"ISOLATION, IDENTIFICATION AND SPECIATION OF FUNGI ASSOCIATED WITH CHRONIC RHINOSINUSITIS FROM SPECIMENS COLLECTED DURING FUNCTIONAL ENDOSCOPIC SINUS SURGERY"

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Endorsement by the Hod, Principal/Head of the Institution

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LIST OF ABBREVIATION

AFS	Allergic Fungal Sinusitis
CRS	Chronic Rhino-sinusitis
FESS	Functional Endoscopic Sinus Surgery
IFS	Invasive Fungal Sinusitis
GMS	Gomorri Methanamine Silver
KOH	Potassium hydroxide
PAS	Periodic Acid Schiff
SDA	Sabouraud Dextrose Agar
LCB	Lactophenol cotton blue

ABSTRACT

Introduction: Chronic rhinosinusitis is defined as an inflammatory condition of the mucosa of nasal cavity and the four paranasal sinuses, lasting more than 12 weeks duration.1

Fungi are being increasingly implicated in etiopathogenesis of rhinosinusitis. Fungal sinusitis is frequently seen in immunocompromised patients having diabetes, malignancy and on steroids, also in 45% of immunocompetent individuals. Fungi are most often under-diagnosed cause of chronic rhinosinusitis. Fungal sinusitis is known to have recurrence and refractory to antibiotic treatment. Its main treatment modality remains repeated Functional Endoscopic Sinus Surgery and antifungal drugs. Identification of fungal species helps in diagnosis and prescribing suitable antifungal to patient & adequate management. Hence the present study is undertaken to isolate and identify fungal species from FESS (Functional Endoscopic Sinus Surgery) samples of patients with chronic rhinosinusitis (CRS).

Aims & Objectives:1. To isolate, identify & speciate the fungi associated with chronic rhinosinusitis from specimens collected during FESS. 2. To classify the fungal sinusitis by histopathology.

Material And Methods: All clinically diagnosed cases of chronic rhinosinusitis in all age groups and of both sexes, attending Otorhinolaryngology OPD and undergoing Functional Endoscopic Sinus Surgery were taken for this study.

70 samples were collected during FESS procedure. Specimen were subjected to Direct microscopy(KOH preparation) using 10% and 20% Potassium hydroxide. They were inoculated onto Sabouroud Dextrose Agar with and without antibiotics

(Chloramphenicol and Cycloheximide).Isolates were identified by standard mycological techniques.

Tissue sections were stained with Haematoxylin and Eosin. The presence of fungal elements were confirmed with special fungal stains (Gomori Methanamine Silver and Periodic Acid Schiff).

Results: Out of 70 samples, 25 were both KOH and culture positive, 39 samples were both KOH & culture negative,5 samples were KOH positive but culture negative. One of sample was KOH negative & culture positive. Commonest age group found to be affected is 21-30 years (40%).Least age group affected is 61-70 year(1.42%). Total male:female ratio is 1.3:1. *A.flavus* was most common isolate(13) .*A.niger* was next common isolate (5),.*C.carionii*(3),*H.dematioides*(1),*S.prolificans*(1),*P.expansum*(1) were the other isolates.

Histopathologically,majority of samples , 25(96%) were of Allergic fungal sinusitis type,while 1(4%) was of Fungal ball/Mycetoma type.

Prevalence of fungal sinusitis based on Culture positive status in present study is 35.17%.

Discussion:

- 70 cases of chronic rhinosinusitis were reported with age range of 6-63 years,most patients were in age range of 21-30 years(40%).
- Male:female ratio was 1.3:1.Most (55/70) patients were from rural background.
- Prevalence of fungal sinusitis based on Culture positive status in present study is 35.17%.

- *A.flavus* was the most common isolate.
- This correlated well with studies done by Klossek *et al*,Michael *et al*,Prateek *et al*,Giri *et al*, Ragini *et al*.

Reports of unusual causes of FRS have been on the rise from India as well as other countries. We have also isolated rare fungi like *Cladosporium carionii*, *Hormonema dematioides, Scedosporium prolificans & Penicillium expansum.*

- Giri *et al*,Baradkar *et al*,Swain *et al*,Premamalini *et al*,Janagond *et al* & Shivprakash *et al* also reported rare causes of FRS in their studies.

  Conclusion: Fungal sinusitis is very common in our country with several contributing factors like hot humid climate, poor hygiene, increased outdoor activities, inhalation of spores and immunosuppression.

Accurate and early diagnosis of fungal sinusitis helps in preventing progression to Invasive fungal sinusitis, which is associated with high morbidity and mortality, and unnecessary administration of antibiotics to the patient. Thus better patient care & management.

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INTRODUCTION

Chronic rhinosinusitis is defined as an inflammatory condition of the mucosa of nasal cavity and the four paranasal sinuses, lasting more than 12 weeks duration.[1]

Fungi are being increasingly implicated in etiopathogenesis of rhinosinusitis. Fungal sinusitis is frequently seen in immunocompromised patients having diabetes, malignancy and patients on steroids, and also in 45% of immunocompetent individuals.[1]

Fungi are uncommon causes of sinusitis, but the incidence of these infections is increasing. Many fungi have been associated with fungal sinusitis, including the *Aspergillus* species, several of the dematiaceous fungi including *Curvularia,Bipolaris*, *Exserohilum* and also the zygomycetes. The etiological agents of fungal sinusitis reported from India vary from those of the western countries, wherein dematiaceous fungi are more common. *Aspergillus* spp are more commonly isolated from the Indian subcontinent.[1]

Fungal sinusitis can be divided into two main types: non invasive and invasive. Non invasive can be further divided into two forms: allergic fungal sinusitis (AFS) and sinus mycetoma/fungal ball, which occurs in immunocompetent patients. This classification is mainly confirmed by histopathologically. AFS should be suspected in individuals with intractable sinusitis and recurrent nasal polyposis. These patients usually have atopy.

Invasive fungal sinusitis, unless diagnosed early and treated aggressively has high mortality rate (80-85%)[1]. Most commonly Zygomycetes are responsible for

Invasive fungal sinusitis, which have tendency for angioinvasion, rapid onset & tissue destruction.

The diagnosis of invasive fungal sinusitis is based on a high index of clinical suspicion in immunocompromised patients with fever, nasal congestion, discharge and facial pain. If fungi are grown from nasal swabs, this adds weightage to the diagnosis, but a biopsy is required for confirmation.

Diagnosis of fungal sinusitis with isolation & identification of the species causing sinusitis is important in the management of rhinosinusitis. It also helps to prevent recurrence of the same.[2]

Patients with noninvasive forms have intractable sinusitis that fail to respond to repeated courses of antibiotics and, unfortunately often results in multiple operations before the diagnosis is confirmed.[2]

Fungal sinusitis is known to have recurrence and refractory to antibiotic treatment. Its main treatment modality remains repeated Functional Endoscopic Sinus Surgery and antifungal drugs. Identification of fungal species helps in diagnosis and prescribing suitable antifungal to patient and for adequate management. .

Hence the present study is undertaken to isolate and identify the fungi associated with patients of chronic rhinosinusitis, from FESS (Functional Endoscopic Sinus Surgery) samples.

AIMS & OBJECTIVES

1. To isolate, identify & speciate the fungi associated with chronic rhinosinusitis from specimens collected during FESS.
2. To classify the fungal sinusitis by histopathology.

REVIEW OF LITERATURE

Definition of Rhinosinusitis:

Rhinosinusitis refers to an inflammatory condition involving the four paired structures surrounding the nasal cavities[5].

