscopy (DNE) Who were clinically diagnosed as a case of Rhino sinusitis were included in the study.

STUDY PERIOD:

The study period was from June 2016 to May 2017.

ETHICAL CONSIDERATION:

Approval was obtained from the Institutional Ethical Committee before the commencement of the study. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

INCLUSION CRITERIA:

All the Patients >15 years of age of both the gender within the study period with

- Symptoms of sinusitis of > 12 weeks duration
- Radiologically proven signs of sinusitis
- clinical features, DNE findings and FESS findings suggestive of fungal involvement were included in this study.
- Patients with underlying immunosuppression and chronic diseases like diabetes, Patients with Asthma and chronic eczema having features of chronic rhino sinusitis.

EXCLUSION CRITERIA:

Patients with symptoms of sinusitis of age<15 years were excluded from the study.

DATA COLLECTION :

Data collection included name, age, sex, address, date of admission, diagnosis at admission, physical examination findings and Demographic profile which include H/O asthma, aspirin allergy, Diabetes mellitus, Chronic eczema/dermatitis, COPD, neoplasm, and immunosuppressive therapy.

STATISTICAL DATA ANALYSIS:

Data entry was made in the Excel software and Analysis was done with SPSS-24 computer package. The categorical variable is expressed in frequency and percentage. The continuous variable is expressed in terms of mean and standard deviation. The associations between variables was found by chi square test and independent sample 't'- test P value <0.05 was considered as statistically significant.

CASE DEFINITIONS:

ALLERGIC FUNGAL RHINOSINUSITIS(AFRS): According to Bent and Kuhn, the Criteria for the Diagnosis of AFRS [20]

Major Criteria

1. Type I hypersensitivity
2. Nasal polyposis
3. Characteristics CT scan findings
4. Presence of eosinophilic mucus
5. Positive fungal smear

Minor Criteria

1. Young individuals
2. Co-existence asthma
3. Unilateral predominance
4. Radiographic bone erosion

5. Fungal culture
6. Charcot leyden crystals
7. Serum eosinophilia.

FUNGAL BALL:

Diagnostic criteria for fungal ball as defined by deShazo [40]

- X-Ray and CT-PNS showing sinus opacification and dense conglomeration
- -Mucopurulent cheesy clay like material seen in a single sinus at the time of diagnosis , commonly affecting maxillary sinus.
- -Histopathological evidence of dense accumulation of fungal hyphae often separate from the sinus mucosa with no evidence of allergic mucin.
- -No evidence of fungal hyphal invasion in the tissue.
- -May show gritty matted appearance grossly on surgery [10]

INVASIVE FUNGAL SINUSITIS:

Diagnostic criteria for invasive fungal infections as defined by deShazo [40]

- Radiological evidence of Sinusitis.
- Histopathological evidence of presence of hyphal forms within the sinus mucosa , submucosa, blood vessel or bone.

CHRONIC INVASIVE AND GRANULOMATOUS INVASIVE SINUSITIS

The disease usually has a time course of >12 weeks.[10] histopathological evidence of hyphal forms within the sinus mucosa ,sub mucosa, blood vessel or bone in association with non-caeseating granuloma containing giant cells differentiate granulomatous invasive from chronic invasive fungal sinusitis In chronic invasive FRS, the patients are subtly immunocompromised, sometimes in association with orbital apex syndrome.[41]

ACUTE INVASIVE FUNGAL SINUSITIS:[41]

The time course is usually <4 weeks, with predominant vascular invasion occurring in an immunocompromised individuals. Histopathological evidence of necrotic reaction having plenty of fungal hyphae with neutrophilic infiltration.

DIAGOSTIC CRITERIA:

Host factors:

- Bronchial asthma, Diabetes mellitus, Aspirin hypersensitivity
- Neutropenia(>500/mm^3 for >10 days) or coexistent AIDS.
- Persistent fever >96 hours refractory to antibiotics
- Recent or current use of immunosuppressive agents or steroids>3 weeks

Microbiological criteria:

- Positive result of fungal culture
- Positive findings i.e presence of fungal filaments in KOH mount.
- Histopathological evidence of presence of fungal hyphae in the tissue section.

Radiological criteria:

- Radiological evidence of sinusitis
- Bony invasion

Other minor criteria:

- Upper respiratory tract infections
- Nose ulceration or eschar
- Periorbital swelling
- Maxillary tenderness
- Perforation of hard palate

SAMPLE COLLECTION:

Sample collection was done according to American Thoracic Society Recommendations for collection of specimen for fungal culture [39]

Biopsy from the sinonasal Polyps and necrotic material during functional endoscopic sinus surgery were collected in two sterile container, one containing sterile 0.85% of NaCl which was transported immediately to the Microbiology lab for mycological processing and the other container containing formalin for histopathological examination and fungal stains. In case if the sample was endoscopic aspirates, it was collected in a sterile syringe for mycological processing only.

CRITERIA FOR REJECTION:

- Improperly labelled samples
- Samples that are transported in unsterile containers
- Samples that have leaked or show signs of dehydration
- Samples received in formalin

PROCESSING OF SPECIMENS:

For mycological processing, the polyp specimen was cut into small pieces in a sterile petridish using sterile scalpel and blade. Then a portion of specimen was put into 0.5 ml of 10% KOH for direct microscopic examination. Then the remaining specimen was inoculated into two sterile

SDA tube containing Gentamicin. One tube was incubated at 37ºc and the other was kept at room temperature.

In case of endoscopic aspirates, a portion was inoculated into SDA tubes as it was done for the polyp. The remaining portion was used for Direct microscopic examination.

DIRECT EXAMINATION:

POTASSIUM HYDROXIDE (KOH) MOUNT PREPARATION [26]:

A small portion of the specimen was placed on a clean grease free glass slide, then a drop of 10%KOH was added and mixed well. If the tissue seems to be hard, it was placed in a test tube containing 10% KOH overnight for complete softening and clearing. A coverslip was placed over it, then the slide was heated gently over the flame but it was not allowed to boil and examined under the microscope in low power and then high power objective using reduced light for the presence of hyphal forms, budding yeast cells, spherules or sclerotic bodies. Irrespective of KOH positivity or negativity, all the samples were processed for fungal culture.

FUNGAL CULTURE [26]:

A minimum of 0.5 ml of the specimen was inoculated onto 2 slants of Sabouraud Dextrose Agar with antibiotics Gentamicin added at a

concentration of 0.5mg. Inoculated tubes were incubated at 25 and 37 ^{O} C. Cultures were examined for expected growth, daily in the first week and twice a week for the subsequent period. Cultures were held for the entire period of 4 weeks before being labeling it as negative for fungal growth.

INTERPRETATION OF FUNGAL CULTURES:

The following features were considered before labelling an opportunistic fungi that are otherwise considered as contaminants or pathogen[25]

- Isolation of same strain in all culture tubes
- Repeated isolation of same strain in multiple specimens
- Immune status of the patient
- Direct microscopic detection of fungal forms
- Histopathological examination revealing the presence of fungal hyphae.

MACROSCOPIC EXAMINATION OF FUNGAL CULTURE [26]:

All isolates were systematically identified

i) Colour and texture of the colony

ii) Presence of any rugousities in the reverse

iii) Colour on the reverse

iv) Presence of pigmentation.

MICROSCOPIC EXAMINATION OF GROWTH:[26]

Various mounting methods done include

1) Tease mount

2) Cellophane tape mount

3) Slide culture technique

1) Tease mount:

A small drop of lactophenol cotton blue (LPCB) was placed on a clean microscopic slide. A small portion of growth was removed midway between the colony and edge. The removed colony was placed on a drop of lactophenol cotton blue on the slide. The growth was teased using a pair of teasing needles so as to have a thin spread out. The coverslip is placed gently at the edge of the drop of LPCB fluid avoiding trapping of air bubbles, then it is viewed under low power and high power of the microscope.[26]

2) Cellophane tape mount:

A drop of mounting fluid was placed on the slide. A 4cm long cellophane tape, which was looped back on itself such that the sticky side facing out. Then it was hold with a forceps on one end and the sticky side was pressed over the surface of the fungal colony . Then tape with the surface containing

fungus was laid facing down onto the slide containing LPCB , so that the tape gets stuck over the slide and it was viewed under the low and high power of the microscope.[26]

3) Slide culture technique: Ridels method:

Requirement: In a 100 mm diameter glass petri dish, a filter paper was placed over which a V-shaped glass rod followed by which a microscopic slide and a coverslip was placed . The whole setup is put into the hot air oven at 160^0C for 120 minutes for sterilization.

Procedure:

A 1 cm square agar was cut aseptically from potato dextrose agar. The agar block was transferred to the slide in the setup. A very small amount of the colony was stabbed to the four sides of the agar block with a sterile needle. A coverslip was placed on the inoculated agar block. Around 1 ml of sterile water was added to the filter paper. Slide culture was incubated at room temperature till good sporulation occurs.

Removing the slide culture:

A small drop of LPCB fluid was placed on a slide. With forceps, the cover slip was carefully removed from the slide culture set and placed

over the mounting fluid . The excess of mounting fluid was removed and the mount was examined under low and then high power of the microscope [26]

Microscopic features to be observed:

i. Fruiting structures: Synnemata, Pycnidia,

Ascocarps (Gymnothecia, Cleistothecia, Perithecia)

ii. Hyphae: Colour whether hyaline hyphae or dark pigmented hyphae as in case of demetiaceous fungi,. Size, Septation, branching Special Structures like rhizoids.

iii. Conidiogenesis: Conidiogenous cell, Proliferation of conidiophores, arrangement of conidial heads .

Cultural and Microscopic characteristics of Zygomycetes:

Colonies of Zygomycetes are greyish in colour which rapidly fills the tube or petriplate giving **a cotton candy** appearance. Differentiation of various genera can be made only by microscopy .

Rhizopus spp:

Culture- At first the colonies are white in colour then it turns to grey or yellowish brown, reverse will be white. They are rapidly growing quickly covers the agar surface.[26]

Microscopy-Hyphae are broad aseptate with numerous stolons running along the mycelia. Sporangiophores are long up to 4 mm, unbranched terminating in a dark round sporangium containing columella and numerous oval shaped sporangiospore. Rhizoids are nodal, seen at the point where the sporangiophore gets attached to the stolon. [26]

Mucor spp

Culture:

At first the colonies are white in colour then it turns to grey or yellowish brown, reverse will be white **,** rapidly fills the entire petriplate resembling cotton candy. [26]

Microscopy:

Hyphae are broad aseptate, branched sporangiphore terminating in a round spore filled sporangium of 50-300µm in diameter. The wall of the sporangium easily gets dissolved liberating the round to oblong spores revealing the collumella and sometimes revealing the collarette at the base of the sporangium. It does not have aphopysis or rhizoids.[26]

Cultural and Microscopic characteristics of Aspergillus species:

Characteristic features [26]	**Aspergillus flavus**	**Aspergillus fumigatus**	**Aspergillus niger**
Colony morphology	Velvety, white at first and later becomes greenish yellow and reverse is brown in colour.	Velvety or powdery, white at first and turns to dark green. Reverse white to tan	Wooly, white at first later turns to black. Reverse white to tan
Microscopic features	Conidiophores are spiny with variable length, enlarges at the tip to form the vesicle.Phialides are biseriate covering the entire vesicle.Phialides produce chains of conidia	Conidiophores are short and smooth. Phialides are uniseriate covering only the upper two-thirds of the vesicle. Phialides produce chains of round conidia.	Conidiophores are long and smooth. Phialides are biseriate covering the entire vesicle.form radiate head. Phialides produce chains of round conidia.

Dematiaceous fungi:

It includes large group of fungi which produces olive, brown coloured colonies due to the presence of melanin in their cell wall. Black fungi which produce AFRS includes Bipolaris, Alternaria, Curvularia, Dreschlaria which are more common in North America[10].

ANTIFUNGAL SUSCEPTIBILTY TESTING:[30]

i) Reference method for Broth dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard(M-38A)

Amphotericin B powder from HiMedia, Mumbai and voriconazole powder from sigma-aldrich were obtained

$$\text{Weight (mg)} = \frac{\text{volume (ml)x concentration (µg/ml)}}{\text{Assay potency (µg/ml)}}$$

$$\text{Volume(ml)} = \frac{\text{weight (mg)x assay potency(µg/ml)}}{\text{concentration (µg/ml)}}$$

Antifungal powders are weighed on an analytical balance

STOCK SOLUTION:

Solvent used is Dimethyl sulfoxide (DMSO) for Amphotericin B and voriconazole. Antifungal Stock solutions were prepared 100 times the highest concentration to be tested , 1600 µg/ml is prepared. Then a series of dilutions at 100 times the final concentration was prepared from the antifungal stock solution in the same solvent. Each intermediate solution was then further diluted to final strength in the test medium, Roswell parker memorial institute-1640,with glutamate and without bicarbonate, in order to avoid dilution artifacts which results from precipitation of compounds due to low solubility in aqueous medium.

Media : RPMI 1640(with glutamine, without bicarbonate, and phenol red as pH indicator), HiMedia, Mumbai.

Inoculum preparation:

All organisms were subcultured onto Potato dextrose agar , incubated at 35ºC for 7 -10 days. The culture was covered with 1 ml of sterile 0.85% saline and a suspension prepared by gently scrapping the colonies. Addition of 1 drop of Tween 20 will help uniform dispersion of conidia. The resulting mixture of conidia and hyphal elements was then transferred to a sterile tube and allowed to settle. Then again suspension was transferred to a screw capped tube and vortexed. The densities of the conidia or the sporangiospore suspensions

were adjusted to a optical density of 0.09-0.11 for A*spergillus* spp and 0.15-0.17 for Rhizopus spp by spectrophotometry at 530nm. Then this suspension was diluted to 1:50 in the standard medium. This will give a density of approximately $0.4x10^4$ to $5x10^4$ CFU/ml when mixed with the antifungal agent.

INCUBATION:

All microtitre plates were incubated at 35ºC. Examination time for Rhizopus: 21-26 hours of incubation and Aspergillus spp: 46-50 hours of incubation.

INTERPRETATION:

Minimum inhibitory concentration is the lowest concentration of an antifungal agent that substantially inhibits growth of the microorganism as detected visually. One growth control well and one sterility control well containing media were also included. Each microdilution well was then given a numerical score as follows;

Score 4 - No reduction of growth

Score 3 - Slight reduction in growth(75 % of growth control)

Score 2 - Prominent reduction in growth(50 % of growth control)

Score 1 - Optically clear or absence of growth

PROCEDURE:

ANTIMICROBIAL SOLUTION						
STEP	**CONCENTRATION (µg/ml)**	**SOURCE**	**VOLUME (ML)**	**SOLVENT (ML)**	**INTERMEDIATE CONCENTRTION (µg/ml)**	**FINAL CONCENTRATION AT 1:50(µg/ml)**
1	**1600**	**STOCK**			**1600**	**32**
2	**1600**	**STOCK**	**0.5**	**0.5**	**800**	**16**
3	**1600**	**STOCK**	**0.5**	**1.5**	**400**	**8.0**
4	**1600**	**STOCK**	**0.5**	**3.5**	**200**	**4.0**
5	**200**	**STEP 4**	**0.5**	**0.5**	**100**	**2.0**
6	**200**	**STEP 4**	**0.5**	**1.5**	**50**	**1.0**
7	**200**	**STEP 4**	**0.5**	**3.5**	**25**	**0.5**
8	**25**	**STEP 7**	**0.5**	**0.5**	**12.5**	**0.25**
9	**25**	**STEP 7**	**0.5**	**1.5**	**6.25**	**0.125**
10	**25**	**STEP 7**	**0.5**	**3.5**	**3.13**	**0.0625**

CLINICAL SIGNIFICANCE:

AMPHOTERICIN:

MIC above 2 µg/ml have been associated with treatment failure and MIC below 2 µg/ml with clinical cure.

VORICONAZOLE:

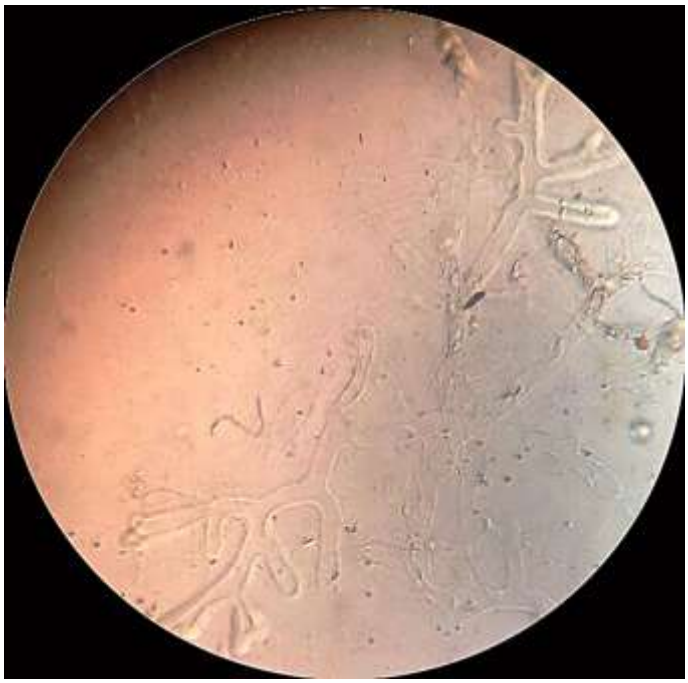
Data are not available to indicate for correlation between MIC and treatment outcome for new triazoles.

ii) E TEST:

Inoculum transmittance was adjusted according to CLSI M38-A protocol as described above for microbroth dilution. Suspensions were applied to the surface of the agar media by using swab applicators i.e RPMI agar for E test. The inoculated plate was allowed to dry for 15 minutes . Estrip for Amphotericin B was applied onto the inoculated RPMI agar**.** . E test was read after 24 hours or when there was sufficient growth to take a reading. Zone diameter was determined were the edge of the ellipse intersects the E strip and it was taken as MIC.

HISTOPATHOLOGICAL EXAMINATION:

All the tissue material taken was sent to Pathology department in a separate sterile container containing saline. Histopathological examination using Eosin and Hematoxylin stain was used in order to see the presence of fungal hyphae in mucosa and vascular invasion. Special stain like Gomori Methenamine Silver stain was also used for identification.



PICTURE:1 KOH Mount showing broad aseptate branching hyaline filaments

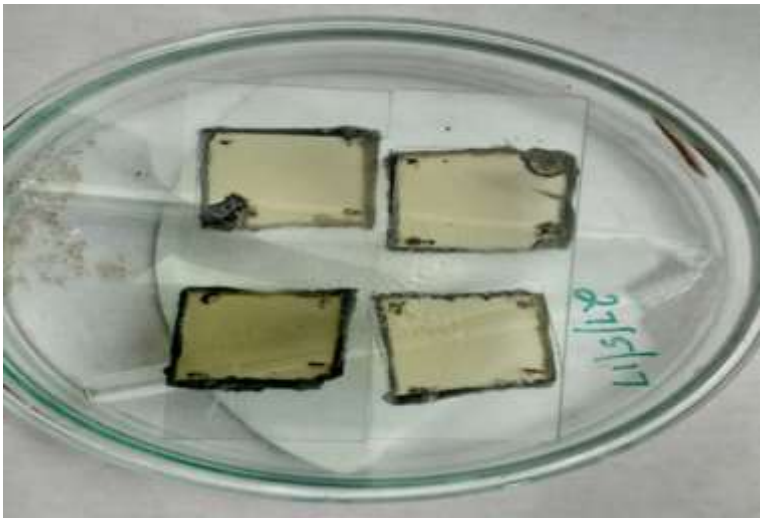
Picture:2 SDA Showing colonies of Aspergillus flavus

Picture:3 SDA Showing colonies of Aspergillus niger

Picture:4 SDA Showing colonies of Penicillium species

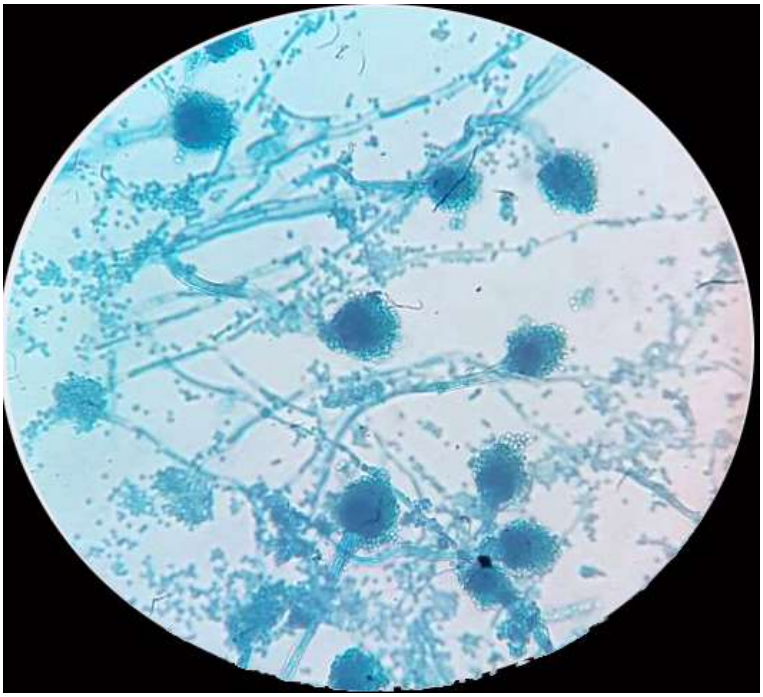
Picture:5 SDA Showing colonies of Aspergillus fumigatus

Picture:6 SDA Showing colonies of Rhizopus species

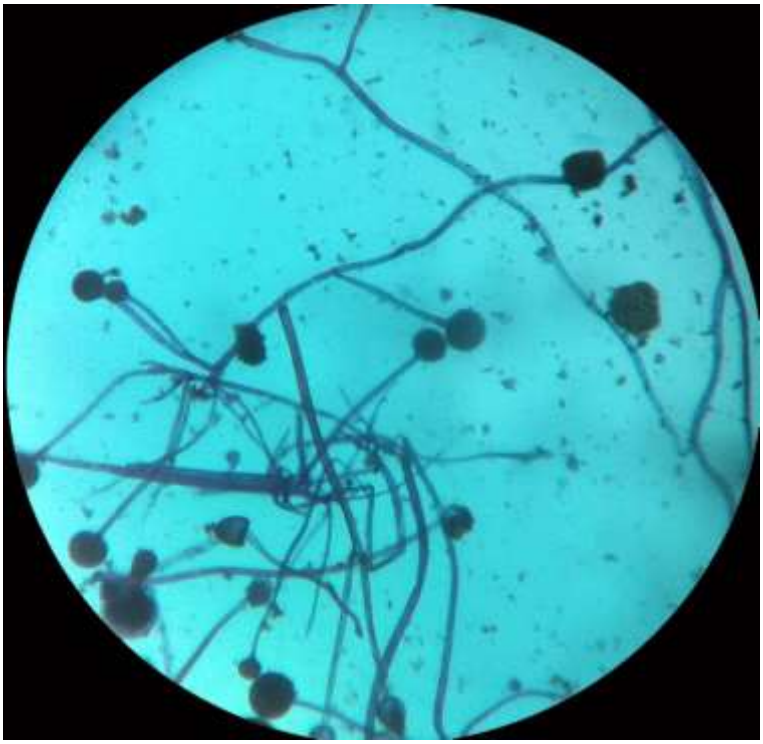


Picture: 7 Slide culture

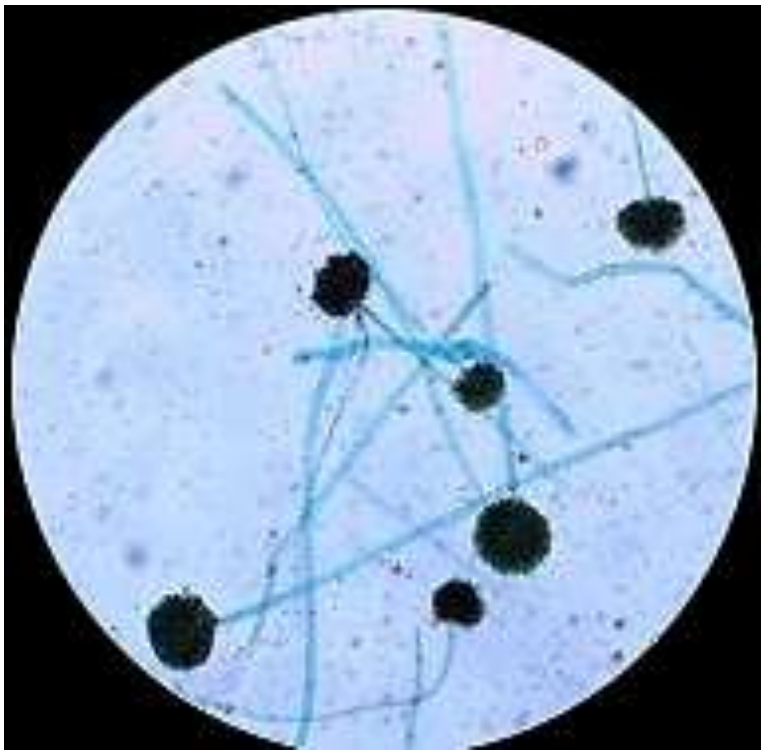
Picture:8 LPCB Picture showing Aspergillus flavus