Acute rhinosinusitis is defined as sinusitis of less than 4 weeks duration[5].

Chronic rhinosinusitis is defined as symptoms of sinus inflammation lasting more than 12 weeks[33].

History:

The history of nasal polyps goes back over 4000 years to Ancient Egypt. Further significant advances were made in Ancient Greece and Renaissance Europe. The first known medical practitioner was an Egyptian rhinologist called Ni-Ankh Sekhmet his picture together with that of his wife were found on a slab in the tomb of the king together with a testimony of royal gratitude which states “he has made his nostrils well” (Brain 1997). It has been shown that the Egyptians were familiar with nasal polyps, which they described as “grapes coming down from the nose”. Treatment included medicaments containing alcohol and it is possible that some of the Egyptian surgical instruments may well have been used to remove polyps (Pahor and Kimura 1991). Hippocrates (460-370 B.C.), the “Father of Medicine” thought that polyp disease resulted from a disturbed equilibrium between the 4 “humours”.

When the humours were too thick it could result in the development of polyps. He developed a technique for removal of polyps, which was included in textbooks as late as that of Voltolini’s in 1888(Vancil 1969). Paulus of Aegina (625-

690 A.D.), a physician in Alexandria, believed that the ethmoid cells were the origin of nasal polyps.

In 1835, Augustino Bassi established that fungi could also cause infections in man. In 1885, fungal sinusitis was first described in French literature by Platauf, he also reported first case of Mucormycosis with rhinocerebral involvement.In 1893,Mackenzie first reported paranasal sinus mycosis. In 1897, Oppe reported *Aspergillus* infection of sphenoid sinus. In 1980, Safirstein first described Allergic Bronchopulmonary Aspergillosis. In 1981, Miller first reported allergic aspergillosis of the paranasal sinuses. In 1983, Katzenstein reported Allergic *Aspergillus* Sinusitis. In 1987,Macmillan first noted *Curvularia lunata* could be found in AFS cases. In 1997,deShazo & colleagues described classification system with criteria for the diagnosis of fungal sinusitis.

The International Society for Human and Animal Mycology (Isham) during its 16th Congress held at Paris constituted Working Group on Fungal Sinusitis under leadership of Prof. A. Chakrabarti[55].

Anatomy of Nose & sinuses

NASAL CAVITY

The nasal cavity is an irregular space between the roof of the mouth and the cranial base. It is wider below than above, and widest and vertically deepest in its central region, where it is divided by a vertical osseocartilaginous septum that is approximately median in position. The bony part of the septum reaches the posterior limit of the cavity.

The nasal cavity communicates with the frontal, ethmoidal, maxillary and sphenoidal paranasal sinuses and opens into the nasopharynx through a pair of oval openings, the posterior nasal apertures or choanae. The latter are separated by the posterior border of the vomer, and each is limited above by the vaginal process of the medial pterygoid plates, laterally by the perpendicular plate of the palatine bone and the medial pterygoid plate, and below by the horizontal plate of the palatine bone .

The adult choana typically measures 2.5 cm in vertical height and 1.3 cm transversely: size is not usually affected by deviations of the nasal septum. The vomerovaginal and palatovaginal canals are found in the roof of this region.

Each half of the nasal cavity has a roof, floor, medial (septal) and lateral walls and a vestibule.

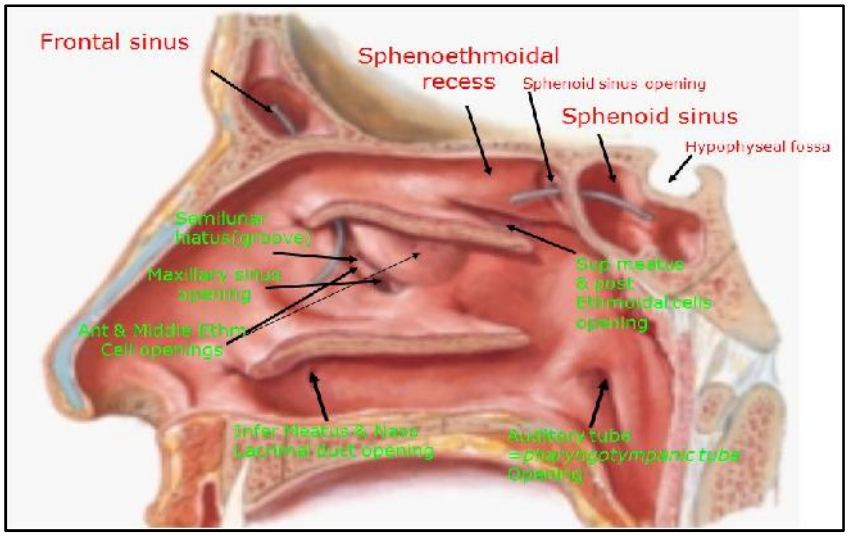


Fig 1: Anatomy of Nasal cavity

PARANASAL SINUSES

The paranasal sinuses are the frontal, ethmoidal, sphenoidal and maxillary sinuses, housed within the bones of the same name. They all open into the lateral wall

of the nasal cavity by small apertures that permit both the equilibration of air between the various air spaces and the clearance of mucus from the sinuses into the nose via a mucociliary escalator. The detailed position of these apertures, and the precise form and size of each of the sinuses, varies enormously between individuals. Respiratory epithelium extends through the apertures of the paranasal sinuses to line their cavities, a feature that unfortunately favours the spread of infections. Sinus mucosa is thinner, less vascular and has fewer goblet cells than nasal mucosa. Cilia are always present in the mucosa near the apertures but less evenly distributed elsewhere within the sinuses.

Most sinuses are rudimentary or absent at birth, but enlarge appreciably during the eruption of the permanent teeth and after puberty, events that significantly alter the size and shape of the face.