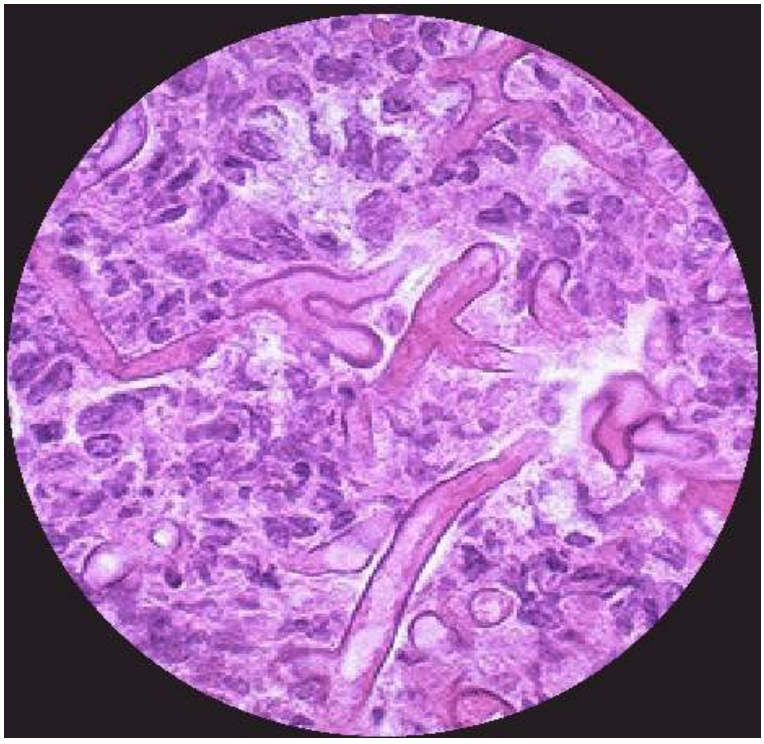
Picture:9 LPCB Picture showing Aspergillus fumigatus



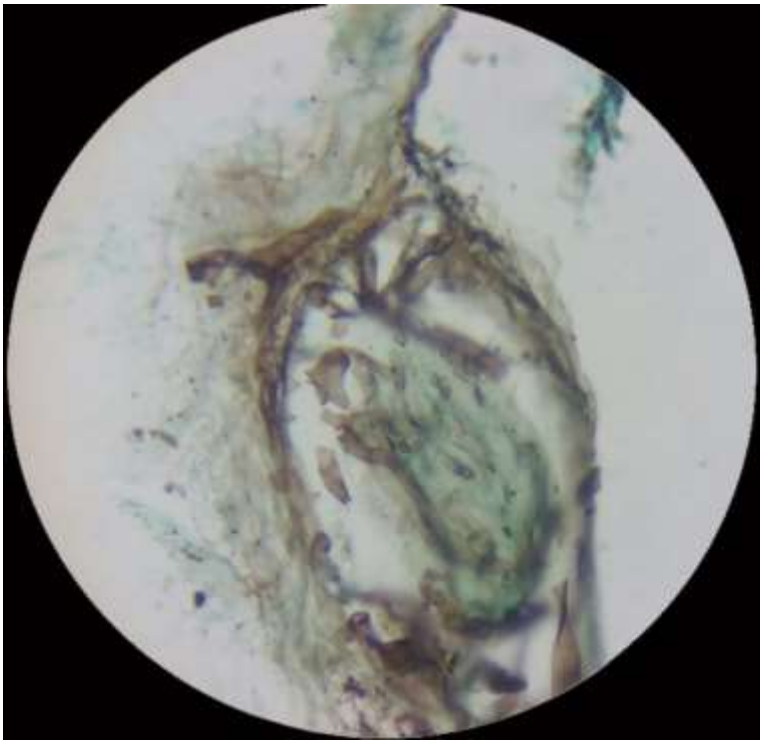
Picture:10 LPCB Picture showing Rhizopus species



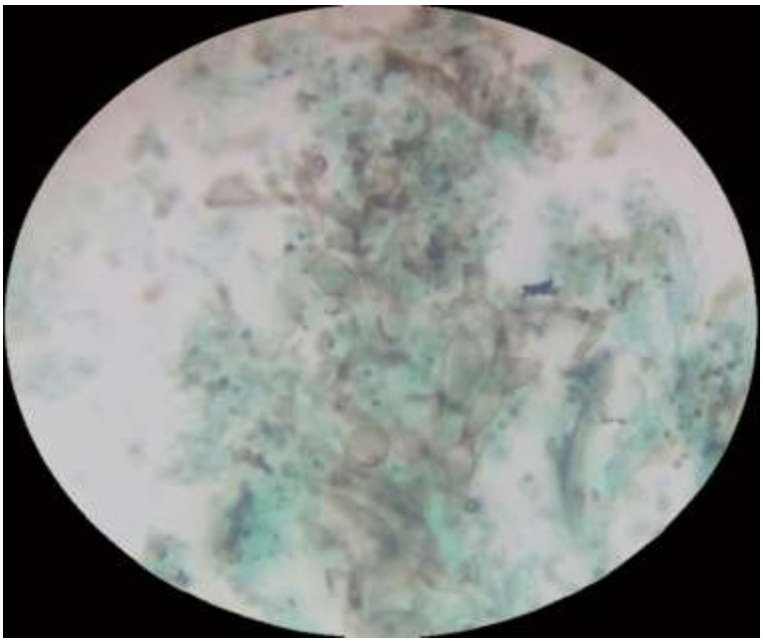
Picture:11 LPCB Picture showing Aspergillus niger



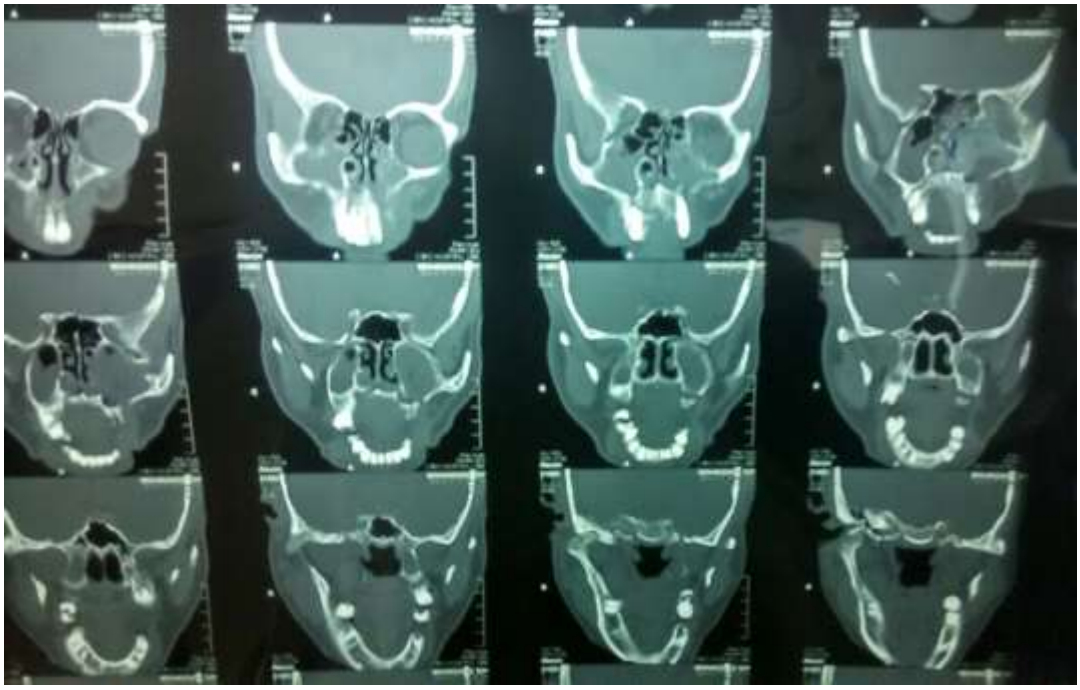
Picture:12 HPE showing broad aseptate wide branching fungal filaments



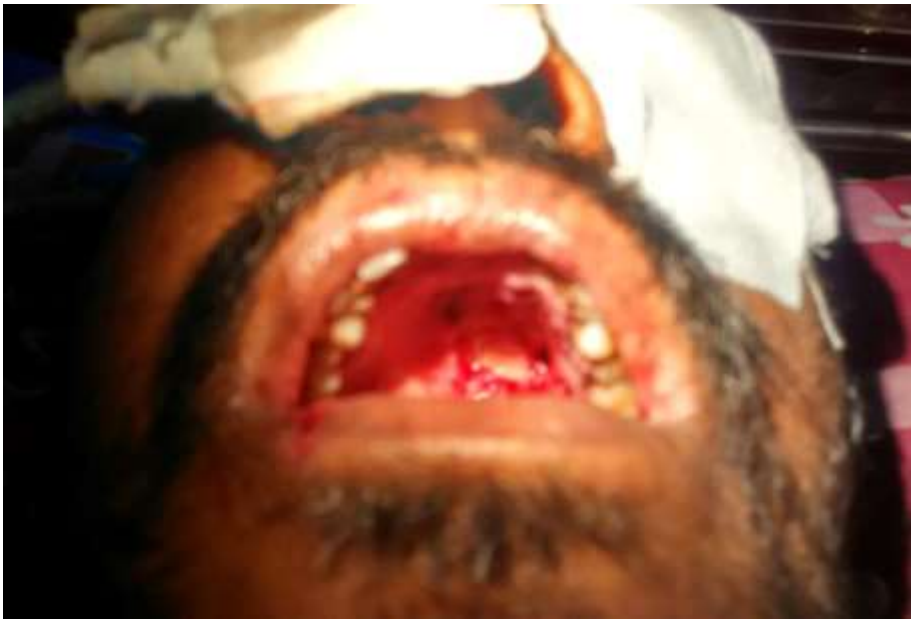
Picture:13 GMS stain showing vascular invasion of fungal filaments



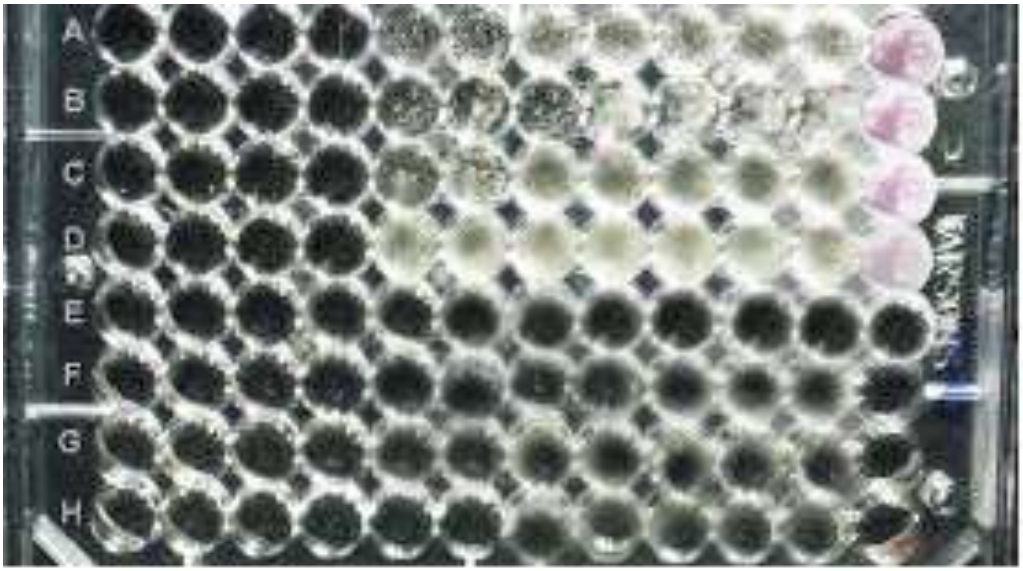
Picture:14 GMS stain showing the presence of Fungal filaments



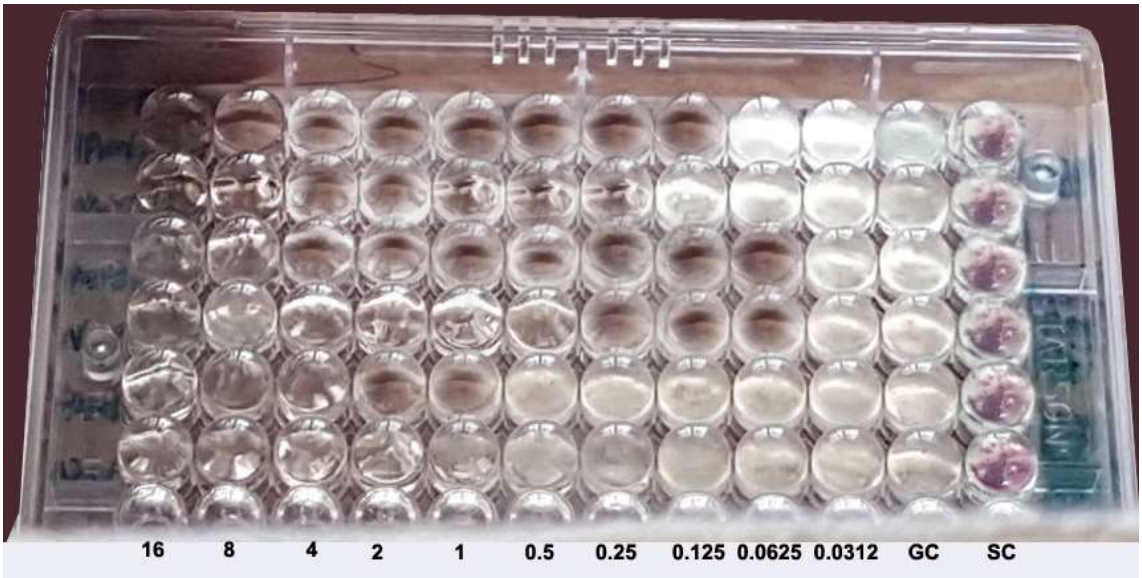
Picture:15 CT-PNS Showing bony erosion



Picture:16 A patient with Acute invasive FRS showing hard palate necrosis



Picture:17 MIC determination by CLSI Microbroth dilution for Rhizopus species using Amphotericin-B



Picture:18 MIC determination by CLSI Microbroth dilution for Aspergillus species species using Voriconazole

Picture:19 MIC Determination of Amphotericin-B by E-strip for Aspergillus niger

RESULTS

RESULTS

A total of 106 patients having features of chronic rhino sinusitis were included in my study period, who underwent Diagnostic nasal endoscopy and Functional endoscopic sinus surgery in the department of ENT, Coimbatore medical college hospital, Coimbatore.