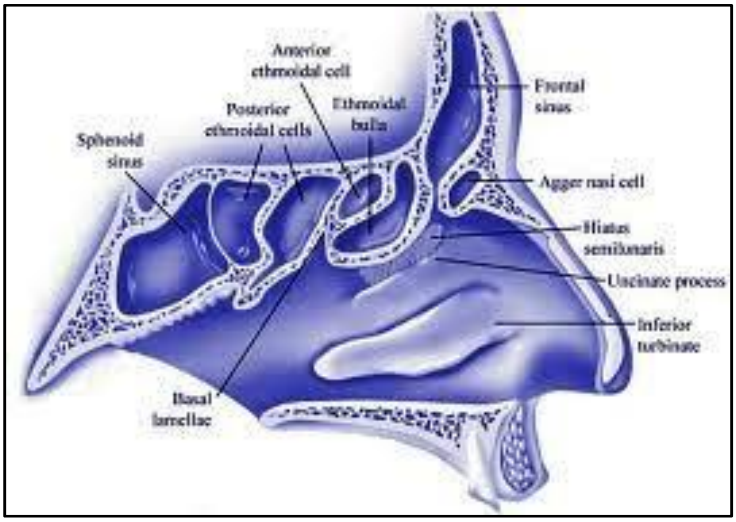


Fig 2: Anatomy of Nasal septum

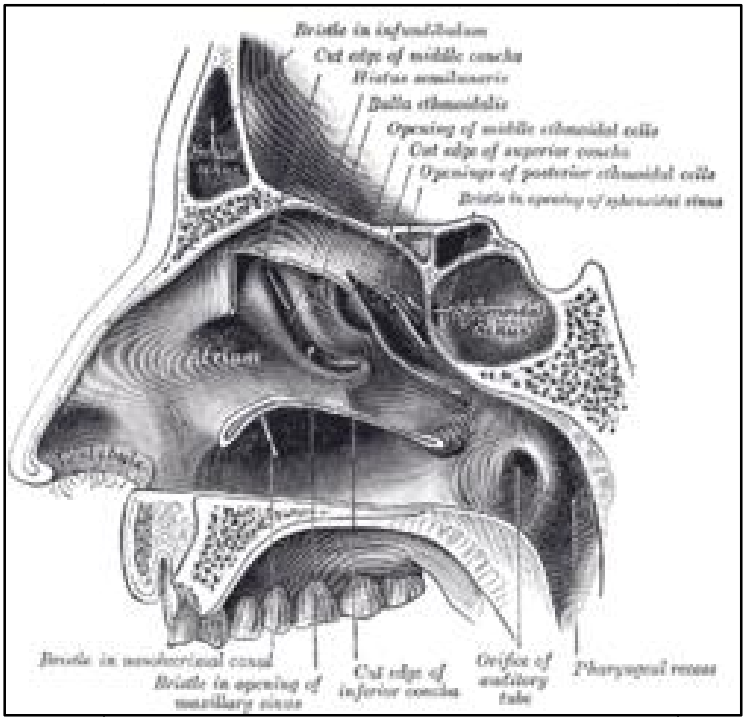


Fig 3: Anatomy of paranasal sinuses

The functions of the paranasal sinuses remain speculative. They clearly add some resonance to the voice, and also allow the enlargement of local areas of the skull while minimizing a corresponding increase in bony mass. It is likely that such growth-related changes serve to strengthen particular regions, e.g. the alveolar process of the maxilla when the secondary dentition erupts, but they may also function in contouring the head to provide visual signals indicating the individual's status in a social context (e.g. gender, sexual maturity and group identity).

FRONTAL SINUS

The paired frontal sinuses are posterior to the superciliary arches, between the outer and inner tables of the frontal bone. Each usually underlies a triangular area on the surface of the face, its angles formed by the nasion, a point 3 cm above the nasion and the junction of the medial one-third and lateral two-thirds of the supraorbital margin. The two sinuses are rarely symmetrical, since the septum between them

usually deviates from the median plane. Each sinus may be further divided into a number of communicating recesses by incomplete bony septa.

The average dimensions of an adult frontal sinus are: height 3.2 cm; breadth 2.6 cm; depth 1.8 cm. Each usually has a frontal portion that extends upwards above the medial part of the eyebrow, and an orbital portion that extends back into the medial part of the roof of the orbit. In 4% one or both sinuses may be hypoplastic or even absent; racial differences have been reported. The prominence of the superciliary arches is no indication of the presence or size of the frontal sinuses. A frontal sinus may extend posteriorly as far as the lesser wing of the sphenoid bone but does not invade it. The morphology of the frontal sinus may be specific enough to allow identification of individuals from radiological evidence for forensic purposes.

The aperture of each frontal sinus either opens into the anterior part of the corresponding middle meatus by the ethmoidal infundibulum either as a frontonasal recess (rather than a duct) or medial to the hiatus semilunaris if the uncinate process is attached to the lateral nasal wall or an agger nasi cell.

The frontal sinuses are rudimentary or absent at birth, generally well developed between the seventh and eighth years, but reach full size only after puberty. They are more prominent in males, and lend the forehead an obliquity that contrasts with the vertical or convex profile typical of children and females. In the presence of a persistent metopic suture, the frontal sinuses develop separately on either side of the suture, which can be helpful in excluding frontal fractures.

Vascular supply, lymphatic drainage and innervation :

The frontal sinuses receive their arterial supply from the supraorbital and anterior ethmoidal arteries. Lymphatic drainage is to the submandibular nodes. The sinuses are innervated by branches of the supraorbital nerves, and the orbital branches of the pterygopalatine ganglia.

SPHENOIDAL SINUS

The sphenoidal sinuses are two large irregular cavities within the body of the sphenoid and therefore lie posterior to the upper part of the nasal cavity. Each opens into the corresponding sphenoethmoidal recess via an aperture high on the anterior wall of the sinus. At birth the sinuses are minute cavities, and their main development occurs after puberty. The average adult dimensions are: vertical height 2 cm; transverse breadth 1.8 cm; anteroposterior depth 2.1 cm. The sinuses are usually separated by a septum that deviates from the midline in 75%, so that they are unequal in size and form. Their lumina may be further partially divided by bony laminae, accessory septa, especially in the region of former synchondroses. Occasionally one sinus overlaps the other above and, rarely, they intercommunicate. Bony ridges, produced by the internal carotid artery, pterygoid canal, maxillary branch of trigeminal and, in 15% of individuals, the optic nerve, may project into the sinuses from their lateral walls. Dehiscences in the osseous walls of either sinus may occasionally leave their mucosa in contact with the overlying dura mater.

The extent of pneumatization of surrounding bone is highly variable. In nearly 50% of skulls, a lateral recess may extend into the greater and lesser wings of the sphenoid or the pterygoid processes, and may even invade the basilar part of the

occipital bone almost to the foramen magnum. A posterior ethmoidal sinus may extend posterosuperior to the relatively smaller sphenoidal sinuses. The sphenoidal sinuses are related above to the optic chiasma and hypophysis cerebri and on each side to the internal carotid artery and cavernous sinus. One or both sinuses may partially encircle the optic canal.

The shape and position of the sphenoidal sinus is of clinical importance in the trans-sphenoidal surgical approach to the hypophysis cerebri. The sinuses may be classified into three main types: sellar, the commonest type, where the sinus extends for a variable distance beyond the tuberculum sellae, presellar, where the sinus occasionally extends posteriorly towards, but not beyond, the tuberculum sellae, conchal, the rarest type, where a small sinus is separated from the sella turcica by 10 mm of trabecular bone. The anterior midline septum often becomes deviated to one side posteriorly: identification of this septation is important prior to surgery.

Vascular supply, lymphatic drainage and innervations:

The sphenoidal sinuses receive their arterial supply from the posterior ethmoidal branches of the ophthalmic arteries and sphenopalatine arteries. Lymph drainage is to the retropharyngeal nodes. The sinuses are innervated by the posterior ethmoidal branches of the ophthalmic nerves, and the orbital branches of the pterygopalatine ganglia.

ETHMOIDAL SINUSES

The ethmoidal sinuses differ from the other paranasal sinuses in that they are formed of multiple thin-walled cavities in the ethmoidal labyrinth. The number and size of the cavities varies, from three large to 18 small sinuses on each side. They lie

between the upper part of the nasal cavity and the orbit, and are separated from the latter by the paper-thin lamina papyracea or orbital plate of the ethmoid (this presents a poor barrier to infection which may therefore spread into the orbit). Pneumatization may extend into the middle concha, or into the body and wings of the sphenoid bone lateral to the sphenoid sinus.

The ethmoidal sinuses are divided clinically into anterior and posterior groups on each side, distinguished by their sites of communication with the nasal cavity. (Cells previously designated as belonging to a middle group are now included with the anterior group.) The anterior and posterior groups are separated from each other by the basal lamella of the middle concha; this may be indented by cells from either group and therefore it forms a rather tortuous barrier between them. In each group the sinuses are only partially separated by incomplete bony septa. The sinuses are of clinical significance at birth because they are susceptible to inflammation. They grow rapidly between the ages of 6 and 8 years and after puberty.