Demographic profile of the study participants had showed a male: female of 1.7 : 1 particularly in the age group of 15-30 years(n=36)34%, majority belonging to Coimbatore(n=89)84% .Most of them had presented with headache(95 %) ,nasal blockade(92%) and nasal discharge(89%).

Out of 106 study participant, 22(Table:3) were diagnosed to have fungal Rhino sinusitis, based on positive KOH findings, fungal culture and histopathological evidence. Thus the overall prevalence was 21%.

Occurrence of rhino sinusitis in general was found to be more in males as compared to females. It was about 76.1% (Table:12) prevalent in males in case of NFRS and 23.9% in FRS .In case of females it was 84.6% in NFRS and 15.4% in FRS. It was found that 15-30 years was the commonest age group for the occurrence of both FRS and NFRS. The mean age of patients with fungal sinusitis was higher (42.45) (Table:11) when compared to NFRS(36.4) and is found to be statistically significant by independent t test.

Gender wise fungal sinusitis was high among males but this was not statistically significant, because the p value was 0.39(Table:11) which is not significant.

Fungi were isolated more among Sino nasal polyp(68%)(n=15)(Table:7). Endoscopic aspirates (14%)(n=3) was found to be poor specimen for isolation and in acute and chronic invasive form, fungi was isolated more in necrotic material(18%)(n=4) .

A broad spectrum of clinical presentation was noticed in patients with FRS(Chart:4) ranging from nasal blockade(100%), headache(91%) and nasal discharge(86%) especially in AFRS to orbital complications(18%), cranial nerve involvement(14%) , CVA(9%) and cavernous sinus involvement(5%) in invasive forms. About 9% of cases had shown vascular invasion especially in invasive form of the disease.

The most common form of mycosis was found to be, AFRS (68%)(Table:4) ,followed by CIFRS(14%). It was found that pansinus involvement was common among FRS(n=14) patients followed by maxillary sinus involvement.

Aspergillus species was the commonest pathogen isolated, About 50% was Aspergillus flavus(n=11), 18% was Aspergillus fumigatus(n=4), and 9% was Aspergillus niger(n=2), Rhizopus spp(n=4) was found to be 18% and 5% was Penicillium spp(n=1).

In about 67%(Table:9) cases of AFRS Aspergillus flavus was isolated .13% isolates of AFRS were Aspergillus niger and Aspergillus fumigatus also had 13% incidence in causing AFRS. Penicillium species was isolated in about 7% cases of AFRS.

In case of Fungal ball, Aspergillus fumigatus was isolated and one out of 3 case(33%)(Table:9) of Chronic invasive FRS showed the presence of Aspergillus fumigatus. Rhizopus species was isolated in 67% (Table:9)of Chronic invasive FRS. Aspergillus flavus was isolated in Chronic granulomatous invasive FRS and Rhizopus species was isolated in all the Acute invasive FRS.

Out of 22 positive cases all are culture proven(n=22), but 95%(Table:10) were KOH(n=21) and 77% were HPE(n=17) proven. This is because when superficial sections are taken for histopathological examinations, fungal filaments could not be demonstratable. About 89% of the samples showed positivity for GMS staining.(chart:9)

Bronchial asthma was found to be the commonest risk factor(Table:7) associated with the occurrence of AFRS(n=8)53% and Diabetes mellitus(n=5) 100% being the most common immunosuppressive condition associated with invasive forms, especially in chronic invasive and acute invasive forms .Statistically it was found to be significant.

Growth of all the isolates on RPMI-1640 and RPMI agar was good which were used for CLSI microbroth dilution method and E-strip method respectively. The mean MIC obtained by broth dilution method was mentioned in Table:14 for Amphotericin-B. The mean MIC obtained by broth dilution method for Aspergillus species using Voriconazole was mentioned in Table:15.

All the isolates of Aspergillus species was found to have MIC of <2 µgm/ml for Amphotericin –B by broth dilution method . As per CLSI guidelines MIC <2µgm/ml was associated with good clinical outcome. Thus all the Aspergillus species isolated was found to be associated with good clinical outcome with the mean MIC of 0.7 µgm/ml for Aspergillus flavus, 0.6 µgm/ml for Aspergillus fumigatus and 0.5 µgm/ml for Aspergillus niger by broth dilution method .

The mean MIC for Voriconazole(Table:15) by broth dilution method for Aspergillus flavus- 0.22 µgm/ml for Aspergillus fumigatus- 0.25 µgm/ml and for Aspergillus niger - 0.25µgm/ml .When compared to Amphotericin-B , the mean MIC was found to be low for the same Aspergillus species tested against Voriconazole. This shows that Voriconazole is having better susceptibility towards Aspergillus species when compared to Amphotericin –B.

In case of Rhizopus species(Table:14) all the isolates was found to have MIC of <2µgm/ml for Amphotericin-B by broth dilution method which is considered as good clinical outcome as per CLSI guidelines. Mean MIC for Rhizopus species was found to be 1µgm/ml.

Penicillium species was found to have MIC of 0.25µgm/ml for Amphotericin-B by broth dilution method.

E-strip test for Amphotericin-B tested against Aspergillus species, Rhizopus and Penicillium species was mentioned in (table:16) and their comparison with reference to CLSI broth dilution method mentioned in (Table 17). For comparison purpose the mean MIC obtained by broth dilution and E-strip method was adjusted to the next higher concentration in order to match the 2-fold dilution scheme. It was found that the MIC obtained by E- strip method lies between ± 2 dilutions of broth dilution method. Overall essential agreement was 100% with E-strip method, and thus it correlates better with broth dilution method.

TABLES Table 1 : Descriptive statistics of the age of the participants

Variable	N	Minimum	Maximum	Mean	Std. Deviation
Age	106	15	67	37.66	12.18

Table 2 : Gender- wise Distribution of the study participant

Variable	Total number	Percentage
Males	67	63.2
Females	39	36.8
Total	106	100.0

TABLE 3 : Type of Sinusitis

Sinusitis	Total number	Percentage
Fungal Sinusitis	22	20.8
Non-fungal sinusitis	84	79

TABLE 4 : Types of Fungal Sinusitis

Sinusitis	**Total number**	**Percentage**
Allergic fungal rhinosinusitis (AFRS)	15	68.18
Fungal ball	1	4.54
Chronic granulomatous	1	4.54
Chronic invasive	3	13.63
Acute invasive	2	9.09
Total	22	100.0

Comparison of the different study variable with the type of sinusitis

TABLE:5 Comparison between Age and Type of Sinusitis

AGE	NFRS	AFRS	Fungal Ball	Chronic Granulomatous	Chronic Invasive	Acute	Total
15-30 years	30	5	1	0	0	0	36
31-40 years	24	3	0	0	0	0	27
41-50 years	19	4	0	0	1	1	25
51-60 years	10	3	0	0	2	1	16
61-70 years	1	0	0	1	0	0	2
Total	84	15	1	1	3	2	106

TABLE:6 GENDER WISE DISTRIBUTION OF PATIENTS WITH FUNGAL RHINOSINUSITIS

Sex	AFRS	Fungal Ball	Chronic Granulomatous	Chronic Invasive	Acute Invasive	Total
Males	9 (56.3%)	1 (6.3%)	1 (6.3%)	3 (18.8%)	2 (12.5%)	16 (73%)
Females	6 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (27%)
Total	15	1	1	3	2	22

Fungal Sinusitis is more common in males when compared with the females.

TABLE:7 DISTRIBUTION OF FUNGAL ISOLATES FROM SINONASAL SPECIMENS

S.NO	SPECIMEN	AFRS n=15	FB n=1	CGFRS n=1	CIFRS n=3	AIFRS n=2	TOTAL n=22
1	ENDOSCOPIC ASPIRATES	3	0	0	0	0	3
2	SINONASAL POLYPS	12	1	1	1	0	15
3	NECROTIC MATERIAL IN SINONASAL REGION	0	0	0	2	2	4

TABLE:8 SITE OF INVOLVEMENT OF FUNGAL SINUSITIS

Site of involvement	Total number	Percentage
Pansinusitis	14	63.6
Maxillary	7	31.8
Ethmoid	1	4.5
Total	22	100.0

Table:9 FUNGAL ISOLATES AMONG RHINOSINUSITIS PATIENTS AND THEIR RELATIVE FREQUENCY IN DIFFERENT FORMS OF FUNGAL SINUSITIS

S.NO	FUNGAL ISOLATES	AFRS n=15	FB n=1	CGFRS n=1	CIFRS n=3	AIFRS n=2	TOTAL n=22
1	A.flavus	10 (67%)	-	1 (100%)	-	-	11
2	A.fumigatus	2 (13%)	1 (100%)	-	1 (33%)	-	4
3	A.niger	2 (13%)	-	-	-	-	2
4	Rhizopus.spp	0	-	-	2 (67%)	2 (100%)	4
5	Penicillum.spp	1 (7%)	-	-	-	-	1

TABLE:10 COMPARISON BETWEEN DIRECT MICROSCOPIC OBSERVATION, HPE AND CULTURE EXAMINATION

AFRS n=15			FB n=1			CGFRS n=1			CIFRS n=3			AIFRS n=2		
KOH	CULTURE	HPE	KOH	CULTURE	HPE	KOH	CULTURE	HPE	KOH	CULTURE	HPE	KOH	CULTURE	HPE
14 93%	15 100%	11 73%	1 100%	1 100	0 0%	1 100	1 100	1 100	3 100%	3 100%	3	2 100%	2 100%	2 100%

Risk Factor Assessment

i) Age

TABLE:11

Type of sinusitis	N	Mean	Std. Deviation	p Value
NFRS	84	36.40	11.62	<0.05
FRS	22	42.45	13.35	

Above table shows that the mean age of patients with fungal sinusitis is higher and is found to be statistically significant by independent t test

ii) Gender

TABLE:12

			NFRS	FRS	TOTAL	X^2	p Value
Sex	Male	Count	51	16	67	1.08	0.39
		Percentage	76.1%	23.9%	100.0%		
	Female	Count	33	6	39		
		Percentage	84.6%	15.4%	100.0%		
Total		Count	84	22	106		

Gender wise fungal sinusitis was high among males but this was not statistically significant

iii) Comorbid Conditions:

TABLE:13 DISTRIBUTION OF COMORBID CONDITION AMONG FRS AND NFRS

Risk Factors	FRS	NFRS	X^2	p Value
Either Asthma or diabetes	15	19	16.61	<0.001
Others/ No risk factors	7	65		
Total	22	84		

The presence of either Asthma or diabetes is definitely a risk factor for fungal sinusitis and this is found to be statistically significant(by chi square method).

TABLE:14 MINIMUM INHIBITORY CONCENTRATION OF AMPHOTERICIN B TO DIFFERENT MOULDS BY BROTH DILUTION METHOD

S.NO	FUNGAL ISOLATES	No. isolates	Amphotericin B		
			MIC> 2µg/ml	MIC< 2µg/ml	Mean MICµg/ml
1	A.flavus	11	11 (100%)	0	(1)0.7
2	A.fumigatus	4	4 (100%)	0	(1)0.6
3	A.niger	2	2 (100%)	0	0.5
4	Penicillum.spp	1	1 (100%)	0	0.25
5	Rhizopus.spp	4	4 (100%)	0	1

TABLE:15 MINIMUM INHIBITORY CONCENTRATION OF VORICONAZOLE TO ASPERGILLUS SPECIES BY BROTH DILUTION METHOD

S.NO	FUNGAL ISOLATES	No.isolates	Voriconazole mean MIC µg/ml
1	A.FLAVUS	11	0.22 (0.25)
2	A.FUMIGATUS	4	0.25
3	A.NIGER	2	0.25

TABLE:16 E-TEST FOR AMPHOTERICIN-B FOR FILAMENTOUS FUNGI

S.NO	FUNGAL ISOLATES	No. isolates	Amphotericin B		
			MIC> 2µg/ml	MIC< 2µg/ml	Mean MICµ g/ml
1	A.flavus	11	11 (100%)	0	0.52 (1)
2	A.fumigatus	4	4 (100%)	0	0.63 (1)
3	A.niger	2	2 (100%)	0	0.126 (0.25)
4	Penicillum.spp	1	1 (100%)	0	0.5
5	Rhizopus.spp	4	4 (100%)	0	1

TABLE: 17 COMPARISION OF MIC VALUES OF AMPHOTERICIN-B E-TEST WITH REFERENCE TO BROTH DILUTION METHOD

S.NO	FUNGAL ISOLATES	NO. OF ISOLATES	MEAN MICµgm/l BY BROTH DILUTION	MEAN MICµgm/l BY E-STRIP
1	A.FLAVUS	11	0.7(1)	0.52(1)
2	A.FUMIGATUS	4	0.6(1)	0.68(1)
3	A.NIGER	2	0.5	0.126(0.25)
4	PENICILLUM.SPP	1	0.25	0.5
5	RHIZOPUS.SPP	4	1	1

The overall essential agreement of Amphotericin-B for E-test with reference to CLSI broth dilution method for the moulds isolated was found to be 100%.

CHART:1 Pictorial Representation of Type of sinusitis

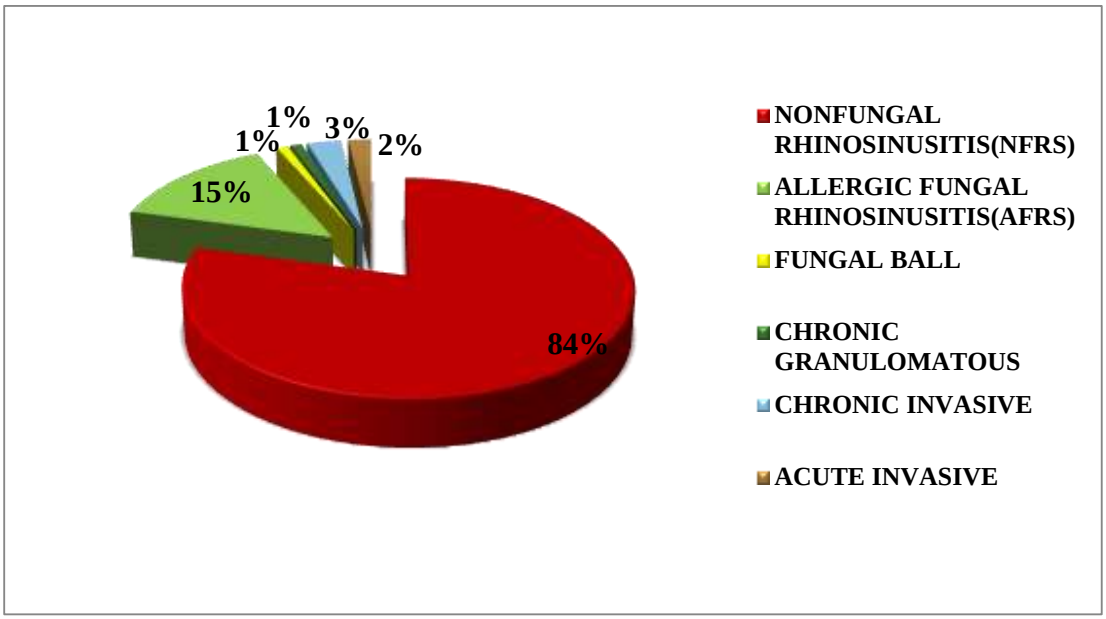


CHART:2 Types of fungal sinusitis

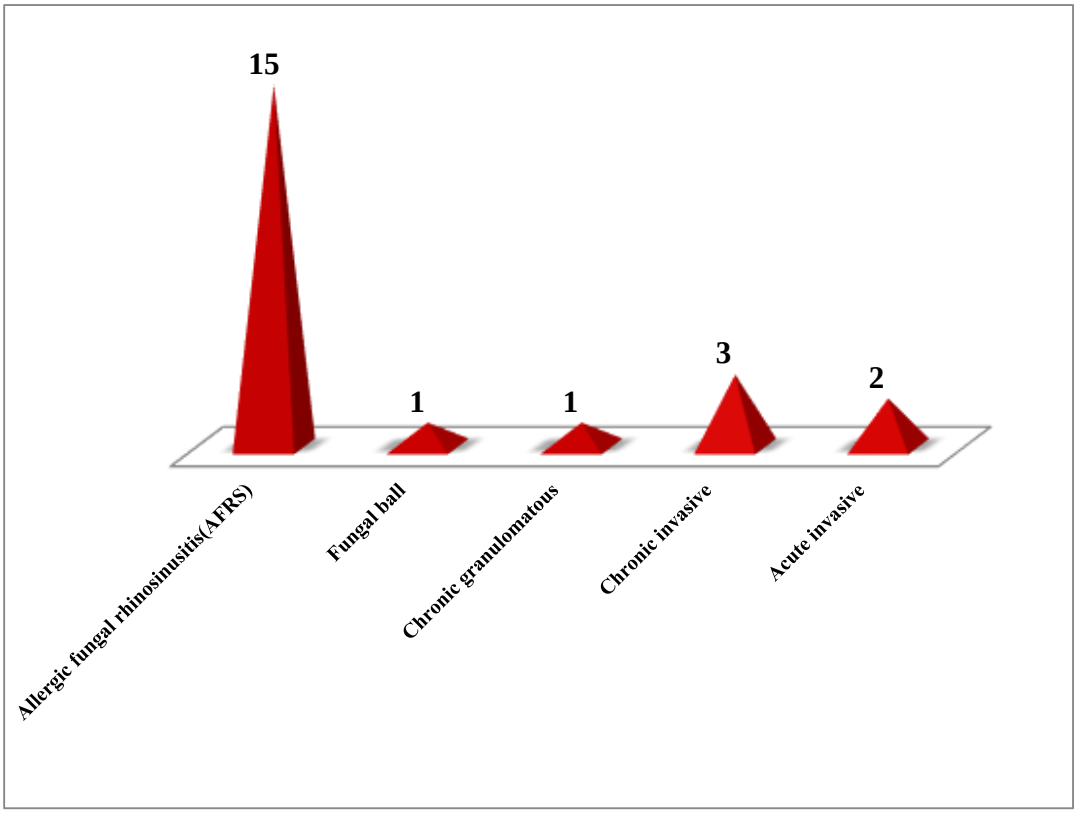


CHART 3 : Specimen Taken

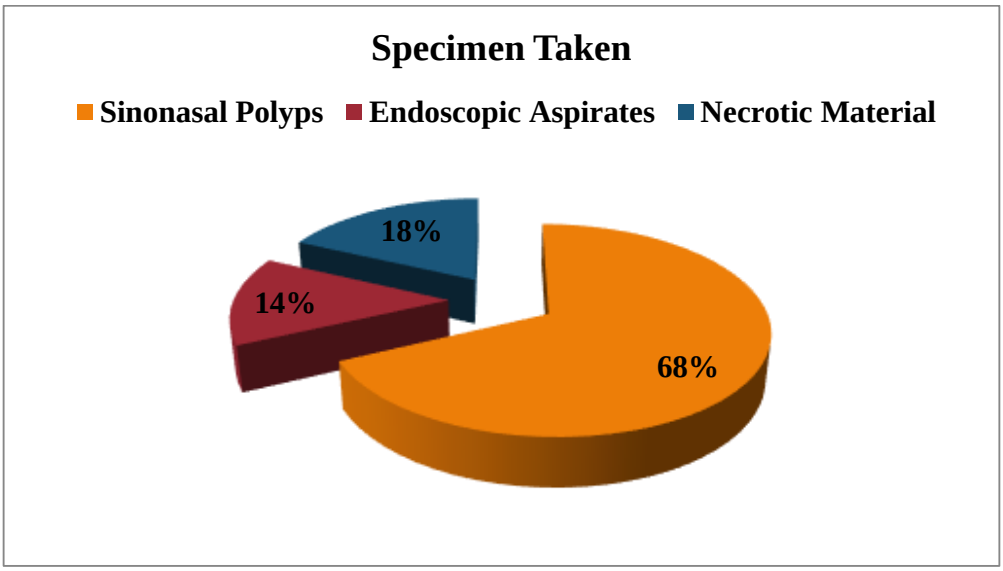


CHART:4 Clinical Presentation of fungal sinusitis

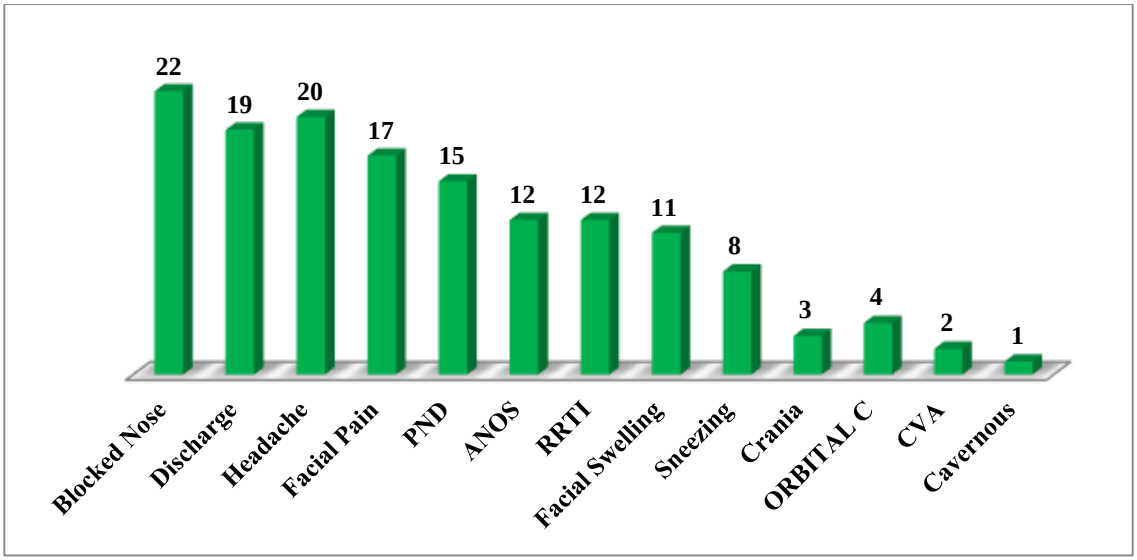


CHART:5 Fungi isolated

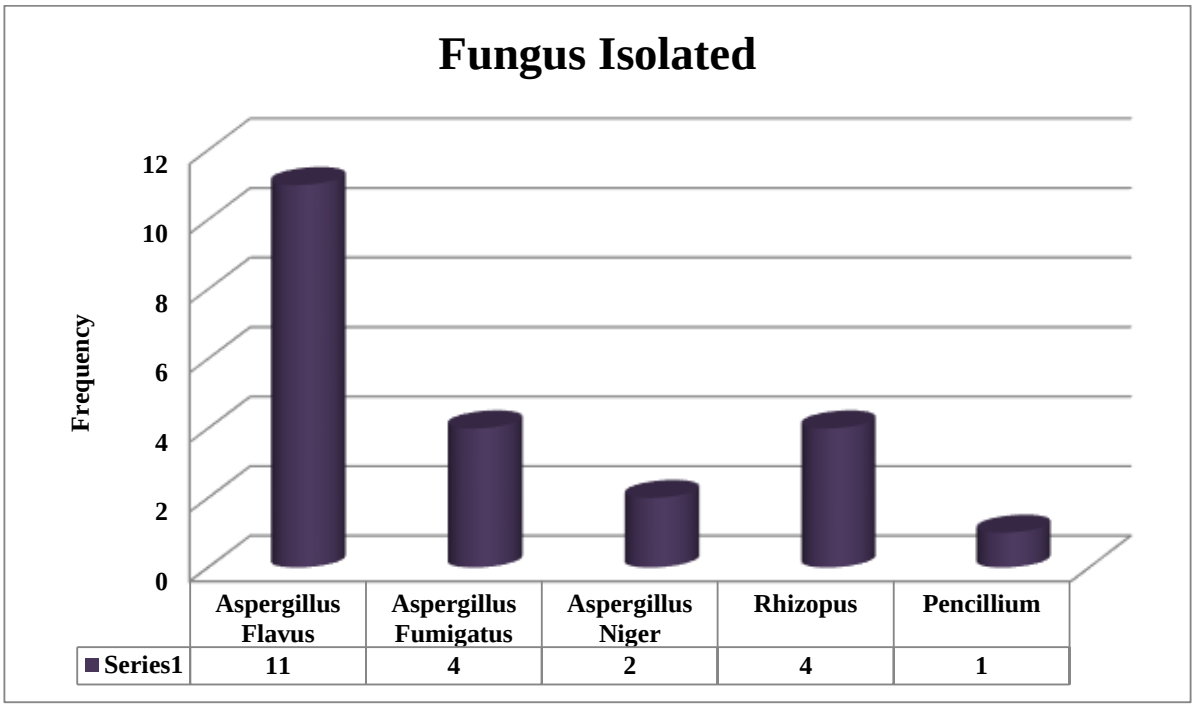


CHART:6 Comparison Between Direct Microscopy, HPE Culture and GMS

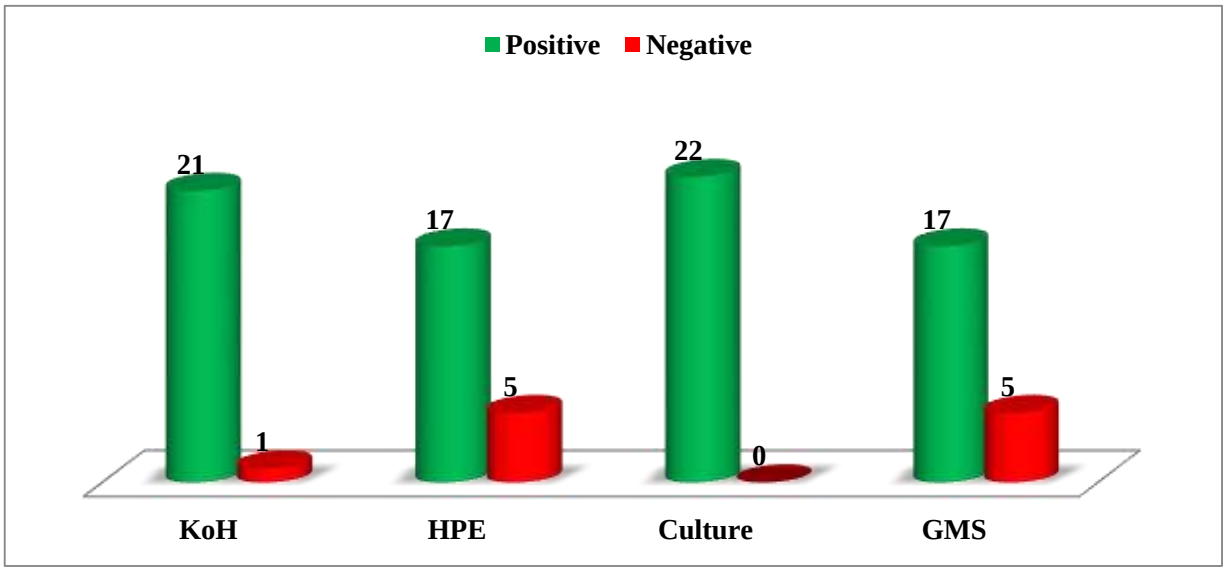


CHART:7 Risk Factors among Fungal Sinusitis patients

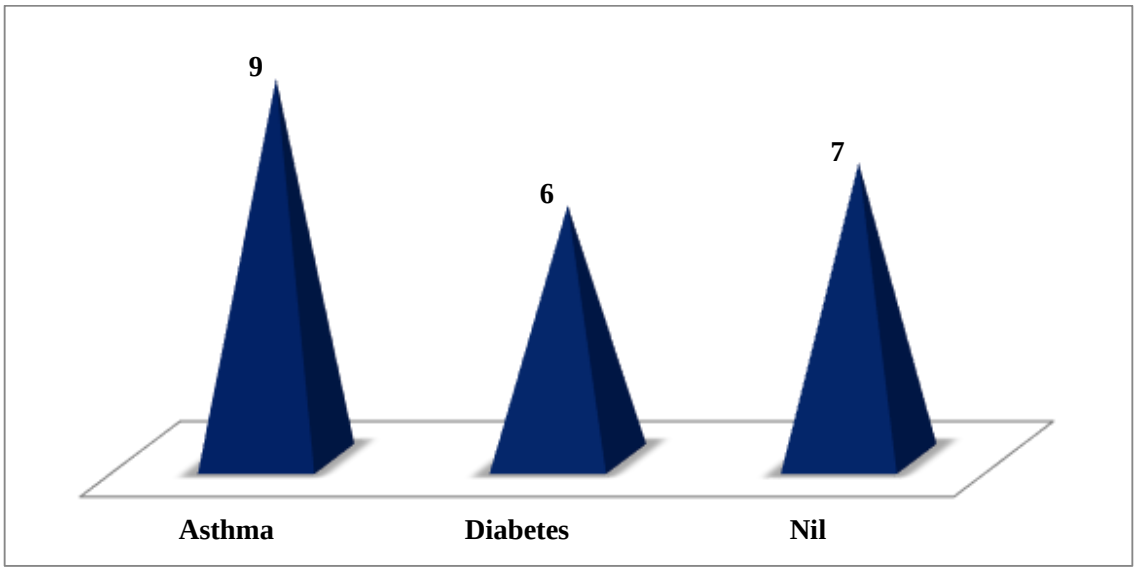


CHART:8 Site Involved in Fungal Sinusitis

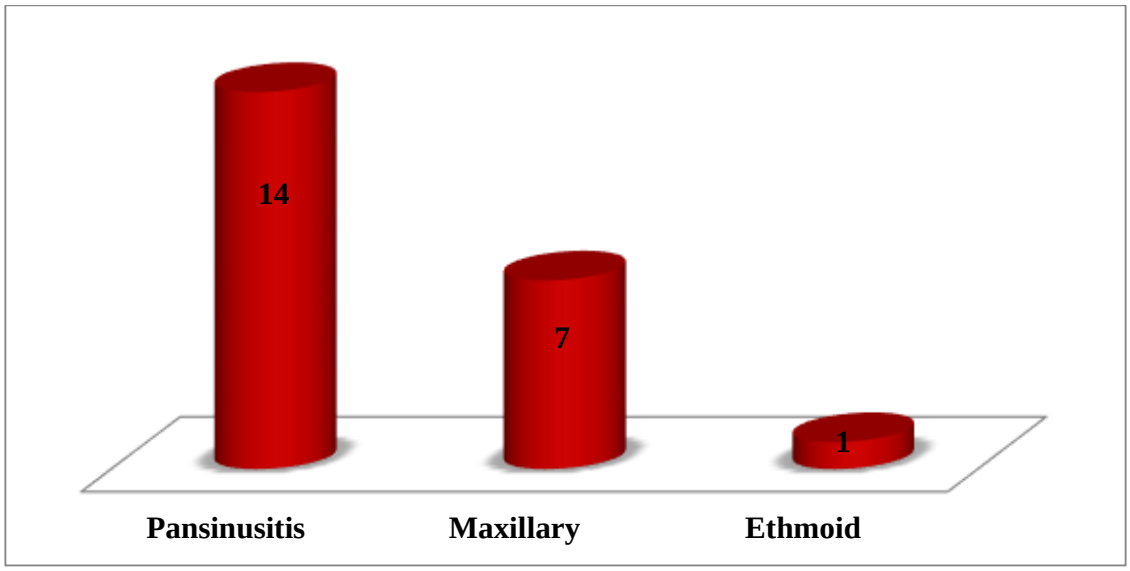


CHART:9 GMS Positivity among fungal sinusitis

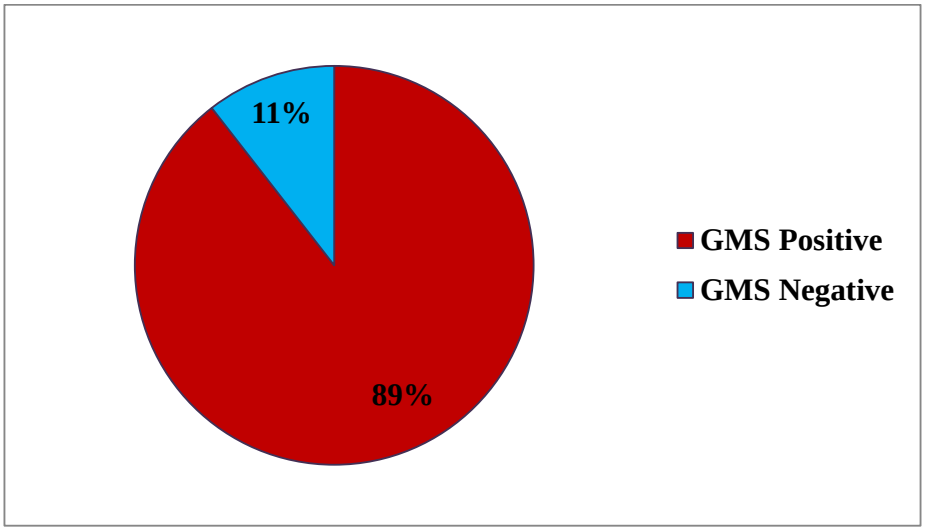
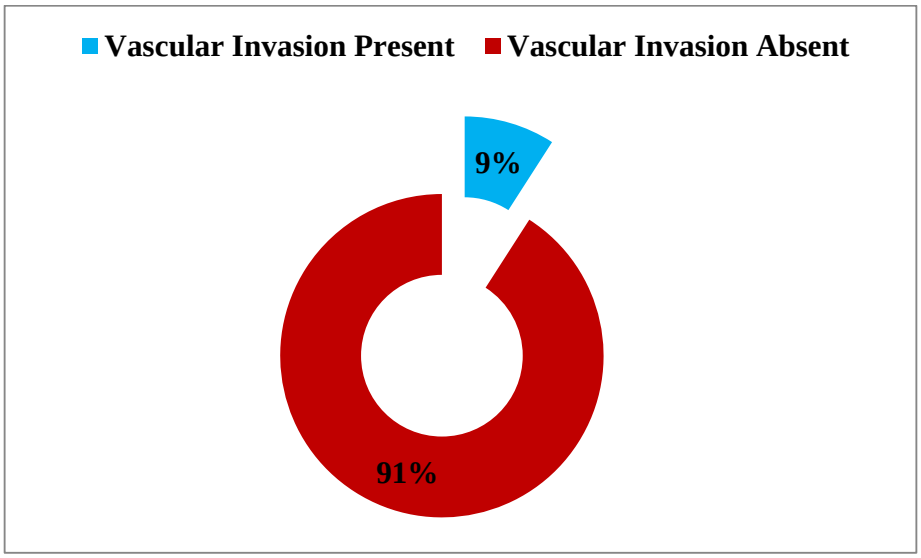


CHART:10 Vascular Invasion among fungal sinusitis



DISCUSSION

DISCUSSION

The incidence of FRS shows a rising trend in the past decade, especially in tropical countries like India. This is because of hot and humid climate in tropical countries. One of the favourable factor for occurrence of FRS. Improved techniques of specimen collection for mycological processing and development of an ideal approach for the diagnosis of FRS, which depends on clinical diagnosis, KOH and culture positivity and histopathological evidence of fungal hyphae in the tissue examined.

This study was conducted among 106 cases of chronic rhinosinusitis fulfilling the inclusion criteria who underwent diagnostic nasal endoscopy and functional endoscopic sinus surgery in the department of ENT, Coimbatore medical college hospital, Coimbatore from June 2016- May 2017.

Prevalence Rate:

In this study the prevalence of FRS based on microscopy, culture and histopathology was found to be 21%(Table:3) .This correlates well with the study conducted by G Banerjee et al. which showed 21% [5] prevalence rate and another study by Shivani et al.[9] also reported 21% .Based on several studies the distribution of FRS was found to range from 0-100% [7]. This wide range of occurrence is due to Geographical variation in the prevalence of fungal spores in the environment.

Gender-wise distribution of FRS:

There was a male preponderance with a male: female of 2.7:1, but statistically the gender difference was not significant(Table:12) (p=0.39). Similar observations made by Shiv Sekar et al 2009[10], who observed a male predominance .

Age-wise distribution of FRS:

The mean age of distribution of FRS was found to be high i.e 42.45 years(Table:11) and it is found to be statistically significant by independent 't' test with a p value of <0.05 , which is more close to the observation made by H.S.Satish et al.[17] in 2012 of 43.81 years as mean age of occurrence. This is because males in middle age group are the one who are more frequently exposed to the external environment and can easily acquire fungal spores.