Anterior group

Peri-infundibular sinuses (anterior ethmoidal air cells) Up to 11 anterior ethmoidal air cells drain into either the ethmoidal infundibulum or the frontonasal duct by one or more orifices. The most anterior group, agger nasi cells, are medial relations of the lacrimal sac and duct and invaginate beneath the agger nasi on the lateral wall of the nasal cavity anteriorly. Larger anterior and middle cells, Haller's cells, may develop medially beneath the orbital floor. The most anterior supraorbital ethmoidal sinus cells may encroach on the frontal sinus.

Bullar sinuses (middle ethmoidal air cells):

There are usually less than three middle ethmoidal air cells. They open into the middle meatus by one or more orifices on or above the ethmoidal bulla.

Posterior group:

Up to seven posterior ethmoidal air cells usually drain by a single orifice into the superior meatus; one may drain into the supreme meatus when present, and one or more into the sphenoidal sinus. The posterior group lies very close to the optic canal and optic nerve: optic nerve injury is a devastating potential complication of endoscopic sinus surgery, particularly if a spheno-ethmoid or Onodi cell is present. The reported incidence of an Onodi cell varies widely (3.5 to 51%), according to racial group: it is more common in non-Caucasians. The Onodi cell is usually regarded as the most posterior ethmoid cell that pneumatizes lateral and superior to the sphenoid sinus and is intimately associated with the optic nerve: it may contain a tuberculum nervi optici, where the optic canal bulges into the wall of the cell.

Vascular supply and innervation :

The ethmoidal sinuses receive their arterial supply from nasal branches of the sphenopalatine artery and the ophthalmic artery. Venous drainage is by the corresponding veins. The lymphatics of the anterior group drain to the submandibular nodes. The sinuses are innervated by the ophthalmic nerves and pterygopalatine ganglia.

MAXILLARY SINUS

The maxillary sinus (maxillary antrum), is the largest of the paranasal sinuses. It fills the body of the maxilla and is pyramidal in shape. The base is medial and forms much of the lateral wall of the nasal cavity. The floor, which often lies below the nasal floor, is formed by the alveolar process and part of the palatine process of the maxilla. It is related to the roots of the teeth, especially the second premolar and first molar, but may extend posteriorly to the third molar tooth and/or anteriorly to incorporate the first premolar, and sometimes the canine. Defects in the bone overlying the roots are not uncommon. The roof of the sinus forms the major part of the floor of the orbit. It contains the infraorbital canal which may exhibit dehiscences. The lateral truncated apex of the pyramid extends into the zygomatic process of the maxilla, and may reach the zygomatic bone, in which case it forms the zygomatic recess which throws a V-shaped shadow over the antrum on a lateral radiograph.

The facial surface of the maxilla forms its anterior wall, and is grooved internally by a delicate canal (canalis sinuosus) which houses the anterior superior alveolar nerve and vessels as they pass forwards from the infraorbital canal. The posterior wall is formed by the infratemporal surface of the maxilla: it contains alveolar canals that may produce ridges in the sinus and that also conduct the posterior superior alveolar vessels and nerves to the molar teeth. The medial wall is deficient posterosuperiorly at the maxillary hiatus, a large opening which is partially closed in an articulated skull by portions of the perpendicular plate of the palatine bone, the uncinate process of the ethmoid bone, the inferior nasal concha, the lacrimal bone, and the overlying nasal mucosa, to form an ostium and anterior and posterior fontanelles. The ostium usually opens into the inferior part of the ethmoidal

infundibulum, and thence into the middle meatus, via the hiatus semilunaris (the hiatus forms the area above the superior edge of the uncinate process). The fontanelles are covered only by periosteum and mucosa and may contain accessory ostia which may be visible on CT. All of the openings are nearer the roof than the floor of the sinus which means that the natural drainage of the maxillary sinus is reliant on an intact mucociliary escalator: the cilia of the sinus mucoperiosteum normally beat towards the ostium.

The maxillary sinus may be incompletely divided by septa; complete septa are very rare. The thinness of its walls is clinically significant in determining the spread of tumours from the maxillary sinus. A tumour may push up the orbital floor and displace the eyeball; project into the nasal cavity, causing nasal obstruction and bleeding; protrude onto the cheek, causing swelling and numbness if the infraorbital nerve is damaged; spread back into the infratemporal fossa, causing restriction of mouth opening due to pterygoid muscle damage and pain; or spread down into the mouth, loosening teeth and causing malocclusion. Extraction of molar teeth may damage the floor, and impact may fracture its walls. Hypoplasia of one maxillary antrum is present in up to 0.3% of the population.

Ostiomeatal complex

The term ostiomeatal complex, or ostiomeatal unit, refers to the area that includes the maxillary sinus ostium, ethmoid infundibulum and the hiatus semilunaris. It is the common pathway for drainage of secretions from the maxillary and anterior group of ethmoidal sinuses; where the uncinate process attaches to the lateral nasal wall the complex also drains the frontal sinus.

Vascular supply, lymphatic drainage and innervations:

The arterial supply of the maxilla is derived mainly from the maxillary arteries via the superior anterior, middle and posterior alveolar branches and from the infraorbital and greater palatine arteries. Veins corresponding to the arteries drain into the facial vein or pterygoid venous plexus on either side. Lymph drainage is to the submandibular nodes. The sinuses are innervated by the maxillary nerves and pterygopalatine ganglia.[4]

Classification & Definition of Rhinosinusitis[5]

Rhinosinusitis refers to an inflammatory condition involving the four paired structures surrounding the nasal cavities.

Although most cases of sinusitis involve more than one sinus, the maxillary sinus is most commonly involved; next, in order of frequency, are the ethmoid, frontal, and sphenoid sinuses. Sinusitis affects a tremendous proportion of the population, accounts for millions of visits to primary care physicians each year, and is the fifth leading diagnosis for which antibiotics are prescribed. It typically is classified by duration of illness (acute vs. chronic); by etiology (infectious vs. noninfectious); and, when infectious, by the offending pathogen type (viral, bacterial, or fungal).

Acute rhinosinusitis, defined as sinusitis of less than 4 weeks duration. It occurs primarily as a consequence of a preceding viral URI (Upper respiratory Infection).

Chronic sinusitis is defined as symptoms of sinus inflammation lasting more than 12 weeks. This illness is most commonly associated with either bacteria or fungi,

Etiology:

The ostial obstruction that results in rhinosinusitis can arise from both infectious and noninfectious causes.

Noninfectious causes include allergic rhinitis (with either mucosal edema or polyp obstruction), barotrauma (e.g., from deep-sea diving or air travel), and exposure to chemical irritants. Obstruction also can occur with nasal and sinus tumors (e.g., squamous cell carcinoma) or granulomatous diseases (e.g., granulomatosis with polyangiitis (Wegener's) or rhinoscleroma), and conditions leading to altered mucus content (e.g., cystic fibrosis) can cause sinusitis through impaired mucus clearance. In ICUs, nasotracheal intubation and nasogastric tubes are major risk factors for nosocomial sinusitis.

Infectious causes of rhinosinusitis:

Viral rhinosinusitis is far more common than bacterial sinusitis, the viruses most commonly isolated are rhinovirus, parainfluenza virus, and influenza virus.

Bacterial causes of sinusitis have been better described. *S. pneumoniae* and nontypable *Haemophilus influenzae* are the most common pathogens, *Moraxella catarrhalis* causes disease in a significant percentage (20%) of children but a lesser percentage in adults. Other streptococcal species and *Staphylococcus aureus* cause only a small percentage of cases, although there is increasing concern about community-acquired methicillin-resistant *S. aureus* (MRSA) as an emerging cause. Anaerobes occasionally are found in association with infections of the roots of premolar teeth that spread into the adjacent maxillary sinuses. The role of *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* in the pathogenesis of

acute sinusitis is unclear. Nosocomial cases commonly are associated with bacteria found in the hospital environment, including *S. aureus, Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumonia* and *Enterobacter* species.

Fungi are also established causes of sinusitis, although most acute cases are in immunocompromised patients and represent invasive, life-threatening infections. The best-known example is rhinocerebral mucormycosis caused by fungi of the order Mucorales, which includes *Rhizopus*, *Rhizomucor*, *Mucor*, *Mycocladus (formerly Absidia)* and *Cunninghamella*. Other hyaline molds, such as *Aspergillus* and *Fusarium* species, are also occasional causes of this disease. Noninvasive disease, is associated with dematiaceous molds such as *Curvularia* or *Bipolaris* species, can present as a number of different scenarios.

Clinical Manifestations:

Most cases of acute sinusitis present after or in conjunction with a viral URI. Common presenting symptoms of sinusitis include nasal drainage and congestion, facial pain or pressure, and headache. Thick, purulent or discolored nasal discharge is often thought to indicate bacterial sinusitis but also occurs early in viral infections such as the common cold and is not specific to bacterial infection. Other nonspecific manifestations include cough, sneezing, and fever. Tooth pain, most often involving the upper molars, as well as halitosis can be associated with bacterial sinusitis.

In acute sinusitis, sinus pain or pressure often localizes to the involved sinus (particularly the maxillary sinus) and can be worse when the patient bends over or is supine. Although rare, manifestations of advanced sphenoid or ethmoid sinus infection can be profound, including severe frontal or retroorbital pain radiating to the

occiput, thrombosis of the cavernous sinus, and signs of orbital cellulitis. Acute focal sinusitis is uncommon but should be considered over the maxillary sinus and fever in patients with severe symptoms, regardless of illness duration. Similarly, patients with advanced frontal sinusitis can present with a condition known as *Pott's puffy tumor*, with soft tissue swelling and pitting edema over the frontal bone from a communicating subperiosteal abscess. Life-threatening complications of sinusitis include meningitis, epidural abscess, and cerebral abscess.

Patients with acute fungal rhinosinusitis often present with symptoms related to pressure effects, particularly when the infection has spread to the orbits and cavernous sinus. Signs such as orbital swelling and cellulitis, proptosis, ptosis, and decreased extraocular movement are common, as is retroorbital or periorbital pain. Nasopharyngeal ulcerations, epistaxis, and headaches are also common, and involvement of cranial nerves V and VII has been described in more advanced cases. Bony erosion may be evident on examination. Often the patient does not appear seriously ill despite the rapidly progressive nature of these infections.

Patients of chronic sinusitis have undergone treatment with repeated courses of antibacterial agents and multiple sinus surgeries, increasing their risk of colonization with antibiotic-resistant pathogens and of surgical complications. These patients often have high rates of morbidity, sometimes over many years.

The management team should include an otolaryngologist to conduct endoscopic examinations and obtain tissue samples for histologic examination and culture. An endoscopy-derived culture not only has a higher yield but also allows direct visualization for abnormal anatomy.

In mild, indolent disease, endoscopic surgery is usually curative , with no need for antifungal therapy. Another form of disease presents as long-standing, often unilateral symptoms and opacification of a single sinus on imaging studies as a result of a mycetoma (fungus ball) within the sinus. Treatment for this condition is also surgical, although systemic antifungal therapy may be warranted in the rare case in which bony erosion occurs.

Treatment of chronic bacterial sinusitis can be challenging and consists primarily of repeated culture-guided courses of antibiotics, sometimes for 3–4 weeks at a time; administration of intranasal glucocorticoids; and mechanical irrigation of the sinus with sterile saline solution. When this management approach fails, sinus surgery may be indicated and sometimes provides significant, albeit short-term, alleviation. Treatment of chronic fungal sinusitis consists of surgical removal of impacted mucus. Recurrence, unfortunately, is common.[5]

Fungal rhinosinusitis

Classification:

Fungal sinusitis can be divided into two main types: non invasive and invasive.

Non invasive can be further divided into two forms: a) allergic fungal sinusitis (AFS) and b) sinus mycetoma/fungal ball, which occurs in immunocompetent patients.[1]

Invasive fungal sinusitis:It is further divided into 3 subtypes-a)Acute invasive, b) Chronic invasive, c) Granulomatous invasive fungal sinusitis.

a) **Allergic fungal sinusitis(AFS):** AFS is one of the non invasive forms of fungal sinusitis, it is characterized by allergic mucin, described as thick & tenacious secretion, tan or brown or black in color, which on H & E stain reveals eosinophils, Charcot Leyden crystal and fungal hyphae seen after GMS stain.[2]

Fungal species most commonly implicated with AFS are *Bipolaris, Alternaria, Curvularia, Rhizopus, Fusarium & Chrysosporium*.[3]

b) **Sinus mycetoma/Fungal ball:** Fungal ball is described as a dense mass of noninvasive, matted fungal hyphae within a paranasal sinus, mostly maxillary & sometimes sphenoid sinus.

Fungi commonly associated with fungal ball are *Aspergillus* & Dematiacious fungi.[2]

Invasive fungal sinusitis is one of the most challenging forms of sinonasal pathology to manage, most commonly presenting in immunocompromised individuals. Diagnosis of invasive fungal sinusitis requires histopathologic evidence of fungi invading nasal tissue: Hyphal forms within the sinus mucosa, submucosa, blood vessels or bone.[28]

It usually occurs in immunocompromised patients with acute onset of fever, cough, nasal mucosal ulceration or eschars, epistaxis and headache.[3]

a) **Acute Fulminant Invasive fungal sinusitis(AFIFS)**:1st described by McGill *et al* in 1980[29]. It is marked by vascular hyphal invasion, haemorrhage & infarction with time course of less than 4 weeks.[30,31]

AFIFS results from the rapid spread of fungi from the nasal and sinus mucosa by way of vascular invasion into the orbit, vessels and parenchyma of the brain. The time course of less than 4 weeks' duration separates acute from chronic disease.[32]

b) Chronic Invasive Fungal Sinusitis(CIFS): It usually occurs in immunocompromised & patients with diabetes mellitus, characterized by tissue necrosis & chronic low grade inflammatory reaction. Extension of this infection from ethmoid sinus into the orbit causes "Orbital Apex syndrome". *Aspergillus fumigatus* is the most common organism involved.

(CISA) is being reported in immunocompetent patients at an increasing rate while most of these cases are being reported from the India subcontinent and middle east but cases are being increasingly encountered from North America and elsewhere.[43]

c) Granulomatous Invasive Fungal Sinusitis(GIFS): It occurs in immunocompetent hosts, commonly in a unilateral sinus. The fungus is invasive but limited to the superficial mucosa. The limited 'micro-invasion' is well contained within surrounding granulomas, with scattered multinucleated giant cells. It is also called as "Indolent fungal sinusitis".