Risk Factors:

Bronchial asthma was found to be an associated risk factor for the occurrence of AFRS and type-II Diabetes mellitus for invasive form of the disease. The presence of either Asthma or Diabetes is definitely a risk factor for fungal sinusitis , which is found to be statistically significant by chi square method , p value <0.001.

Clinical Features:

More than 80% (chart:4) of the patients with FRS in this study presents with nasal block, nasal discharge, and headache. A study by H.S.Satish et al.[17] in 2012 have also reported the same clinical findings in > 80% of the patients. Thus FRS very often has the same clinical presentation as that of Bacterial sinusitis, and it can be easily misdiagnosed.

Cranial nerve involvement, orbital complications and cerebrovascular accidents are most frequently observed in chronic invasive forms. This is because of chronicity, the fungal spores may germinate to form filaments which can invade the adjacent tissues. Chronic inflammation also causes bony erosion due to pressure necrosis and extends beyond the confines of the sinus cavity.

Fungal isolation from different specimens:

Fungi are isolated more in the sinonasal polyps (68%) (Table:7) (Chart:3). Since fungi are ubiquitous, they can easily colonise the sinus cavity. It is yet to be determined whether fungal infection leads to the formation of polyp or already existing polyp is superseded by fungal spores. But most of the studies have shown that fungal infection leads to the formation of polyps.

Fungal identification by culture, microscopy and Categorization by HPE:

All the 22 (Table:10) cases of FRS were culture proven, but 95% were KOH and only 77% were HPE proven. This is because when superficial sections are taken for HPE, fungal hyphae could not be demonstrated or that portion of tissue sent for HPE may lacks fungal hyphae. Categorization of FRS has both prognostic and management implications and it is based on the clinical presentation and histopathological findings.

Allergic fungal Rhinosinusitis:

AFRS which constitutes about 68% (Table:4, Chart:2), was found to be the commonest form of FRS in this study, which was close to the results obtained by Anadhilakshmanan et al. in their study(66%)[3]. A study by Rajiv C Michael et al[.2] also showed the occurrence of AFRS to be about 63% of the total FRS patients. Since India is a tropical country with hot and humid climate, the fungal spore counts exceeds pollen counts by 100 folds [44] and there is a strong association between fungal allergy and bronchial asthma. In this study also bronchial asthma seems to be the commonest risk factor accounting for 53% of cases of AFRS associated with bronchial asthma.(chart:7).

Aspergillus flavus constitutes about 67%(Table:9) of total isolates causing AFRS, followed by Aspergillus fumigatus which constitutes about

13%(Table:9) Most of the studies from Indian subcontinent had shown that Aspergillus flavus and fumigatus are the common isolates causing AFRS [5], which is entirely different from the western literature which showed the presence of dematiaceous fungi like Bipolaris, Curvularia lunata, Alternaria species etc. This difference is mainly due to the difference in the distribution of fungi in geographical area.

Fungal ball:

Patients with Fungal ball often presents with unilateral nasal obstruction, nasal discharge and polyp often involving maxillary sinus. Fungal ball showed a prevalence of 4.5% (Table:4) in this study and Aspergillus fumigatus was isolated in maxillary sinus, this was similar to the findings obtained by Das et al.[44] which showed 4% incidence of Fungal ball. Study by Dufour et al.[45] showed occurrence of fungal ball in unilateral maxillary sinusitis, where Aspergillus fumigatus was isolated. Though the diversity in isolation of different fungi in various geographical areas was attributed to the climatic condition, the difference in the presentation of FRS are yet to be defined [11].

Acute Invasive FRS:

Out of 22 cases of FRS, 6 were found to be invasive FRS. Rhizopus species was isolated in 2 cases(Table:4) from the necrotic material in the hard

palate and involving left maxillary sinus who were diagnosed to have Acute invasive FRS and both were found to have diabetic ketoacidosis. One out of two cases of acute invasive FRS had presented with facial swelling, halitosis, CVA and altered sensorium. This is because of vascular invasion of the fungi causing ischemic stroke with cerebral infarction. Though the patient was started on systemic Amphotericin-B, the patient succumbed to illness. This is because the members of zycomycetes family have some of the specific pathogenic factors like rapid growth, high affinity to blood stream, production of certain enzymes like lipases, proteases and having efficient iron transport system [46]. Vascular invasion was found to be 9% in this study(chart:10).

Chronic invasive FRS:

3 out of 22 cases (Table:4) of FRS were diagnosed to have CIFRS which accounts for 14% of total FRS cases. This correlates well with the study by Hardik shah et al. 2013, which showed 10% , where Aspergillus flavus followed by fumigatus was isolated. But in contrast, Rhizopus species was found to be the prime etiological agent accounting for 67%(Table:9), followed by Aspergillus fumigatus accounting for 33% of CIFRS in this study. A study done in Thailand in 1998-2008 had shown that Rhizopus species was the commonest fungi causing CIFRS [13].

Chronic granulomatous FRS:

CGFRS ,Fungal granuloma was diagnosed in one case(Table:9) 4.5%, and Aspergillus flavus was isolated. A study by Ravinder kaur et al.2013-2014 also showed Aspergillus flavus was the commonest isolate from CGFRS[13].Most of the studies have shown Aspergillus flavus was the common isolate in CGFRS. The presence of noncaeseating granuloma differentiates CGFRS from CIFRS.

Antifungal susceptibility testing:

In this study Antifungal susceptibility testing was done using Amphotericin-B for all the isolates and voriconazole for Aspergillus species by CLSI broth dilution method and MIC determination of Amphotericin-B for all isolates by E-strip method. Taking CLSI as a reference method the MIC obtained by E-strip was compared and Essential agreement was determined.

Roswell parker memorial institute (RPMI-1640) media was used for BDM and RPMI agar was used for E-strip method.

In this study all the Aspergillus, Rhizopus and penicillium isolates were found to have MIC of < 2 µgm/ml for Amphotericin-B which is associated with good clinical outcome [30]. This correlates well with the study

conducted by O C Abraham et al , where the mean MIC for Amphotericin-B was 2µgm/ml [48] for Aspergillus species(Table:14).

It was also observed that Voriconazole had low mean MIC of 0.22µgm/ml for Aspergillus flavus, 0.25µgm/ml for Aspergillus fumigatus and 0.25µgm/ml for Aspergillus niger when compared to MIC obtained for Amphotericin-B which showed 0.7µgm/ml for Aspergillus flavus, 0.6µgm/ml for Aspergillus fumigatus and 0.5µgm/ml for Aspergillus niger(Table:14,15). These findings are similar to that observed by O C Abraham et al [48], which also showed that azoles especially Voriconazole had better invitro susceptibility when compared to Amphotericin-B for Aspergillus isolates.

In India, recently there is an increasing trend in the development of resistant strains of Aspergillus species towards Amphotericin-B due to the Wide range of use of the drug. In Aspergillus flavus due to the efflux of the drug, alteration in cell wall composition and increased transcription of Af1MDR1 gene. There is an intrinsic resistance of Amphotericin-B towards Aspergillus terreus because of less ergosterol content in its cell membrane and production more catalase which plays a major role in the development of resistance.[43]

The mechanism of resistance to Voriconazole by Aspergillus species was found to be due to amino acid substitution, because of mutation in azole-target-enzyme gene cyp51A. This results in over expression of these

gene and drug efflux gene and upregulation of homeostatic stress response pathways contributing to azole resistance in Aspergillus fumigatus. T788G missense mutation was responsible for resistance exhibited by Aspergillus flavus towards Voriconazole.[43]

Since the usage of Echinocandins group of antifungal agents like Caspofungin is very low in India, its resistance by Aspergillus species has not been reported in India.[43]

The mean MIC obtained by E-strip(Table:16,17) was found to have ± 2 dilution scheme of reference BDM for Amphotericin-B of all the 22 isolates obtained. Thus the essential agreement was found to be 100%. E-test correlates well with the reference BDM. A similar findings was also observed by Prashanth gupta et al.[43] , which also showed better reproducibility with E-strip.

SUMMARY

SUMMARY

- In this study 106 patients who were diagnosed as CRS, are included and specimen like endoscopic aspirates , necrotic material and polyps were taken during diagnostic nasal endoscopy and FESS procedure.
- All the specimen were subjected to standard mycological processing including direct microscopy and fungal culture. A portion of the tissue specimen was sent to pathology for HPE and fungal staining(GMS).
- The prevalence of FRS was about 21%.
- Categorization was done based on the HPE findings.
- Non invasive form was more prevalent than invasive FRS. In that, AFRS accounts for 68% of total cases of FRS. Fungal ball in 5%, Chronic granulomatous FRS in 5% , Chronic invasive in 14%, and Acute invasive FRS in 9% of cases.
- Aspergillus flavus was isolated in about 67% cases of AFRS, followed by Aspergillus fumigatus (13%), Aspergillus niger (13%) and Penicillium (7%).
- Rhizopus species was the common isolate among the invasive form. About 67% cases CIGFRS showed Rhizopus species and in about 100% of AIFRS Rhizopus was isolated . One case of CIFRS showed the etiological fungi to be Aspergillus fumigatus.

- Aspergillus flavus was isolated in CGFRS and Aspergillus fumigatus was isolated in Fungal ball.
- Head ache, nasal obstruction and nasal discharge was the common clinical presentation which are often misdiagnosed as bacterial sinusitis.
- Life threatening complications like cavernous sinus thrombosis, CVA, cranial nerve involvement are observed in Acute invasive FRS.
- Pansinus involvement (64%) was the more common site of occurrence of FRS.In case of Fungal ball unilateral maxillary sinus involvement was observed.
- Sinonasal polyps were found to be the ideal specimen for isolation of fungi.
- All the 22 isolates were culture proven, 95% were KOH proven and 77% were HPE proven.
- Fungal stain (GMS) was found to be positive in 89% of the specimens.
- There was no difference in gender wise distribution of cases of FRS because the p value(0.39) obtained was not statistically significant.
- The mean age of occurrence of FRS was found to be high i.e 42.45
- Bronchial asthma was the risk factor associated with AFRS(53%) and diabetes mellitus(100%) in case of invasive FRS.
- RPMI-1640 media was used for doing antifungal susceptibility for CLSI microbroth dilution method and RPMI agar for MIC

determination by E-strip method. All the isolates were grown well in both the media.

- All the isolates are found to have a MIC of <2µgm/ml for amphotericin-B by both CLSI microbroth dilution and E-strip method which is associated with good clinical outcome as per CLSI guidelines.
- When compared to Amphotericin-B, Voriconzole had low MIC for Aspergillus species. Thus azole group of drugs are having better invitro susceptibility than Amphotericin-B.
- E-strip method when compared to CLSI microbroth dilution method showed that it has good reproducibility i.e the variation in MIC lies between one ± 2 dilution scheme of reference method with 100% essential agreement.

CONCLUSION

CONCLUSION

Rhinosinusitis is a common disorder affecting 20% of the population. Chronic form accounts for more than 90% of cases. Fungi are found to be the important cause of CRS.

Suspicion of FRS should be kept in mind, in those cases of CRS who are not responding to standard antibiotics therapy. Each and every category of FRS has unique geographical distribution, host related risk factors, clinical presentation, etiological agent and treatment modality. So it is essential to study the prevalence of FRS and continuous monitoring should be done in order to identify the new emerging pattern of FRS and etiological fungi. Thus FRS has wide range of prevalence. Here the prevalence rate was found to be 21%.

Non invasive form was common among the study population in particular AFRS with Aspergillus flavus being the common isolate and bronchial asthma was the associated risk factor. Rhizopus species was the common isolate among invasive FRS. Though the occurrence of invasive form is relatively low, most of the life threatening complications like cavernous sinus thrombosis, brain abscess CVA are encountered frequently in Acute invasive form , often Diabetes mellitus is being the common risk factor.

The presence of fungal elements in KOH mount and HPE is considered as more significant than fungal culture alone.

Recently there are evidence showing emerging resistance to commonly used antifungal agents . So it is important to study about Antifungal susceptibility and all the isolates are found to be sensitive to both Amphotericin-B and Voriconazole by CLSI BDM and E-test. It was also found that voriconazole had a better invitro susceptibility than Amphotericin-B for Aspergillus species.

E-strip method of determining MIC was also carried out using Amphotericin-B . Taking CLSI micro broth dilution method as a reference, E-strip was found to have a better reproducibility.

Since CLSI reference micro broth dilution method is cumbersome, time consuming ,E-strip can be used which has good essential agreement.

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ANNEXURES

STATEMENT OF CONSENT

I, _____, do hereby volunteer and consent to participate in this study being conducted by Dr. N.Vandarkuzhali I have read and understood the consent form (or) it has been read and explained to me thoroughly. I am fully aware of the study details as well as aware that I may ask questions to him at any time.

Signature / Left Thumb Impression of the patient

Station: Coimbatore

Date:

Signature / Left Thumb Impressionand Name of the witness

Station: Coimbatore

Date:

ஒப்புதல்படிவம்

நோயாளியின்பெயர்:
பாலினம் : வயது:
பெற்றோர்பெயர் :
முகவரி :

அரசுகோவை மருத்துவக்கல்லூரியில் நுண்ணுயிரியல் துறையில் பட்ட மேற்படிப்புபயிலும்மாணவி மரு. ந.வண்டார்குழலி அவர்கள் மேற்கொள்ளும் பூஞ்சைகல்லால் ஏற்படும் சைனஸ் பிரச்னை பற்றிய பரிசோதனை ஆய்வில்செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகளை தெரிவுபடுத்திக்கொண்டேன் என்பதை தெரிவித்துக்கொள்கிறேன். இந்தஆய்வில் நான் முழு சம்மதத்துடனும், சுயசிந்தனையுடனும் கலந்து கொள்ளசம்மதிக்கிறேன்.இந்த ஆய்வில் என்னைப்பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக்கொள்கிறேன். எந்தநேரத்திலும் இந்த ஆய்விலிருந்து நான் விலகிக்கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம்:

தேதி: கையொப்பம்/ ரேகை

A study on the prevalence of fungal isolates among the chronic Rhinosinusitis patients at CMCH .

Patient Proforma:

Name:

Age/Sex:

Address:

Occupation:

Presenting Complaints:

Past History:

History of Diabetes/Neoplasm/Chronic Medications (immunosuppressive agents).

History of previous surgeries for the same complaints :

Previous Diagnosis:

Surgery detail :

Culture reports :

Biopsy reports :

History suggestive of Allergic rhinitis: Yes /No.

Clinical findings:

Nasal discharge: Yes/No, bilateral/unilateral-right/left

Type of discharge:Allergic mucin/mucopurulent/purulent/blood stained.

Polyp: Yes/No,bilateral/unilateral-right/left

Orbital cellulitis: Yes/No

Cranial Nerve palsy:Yes/No

Cranial nerve palsy details:

Other findings if any:

Provisional diagnosis:

Diagnostic Nasal Endoscopic Findings: RT
LT

CT Findings:

Routine Investigations:

Surgery Details:

Surgery done: Date of surgery:

Surgery Findings:

Specimen Details: Date of Collection:

Type of specimen- DNE/FESS.