Pathophysiology

AFS is one of the two nontissue- invasive fungal sinusitis along with fungal balls. It is similar to Allergic Bronchopulmonary Aspergillosis which is postulated to Type I and Type III hypersensitivity reaction to fungal antigen. However, in AFRS, it is mainly Type I hypersensitivity which has been postulated in its pathophysiology (Type III reaction is doubtful, since immune complexes have not been demonstrated). According to the theory developed by the team at UT South-Western,[6-8] fungi enter

the nose and sinuses, triggering Type I and III responses; this inflammation results in: production of allergic mucin, stasis of secretions and obstruction of sinus ostia. The trapped fungi continue to stimulate the immune system, and the cycle repeats itself. Over time, massive polyposis develops, and fungal mucoceles distort the sinonasal anatomy.[9]

This theory finds several lines of support; however, the findings from the series of Ponikau et al (1999) question this theory.[10]

From their observations, they concluded that Type I hypersensitivity is not important in the pathophysiology of AFRS, and should not be a diagnostic criterion. They proposed the alternative term eosinophilic fungal sinusitis to describe this disease.[10]

These fungi were thought to be non cross-reactive, but recently an 18-kD protein has been isolated, which has been proposed to be a 'fungal pan allergen'.[11]

The disease is extramucosal, however, secondary bacterial infection may supervene, simulating an acute exacerbation of underlying chronic sinus disease.

The natural course of AFRS is characterized by frequent recurrences, irrespective of the treatment modality.

Kupferberg et al (1997) noted universal recurrence in the absence of vigorous postoperative medical treatment, and they noted, that even with medical treatment, recurrence was common.[12]

The hallmark of AFRS is the allergic mucin. Allergic mucin can be described as a thick and tenacious secretion, the color ranging from tan to green, brown or

black, which on H and E staining reveals eosinophils, Charcot Leyden crystals, and possibly fungal hyphae which can be seen after staining with Gomori Methanamine Silver stain. The Fontana Masson stain specifically stains melanin, which is characteristic of dematiaceous fungi, and thus helps to differentiate it from septate fungi. The mucin tends to become more tenacious and scanty after treatment with steroids. This mucin is essentially a proinflammatory mass of pyknotic eosinophils and their components, including their degranulation products which include the major basic protein(MBP).[13]

This MBP is a known proinflammatory and mucosal epithelial cell-toxic molecule, which plays an important role in asthma.[14]

Also, an 18-kD IgE fungal allergen has been identified within the mucin, which has been postulated to be a fungal pan-allergen, which besides being recognized by the patient's serum, also reacts with many different commercial mold preparations.[11]

Sometimes, this mucin is also encountered in patients with rhinosinusitis, with their clinical profile very similar to that of , but without actual AFRS. This mucin is negative for fungal hyphae on histolopathology with negative fungal cultures. They may also show the characteristic hyper attenuating shadows on CT as in AFRS. Some of them may not be atopic, but may have NSAID hypersensitivity. This entity has been termed as eosinihilic mucin rhinosinusitis (EMRS) by Ferguson (2000). The disease is usually bilateral (in about 93% cases), and patients of EMRS have a greater incidence of coexistent asthma than seen with AFRS, besides also having a lower serum IgE level as compared to the latter.[14-15]

AFRS is the most common form of fungal rhinosinusitis.[16]

AFRS is usually seen in the 3rd to 5th decade of life. Since fungi thrive in warm and humid climates, AFRS is commonly seen more in tropical climates such as that seen in India. It is seen in atopic individuals with a normal immune system. Younger children present in a similar fashion as adults. Asthma is seen to be associated in 33-50% patients, and many of these have aspirin hypersensitivity. About 60% AFRS patients will give a history of allergic rhinitis, but of those who undergo allergy testing, 70-90% show evidence of atopy.[17]

Fungus balls follow a slow, benign course. Patients may have symptoms for months or years before a diagnosis is confirmed.[22]

For a fungus ball to form, fungal hyphae and spores must get trapped in a paranasal sinus and conditions must support their growth. These conditions develop when some pathology disrupts the normal mucociliary clearance and/or obstructs the sinus ostium as is seen in acute or chronic rhinosinusitis.[23,24]

When this occurs, the fungal spores germinate within the sinus cavity and the growth of hyphae further impairs clearance of the fungi and growth proceeds within the sinus cavity.[24]

Invasive sinus Aspergillus infection has been reported in the last decade with increased frequency, most commonly in the setting of hematologic malignancy, neutropenia, HIV infection and other states of immunosuppression.[26,27]

Invasive and noninvasive syndromes of fungal sinusitis share many features.

They may occur in immunocompetent or immunocompromised persons, may have an acute or chronic course, and may extend beyond the thin walls of the sinuses into the orbit, structures of the eye and the brain.

Clinical Presentation

- Symptoms of AFRS
- History of nasal polyposis (incidence is nearly 100%)
- History of previous surgery (indicating recurrence)
- Patient may have documented atopy
- Proptosis is common in children with AFRS
- hypertelorism due to expansion of bony labyrinth
- 75% patients have history of expelling dark colored rubbery nasal casts
- These symptoms are besides those which may be seen in any sinonasal inflammatory pathology

Patients with fungus balls exhibit neither evidence of immunocompromise nor an increased incidence of atopy to explain the development of this condition.[22]

They occur most commonly in the maxillary or sphenoid sinuses however, they are also reported to occur in the frontal or ethmoid sinuses in literature. They usually affect a solitary sinus but, may occasionally involve two contiguous sinuses.[23]

Symptoms are similar to those seen in chronic rhinosinusitis secondary to inflammation or bacterial infection. These include nasal obstruction, nasal discharge, cacosmia, facial pain with a history of these symptoms being refractory to medication. Symptoms are usually of long duration(months to years). Occasionally the patient

may present with unusual symptoms such as epistaxis, visual disturbances, convulsions, fever, cough, and proptosis.[25]

Sometimes, the patient may be asymptomatic and the fungus ball may be an incidental finding. 10% of patients have associated nasal polyps.[24] which are infact, a nonspecific response to a variety of inflammatory conditions. Approximately 50% of individuals give a history of some endodontic treatment being done prior to maxillary sinus fungus ball indicating it could be a predisposing factor.[24,25]

Fungus balls are rarely known to cause bone remodeling with widening of the affected sinus and distortion of anatomy. They may also cause bone erosion. Rarely, if during the infection, the immunity of the host declines, a fungus ball may become invasive. Characteristic imaging findings and histopathologic examination confirms the diagnosis. At surgery, thick inspissated debris forms a mass which fills the sinus cavity[22].

Several studies have investigated the signs and symptoms of AFIFS to determine the subset of patients who require a more aggressive diagnostic investigation. In the immunocompromised patient population, the presence of fever of unknown origin after 48 hours of appropriate broad- spectrum intravenous antibiotic or the presence of localizing sinonasal symptoms should prompt imaging studies and nasal endoscopy.[32,33,34]

Diagnosis

Bent and Kuhn's diagnostic criteria (1994) for AFRS:

In 1994, they proposed major as well as minor diagnostic criteria; the major criteria include:

- Type I hypersensitivity
- Nasal polyps
- Characteristic CT scan findings
- Positive fungal stain or culture
- Allergic mucin with fungal elements and no tissue invasion.[17,18]

Fungus balls are known to occur in normal immuno-competent individuals and are usually found unexpectedly during the treatment of chronic bacterial rhinosinusitis. They are seen to occur in older individuals (60-70 years), usually triggering nonspecific symptoms of chronic rhinosinusitis such as nasal obstruction, postnasal discharge and facial pain.[19]

The average age reported in an American retrospective series was 64 years, ranging from 28-86 years.[20]

A similar age range was reported in a series by both deShazo (1997) and Klossek (1997), the youngest reported age being 18 years. There is a considerable female preponderance with almost all series reporting a female incidence of approximately 64%.[21]

The physical findings in AFIFS can be subtle, but the most consistent finding is an alteration in the appearance of the nasal mucosa. Mucosal discoloration can be variable, and may be gray, green, white or black. Discoloration, granulation and ulceration typically replace the normal pale-pink mucosa. White discoloration indicates tissue ischemia secondary to angiocentric invasion, whereas black discoloration is a late finding of tissue necrosis.