Macroscopic appearance of specimen:

Direct KOH mount findings:

Fungal culture :

Colony appearance: Date:

LPCB mount: Date:

Slide culture: Date:

Histopathological examination findings: Date:

Antifungal susceptibility test results: Date:

Final Diagnosis:

MASTER CHART

S.No	Name	age	sex	place	Risk factor	C/F	Site	side	Specimen	KoH	Culture proven	HPE Proven	GMS	vas inv	type	broth dilution		E-strip
																Amphotericin-B	Voriconazole	Amphotericin-B
1	Rajagopal	60	m	cbe	no	1,3,4,5,8,9	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
2	Manoharan	50	m	cbe	no	1,3,5	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
3	Jagadeswari	58	f	Tirupur	no	1,2,4,6,9	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
4	Rajkamal	27	m	cbe	no	2,3,5,8,9	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
5	Saravanan	25	f	cbe	asthma	1,2,3,5	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
6	Manojkumar	23	M	cbe	no	1,2,3,5,6,9	ethmoid	U/L	polyp	Neg	neg	neg	neg	Neg	NFRS	-	-	-
7	Robinson	40	m	cbe	DM	2,3,4,6	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
8	Murugan	39	m	cbe	Steroid intake	2,3,4,5,8,9	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
9	Divya	18	F	Tirupur	asthma	1,3,6,9	Max	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
10	Ramalingam	40	F	cbe	No	2,3,4,5,6,9	Pan	B/L	Polyp	Neg	neg	neg	neg	Neg	NFRS	-	-	-
11	Dineshkumar	20	m	karur	no	1,3,4,5	Max	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-

12	Ponnusamy	45	m	cbe	Asthma	1,2,3,4,5,6,7,8,9	pan	B/L	MM-discharge	pos	Asp.flavus	neg	neg	Neg	AFRS	1	0.25	0.256
13	Nagaraj	22	m	cbe	no	2,3,4,9	pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
14	Chandran	15	m	cbe	no	1,2,3,6	Pan	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
15	Saravanakumar	26	m	cbe	no	2,3,4,6,9	Max	B/L	MM-discharge	Neg	neg	neg	neg	Neg	NFRS	-	-	-
16	Isak	42	m	cbe	no	1,2,3,4,5,7,8,9	Max	B/L	MM-discharge	pos	A.niger	neg	neg	Neg	AFRS	0.5	0.25	0.25
17	Suguna	55	f	cbe	no	1,2,3,4	Ethmoid	B/L	Polyp	pos	Asp.flavus	pos	Pos	Neg	AFRS	0.5	0.25	1
18	Indrani	57	f	cbe	no	1,2,3,4,5,8,9	Max	B/L	Polyp	Pos	Asp.flavus	pos	pos	Neg	AFRS	1	0.25	0.032
19	Rajakumari	38	f	Tirupur	no	1,6,8,9	Max	B/L	Polyp	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
20	Vidhyasagar	42	m	cbe	asthma	2,3,5,6	Pan	B/L	Polyp	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
21	Selvarajan	32	m	cbe	no	1,2,3,4,5,6,8,9	Max	B/L	Polyp	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
22	Yahoob	36	M	cbe	asthma	1,3,5,9	Pan	U/L	Polyp	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
23	Rangammal	67	2	cbe	Asthma	1,2,3,9	Max	B/L	MM-discharge	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
24	Selvi	25	f	erode	No	1,2,3,5,9	Pan	B/L	MM-discharge	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
25	Sivakumar	45	m	tirupur	Asthma	1,2,3,5,9	Pan	B/L	MM-discharge	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
26	Suriya	19	m	cbe	No	1,2,3,	Pan	B/L	MM-	Neg	Neg	neg	neg	Neg	NFRS	-	-	-

						5,9			discharge									
27	Dhanalakshmi	28	f	cbe	Steroid intake	1,2,3,5,9	pan	B/L	MM-discharge	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
28	Radhamani	40	f	cbe	Asthma	1,2,3,4,5,8,9	Pan	B/L	polyp	pos	Asp.flavus	pos	pos	Neg	AFRS	0.5	0.25	0.128
29	Jeeva	21	m	cbe	No	1,2,3,5,9	Pan	B/L	polyp	pos	Asp.flavus	pos	Pos	Neg	AFRS	1	0.0625	0.256
30	Gandhi	51	m	krishnagiri	DM	1,2,3,4,5,8	Max	B/L	MM-discharge	Neg	Neg	neg	neg	Neg	NFRS	-	-	-
31	Mohandas	54	m	tirupur	DM	1,2,8	Max	U/L	Necrotic material	pos	Rhizopus	pos	pos	Neg	CIFRS	1	-	0.5
32	Giri	44	m	cbe	DM	1,3,4,5,6,7,8,9	Pan	B/L	Polyp	Pos	Asp.fumigatus	pos	pos	Neg	CIFRS	0.5	0.25	0.256
33	Ellango	54	M	cbe	DM	1,2,3,8,10,11,12,13	Pan	B/L	Necrotic material	Pos	Rhizopus	pos	pos	Pos	AIFRS	1	-	2
34	Vellammal	40	f	cbe	Asthma	1,2,3,5,6,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
35	Thangammal	32	f	cbe	No	1,2,3,5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
36	Velusamy	48	m	Tirupur	No	1,2,3,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
37	Pavainandini	22	f	cbe	No	1,2,3,4,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
38	Sentamil	24	f	cbe	No	1,2,3,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
39	Madhavan	53	m	cbe	No	2,3,5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
40	Moorthy	44	m	cbe	No	1,3,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-

41	Veerbathran	27	m	cbe	No	1,2,3, 4,5,6, 9	Max	U/L	Polyp	Pos	Asp.f umig atus	Neg	Neg	Neg	FB	1	0.25	1
42	Veeramuthu	57	m	cbe	DM	1,10, 11	Max	U/L	Necrotic material	Pos	Rhizo pus	Pos	Pos	Neg	CIFRS	1	-	0.5
43	Soundarajan	48	m	cbe	No	1,2,3, 4,5,6, 9	Pan	B/L	Polyp	pos	Asp.fl avus	Pos	Pos	Neg	AFRS	0.25	0.25	0.5
44	Rajasekar	23	M	cbe	No	1,2,3, 6,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
45	Senthilkuma r	20	m	Tirupu r	No	1,3,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
46	Thenmozhi	29	f	cbe	No	1,2,3, 5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
47	Rajaguru	27	m	cbe	No	1,2,3, 4,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
48	Jenifer	23	f	cbe	No	1,2,3, 9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
49	Jeevajothi	34	f	cbe	Asthm a	1,2,3, 5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
50	Rajeshwari	25	f	cbe	No	1,2,3, 4,5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
51	Vasanthi	54	f	cbe	DM	1,2,3, 4,5,6, 7,8,9	Pan	B/L	MM-discharge	Pos	Asp.fl avus	Neg	Neg	Neg	AFRS	0.5	0.25	1
52	Tamilvani	20	f	cbe	Asthm a	1,2,3, 4	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
53	Bhuvanesh wari	35	f	cbe	No	1,2,3	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
54	Karthi	38	m	cbe	No	1,2,3, 4,5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
55	Devi	34	m	cbe	No	1,2,3, 4,5,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
56	Rukmani	66	M	cbe	No	1,3,4,	Max	U/L	Polyp	neg	neg	neg	neg	Neg	NFRS	-	-	-

						7,8,11												
57	Sanathkumar	24	m	Tirupur	No	1,2,3,4,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
58	Sivakumar	35	m	cbe	No	1,2,3,4,5,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
59	Murali	28	m	cbe	No	1,2,3,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
60	Seetha	38	f	cbe	Asthma	1,2,3,4,5,6,7,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
61	Srikanth	34	m	cbe	No	1,2,3,5,6,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
62	Chandru	28	m	cbe	No	1,2,3,4,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
63	Seetha	22	m	Gopi	Asthma	1,2,3,4,5,6,7,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
64	Chellamal	56	m	Tirupur	No	1,2,3,4,6,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
65	Muthukrishnana	28	m	cbe	No	1,2,3,4,5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
66	Krishnan	44	m	cbe	Asthma	1,2,3,5,6,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
67	Kamala	32	f	cbe	No	1,2,3,4,5,6,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
68	Lavanya	21	f	cbe	No	1,2,3,4,5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
69	Rajam	42	f	cbe	No	1,2,3,4,5,6,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
70	Shanthi	40	f	cbe	No	1,2,3,4,5,6,	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-

						8,9											
71	Subramani	50	m	cbe	DM	1,2,3, 4,7,8, 10,11, 12	Max	U/L	Necrotic material	Pos	Rhizo pus	pos	Pos	Pos	AIFRS	1	-	1
72	Mani	41	m	cbe	Asthm a	1,2,3, 4,5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
73	Selvam	40	m	cbe	No	1,2,3, 4	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	
74	Mahendran	27	m	cbe	No	1,2,3, 4,5,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
75	Pattammal	54	f	cbe	No	1,2,3, 4,5,6, 8	pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
76	Rehman	41	m	cbe	no	1,2,3, 4,6,7	Pan	B/L	POLYP	POS	A.fla vus	pos	pos	Neg	CGIFR S	0.5	0.25	0.5
77	Nagalakshm i	48	f	cbe	No	1,2,3, 4,5,6, 8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
78	Rajagopalan	58	m	cbe	Asthm a	1,2,3, 4,5,6, 7,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
79	Dinesh	25	m	cbe	No	1,2,3, 4	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
80	Nallakumar	42	m	cbe	No	1,2,3, 4,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
81	Sivakumar	51	m	cbe	No	1,2,3, 4,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
82	Ragavan	30	m	cbe	Asthm a	1,2,3, 4,5,6, 7,9	Pan	B/L	polyp	pos	Asp.fl avus	pos	pos	Neg	AFRS	1	0.25	1
83	Rathnakuma r	28	m	cbe	No	1,2,3, 4,6,8, 9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-

84	Sangeetha	36	f	cbe	No	1,2,3,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
85	Kanagarani	42	f	cbe	Asthma	1,2,3,4,5	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
86	Muniraj	35	m	cbe	No	1,2,3,5,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
87	Paramesh	33	m	Tirupur	No	1,2,3,4,5,6,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
88	Sathasivam	21	m	cbe	Asthma	1,2,3,4,5,7	Pan	B/L	polyp	Pos	Penicillium.spp	Neg	Neg	Neg	AFRS	0.25	-	0.5
89	Rosy	34	f	cbe	No	1,2,3,4,5,6,7,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
90	Aishwarya	24	f	gopi	Asthma	1,2,3,5,7	Pan	B/L	polyp	pos	Asp.fumigatus	pos	pos	Neg	AFRS	0.5	0.25	1
91	Jeyashree	28	F	erode	No	1,2,4,6,7,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
92	Murugesan	47	1	cbe	Asthma	1,2,3,4	Pan	U/L	Polyp	pos	Asp.fumigatus	pos	pos	Neg	AFRS	0.5	0.25	0.256
93	Senthilkumar	51	m	cbe	No	1,2,3,4,5,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
94	Selvarajan	56	m	cbe	Asthma	1,2,3,4,5,6,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
95	Pasupathy	45	m	cbe	No	1,2,3,5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
96	Annamalai	39	m	cbe	Asthma	1,2,3,4,5,6,7,8,9	Pan	B/L	polyp	pos	A.niger	pos	pos	Neg	AFRS	0.5	0.25	0.002
97	Balakrishna	41	m	cbe	No	1,2,3,	Pan	B/L	MM-	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-

	n					4,5,6, 7,8			discharge								
98	Poornima	23	f	Tirupur	Asthma	1,2,3, 4,5,7, 8,9	Pan	B/L	polyp	pos	Asp.flavus	pos	Pos	Neg	AFRS	0.5	0.25	1
99	Daulath	50	f	cbe	Asthma	1,2,3, 4,7	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
100	Vasugi	48	f	cbe	No	1,2,3, 4,5,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
101	Anbu	36	m	cbe	Asthma	1,2,3, 4,5,6	Pan	B/L	Polyp	Neg	Asp.flavus	pos	pos	Neg	AFRS	1	0.0625	0.002
102	Rathnasamy	47	m	cbe	No	1,3,4, 5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
103	Jerin sithara	36	F	cbe	No	1,2,6, 7,8	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
104	Kamalam	49	f	cbe	No	1,2,3, 4,5,6, 7,8,9	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
105	Jothi	48	f	cbe	No	1,2,3, 4,5,6	Pan	B/L	MM-discharge	Neg	Neg	Neg	Neg	Neg	NFRS	-	-	-
106	Bharathan	35	M	cbe	No	1,2,3, 4,5,6, 7	Pan	U/L	Polyp	Pos	Neg	Neg	Neg	Neg	NFRS	-	-	-

CLINICAL FEATURES(C/F):

1-Nasal blockage 4-Facial pain 7-Facial swelling 10-Cranial nerve involment 13-Cavernous sinus involvement

2-Nasal discharge 5-Postnasal discharge 8-Anosmia 11-Orbital complications

3-Headache 6-Sneezing 9-Recurrent res.tract infection 12-CVA

KEYWORDS TO MASTER CHART

M	Male
F	Female
Cbe	Coimbatore
pos	Positive
neg	Negative
U/L	Unilateral
B/L	Bilateral
Pan	Pansinus
Max	Maxillary sinus
MM	Middle Meatus
NFRS	Non fungal rhinosinusitis
AFRS	Allergic fungal rhinosinusitis
CGIFRS	Chronic granulomatous invasive fungal srhinosinusitis
CIFRS	Chronic invasive fungal srhinosinusitis
FB	Fungal Ball
AIFRS	Acute invasive fungal rhinosinusitis