In a series by Gillespie et al[34] mucosal abnormalities were seen most commonly on the middle turbinate, followed by the septum, palate and inferior turbinate. Anesthetic regions of the face or oral cavity are features of early invasive process and may precede the development of objective changes in the mucosa. Decreased nasal mucosal bleeding or sensation should also be noted because they may be signs of fungal invasion.

Park et al [35] discovered that bedside endoscopic findings did not correlate with intraoperative endoscopy because of a large amount of debris in the nasal cavity that was not removed during bedside examinations and the relative noncompliance of the pediatric patients.

Examination under general anesthesia was recommended for nasal endoscopic examination and directed biopsies of suspicious lesions, the middle and inferior turbinate.

Histopathology And Fungal Culture

Fungus balls are essentially noninvasive and extramucosal fungal infestations without any granulomatous reaction. Routine hemotoxylin and eosin stains can demonstrate the presence of fungus but, special stains such as the gomori methenamine silver (GMS) are helpful in diagnosing the Aspergillus species.

Intraoperatively, the gross appearance of the fungus is gritty or cheesy and clay-like, breaking up into fragments, the color of which ranges from brown to black to green or yellow.

Stainable hyphae are not present in the mucosa of patients with chronic bacterial sinusitis; they are present solely in mucopurulent material within the sinus

in noninvasive disease. Hyphae penetrate the sinus mucosa into submucosa blood vessels or bone in invasive disease.[2]

In patients with suspicion of AFIFS culture and microscopic examination of the specimen can be done. The potassium hydroxide—calcofluor white method can be used immediately on culture aspirate material to reach to an early diagnosis so that therapy can be started at the earliest. This highly sensitive technique uses potassium hydroxide to dissolve human material, and an optic brightener (calcofluor white) that binds to the cell wall of the hyphae. Fungal cell walls, including septations, fluoresce when viewed using a fluorescence microscope.[37]

Microbiology:

Members of the dematiaceous family are most commonly implicated in AFRS, i.e. *Bipolaris spicifera, Drechslera,Alternaria, Curvularia, Excerohilum, Rhizopus, Fusarium* and *Chrysosporium*.

The causative fungi in fungal ball/mycetoma include *Aspergillus fumigatus, Aspergillus flavus, Alternaria* Sp and *Pseudallescheria Boydii*. Only 23-50% cultures result in fungal growth.[21]

Most commonly Zygomycetes are responsible for Invasive fungal sinusitis, which have tendency for angioinvasion, rapid onset & tissue destruction.

Identification of fungal culture:

Aspergillus flavus[72]*:A.flavus* grew very fast. On Sabouraud's dextrose agar, after 5 days at 25°C, the colony was yellow to green, powdery. The reverse was pale to yellowish.

Microscopic morphology:

Lactophenol cotton blue mount showed septate hyphae with rough, colorless conidiophores. Conidiophores end in vesicle, which was globose and the entire surface was covered (radiating) with one series or two series of sterigmata (uni- or biseriate). Conidia measuring 3 – 6 µm, produced from these sterigmata are round, rough walled and in chains. .

Differentiation from other Aspergillus species:

A.flavus was differentiated from other Aspergilli by yellow – green colonies, rough walled conidiophores, radiating conidial heads with uniseriate or biseriate sterigmata.

Aspergillus niger*[72]*:

Macroscopy:

A. niger a fast growing fungus. On Sabouraud's dextrose agar, after 5 days at 25°C, the initial growth was white, becoming black later on giving "salt and pepper appearance" and reverse turning pale yellow. Good growth was seen at 37°C.

Microscopic morphology:

Lactophenol cotton blue mount showed septate hyphae with smooth-walled, simple conidiophores measuring up to 1 mm in length. Conidiophores end in vesicle, which was globose and entirely covered (radiating) with two series of sterigmata (biseriate). Conidia produced from these sterigmata were brown to black, round, rough walled, and in chains measuring 4 – 5 µm in diameter.

Differentiation from other molds:

A. niger was differentiated from other Aspergillus species by its rapid growth, black colonies, microscopically biseriate, radiating heads with black, round, rough conidia.

Cladosporium carionii*[72,73]*:

Macroscopy:

Colonies are slow growing, reaching 3-4 cm in diameter after one month, with a compact suede-like to downy surface and are olivaceous-black in color.

Microscopy:

Microscopy shows ascending to erect, olivaceous-green, apically branched, elongate conidiophores producing branched acropetal chains of smooth-walled conidia. Conidia are pale olivaceous, smooth-walled or slightly verrucose, limoniform to fusiform, 1.5-3.0 x 2.0-7.0 µm in size. Bulbous phialides with large collarettes and minute, hyaline conidia are occasionally formed on nutritionally poor media. Maximum growth temperature 35-37°C.

Conidia are smaller and comprise heavily branched systems which fall apart much more easily than in the other *Cladophialophora* species.

Microscopic Examination of Culture[55,57]:

a) **Tease Mount**: For preparing a mount, a portion of fungal growth was removed with a sterile spud and was teased on a clean glass slide in a drop of LPCB stain using 2 sterile teasing needles. A cover slip was placed and examined under the microscope.

Slide culture: A microscopic slide was placed on a bent glass rod at the bottom of a sterile petri dish along with 1-2 cover slips and a filter paper. Petri dishes were closed with their lid, wrapped with craft paper and sterilized using hot air oven at 160°C for

1 hour. Block of 1x1cm of Sabourauds Dextrose agar with a depth of 4mm was cut using sterile scalpel blade on a template. The block was transferred to the surface of the sterile glass slide. The fungal strain to be identified was inoculated at four sides of the agar block with the sterile spud. The inoculated block was covered with sterile cover slip and incubated at 25^0C. A little sterile distilled water was added on the filter paper to avoid drying of agar. When growth appears, a drop of LPCB was placed on a slide and cover slip from block was placed over it. Likewise drop of stain was placed on glass slide of the slide culture after removing agar block; fresh cover slip was applied over it and examined under the microscope.

Several factors underlie the dichotomous geographic distribution of Aspergillus species. Although both species are considered ubiquitous saprophytic organisms.[46,47]

A. fumigatus appears to be particularly tolerant to variations in temperature48 and has been detected in greater concentration in cooler air samples of Europe and North America.[48-50]

A. flavus is the most commonly isolated species from the environmental samples in areas where granulomatous fungal sinusitis predominates[51] probably due to the tropical climate which also promotes a microaerophilic sinus environment conducive to the growth of *A. flavus*.[26]

According to study by *Michael R. et al* out of 211 culture positive fungal sinusitis, 63% had allergic & 34% had invasive fungal sinusitis, *Aspergillus flavus & Rhizopus arrhizus* were the most common causative agents, respectively.[1]

Another study by *Ragini T. et al* out of 47 patients clinically diagnosed with sinusitis, fungal cultures were positive in 10(21.3%), *Aspergillus, Fusarium, Rhizopus, Candida albicans & Bipolaris* were isolated in these cases.[3]

Another study by *Andrew M. et al* seven fungi were discovered at very high concentrations in Chronic rhinosinusitis patients including *Alternaria alternate, Cladosporium cladosporioides* types 1 & 2, *Cladosporium herbarum, penicillium brevicompactum, penicillin crustosum and Penicillium chrysogenum* type 2.[52]

Histopathology:

Histopathologic evaluation of the suspected tissue, however, is typically the most critical to making the diagnosis. Permanent section with the Gomori methenamine-silver stain uses deposition of silver onto the fungal cell wall and, because it can detect even a single cell, it undoubtedly is the most sensitive of the commonly used histologic stains. No histologic specimen should be considered to be negative for fungus unless a silver stain has been performed.[29]

Fungal disease is determined to be invasive if it meets the following criteria: (1) hyphal forms within the submucosa, with or without angiocentric invasion and (2) tissue necrosis with minimal host inflammatory cell infiltration. [38]

Frozen section allows for a timely diagnosis, and, if positive, appropriate antifungal therapy and extended surgical resection can be initiated without delay.[38]

Mucormycosis fungal elements are broad, ribbon-like, irregular, and rarely septated, whereas the Aspergillus species demonstrate more narrow hyphae with regular septations and 45° branching.[29,23]

Aspergillus species can be angioinvasive, but it is not the obliterative invasion seen with mucormycosis.

Radiological features:

CT of paranasal sinuses is usually obtained during the workup of immunocompromised patients who have fever or sinonasal symptoms, usually before evaluation by an otolaryngologist. Severe unilateral thickening of the nasal cavity mucosa has been shown to be the most consistent finding on CT, suggestive of underlying invasive fungal sinusitis.[36]

The fungal material is commonly associated with dense polyposis and calcification that results in areas of focal or diffuse radiodensity on computed tomographic (CT) imaging of the sinuses and decreased signal intensities on T1 and T2-weighted magnetic resonance imaging (MRI). Invasive fungal sinusitis can be distinguished from noninvasive disease with the use of clinical criteria that include radiologic diagnosis of sinusitis and histopathological examination of tissue from sinuses.

Radiologic findings associated with fungal sinusitis include those also seen with isolated bacterial sinusitis, such as air fluid levels or more than 8 mm of mucoperiosteal thickening, and those more specific for fungal sinusitis, such as calcifications and loss of bony sinus margins. [27]

The earliest evidence of AFIFS on CT scan could be the infiltration of the periantral fat planes.[37]

CT scans are helpful in defining individual variations in sinus architecture and possible periorbital and intracranial spread which helps to support the diagnosis of AFIFS.

MRI is superior to CT in delineating the intracranial extent of the disease and it may have a role in evaluating patients who demonstrate signs of intracranial invasion: Mental status changes, orbital apex syndrome, seizure or stroke.

Early aggressive endoscopic sinonasal debridement should be performed on all patients who have biopsy-proven disease or on any patient suspected of having fungal invasion. Radical resections (radical maxillectomy, craniofacial resection and orbital exenteration) to remove disease outside the sinonasal cavity rarely achieve negative margins or improve long-term survival.[33,39]

Endoscopic sinus debridement slows the progression of the disease, reduces the fungal load and provides a specimen for culture and histopathologic diagnosis.[33]

Debridement of the involved sinuses or structures is extended until clear bleeding margins are exposed.

In a large retrospective review by Parikh,[41] the overall mortality rate directly as a result of invasive fungal sinusitis was found to be 18%. When examining each specific disease subgroup, the mortality rate from invasive fungal sinusitis among diabetic patients (40%) was significantly higher than in patients who had hematologic malignancy (11%), chronic steroid users (33%), and solid organ transplant patients (0%).

This disparity can be due to a greater incidence of Mucor over Aspergillus affecting diabetics and a delay in diagnosis resulting in more advanced disease at presentation in this subgroup of patients.[40]

MATERIAL AND METHODS

The present study of fungal sinusitis was carried out in the Department of Microbiology, KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre Belgaum, over a Period of one year from January 2013 to December 2013. All clinically diagnosed cases of chronic rhinosinusitis in all age groups and of both sexes,attending Otorhinolaryngology OPD & undergoing Functional Endoscopic Sinus Surgery were taken for this study.

Written informed consent was taken from all the patients prior to surgery.

Study design:

Cross-sectional study

Inclusion Criteria:

Patients of chronic rhinosinusitis undergoing Functional Endoscopic Sinus Surgery at KLE`s Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum.

E*x*clusion Criteria:

Patients who are not clinically diagnosed as chronic rhinosinusitis,viz patients of

- Cerebrospinal fluid (CSF) leak closure
- Orbital decompression (eg, Graves ophthalmopathy)
- Optic nerve decompression
- Dacryocystorhinostomy (DCR)

- Choanal atresia repair
- Foreign body removal
- Epistaxis control, but undergoing FESS surgery.

Statistical Analysis:

Will be done using percentage.

Sample calculation: $4pq/ d^2$

p= prevalence=21.3% (percentage of culture positive cases for fungal sinusitis)[3]

q= 78.7% (100-p)

d= 10% absolute error.

$n=4 * 21.3 * 78.7 / 10^2$

$n^{=67.05} \sim 68$

Specimen Collection:

The samples collected from OT for diagnosis of chronic rhinosinusitis during FESS procedure are:

1. Allergic mucin,
2. Exudate from the nasal mucosa,
3. Tissue biopsy,
4. Nasal polyps and debris.

Tissue biopsy is collected in sterile containers of capacity 50 ml containing 5-10 ml of normal saline.

Allergic mucin, exudates and debris are collected in empty sterile container of capacity 50 ml.

Tissue/sample for histopathological examination is collected in 10% formalin.

Direct Examination:

Potassium Hydroxide (KOH) Preparation:

Tissue samples are ground with sterile mortar & pestle and teased with sterile teasing needle. Tissue biopsy & nasal polyp was placed in a 5ml sterile test tube containing 0.5 ml of 20% KOH on, and the tube was gently heated by passing over the flame 3-4 times. After overnight incubation at 37^0C, a drop of specimen was placed on clean glass slide, a cover slip was placed over it & examined, first under the low power of microscope and then under the high power to look for the presence of hyphae or arthrospores.

The samples of allergic mucin, exudates & debris were placed in few drops of 10% KOH on a clean glass slide, gently heated by passing over the flame 3-4 times till the slide becomes just warm, checked by touching with back of palm. After 15-20 minute a cover slip was placed over it & examined under microscope.

The hyphal forms were differentiated from epidermal cell outlines, cotton and vegetable fibers.

Culture:

The specimens collected were inoculated on to Sabourauds Dextrose agar & Sabourauds Dextrose agar containing Chloramphenicol (50mg/l) and Cycloheximide (500mg/l); irrespective of demonstration of fungal elements on KOH mount. Each sample was inoculated into a pair of tubes. One tube with antibiotic and other without antibiotic and were incubated at 27^0C (room temperature). The cultures were examined daily during first week and weekly thereafter, for a period of 4 weeks. Slopes showing no growth after 4 weeks were discarded. If growth was obtained on Sabourauds Dextrose agar, identification was made based on colony morphology, microscopic appearance and other relevant tests.

Macroscopic Examination of Culture:

The growth on Sabourauds dextrose agar was observed to study the colony morphology, the pigmentation of the colony, the reverse of the colony, the texture of the surface, the topography and the appearance of growth.

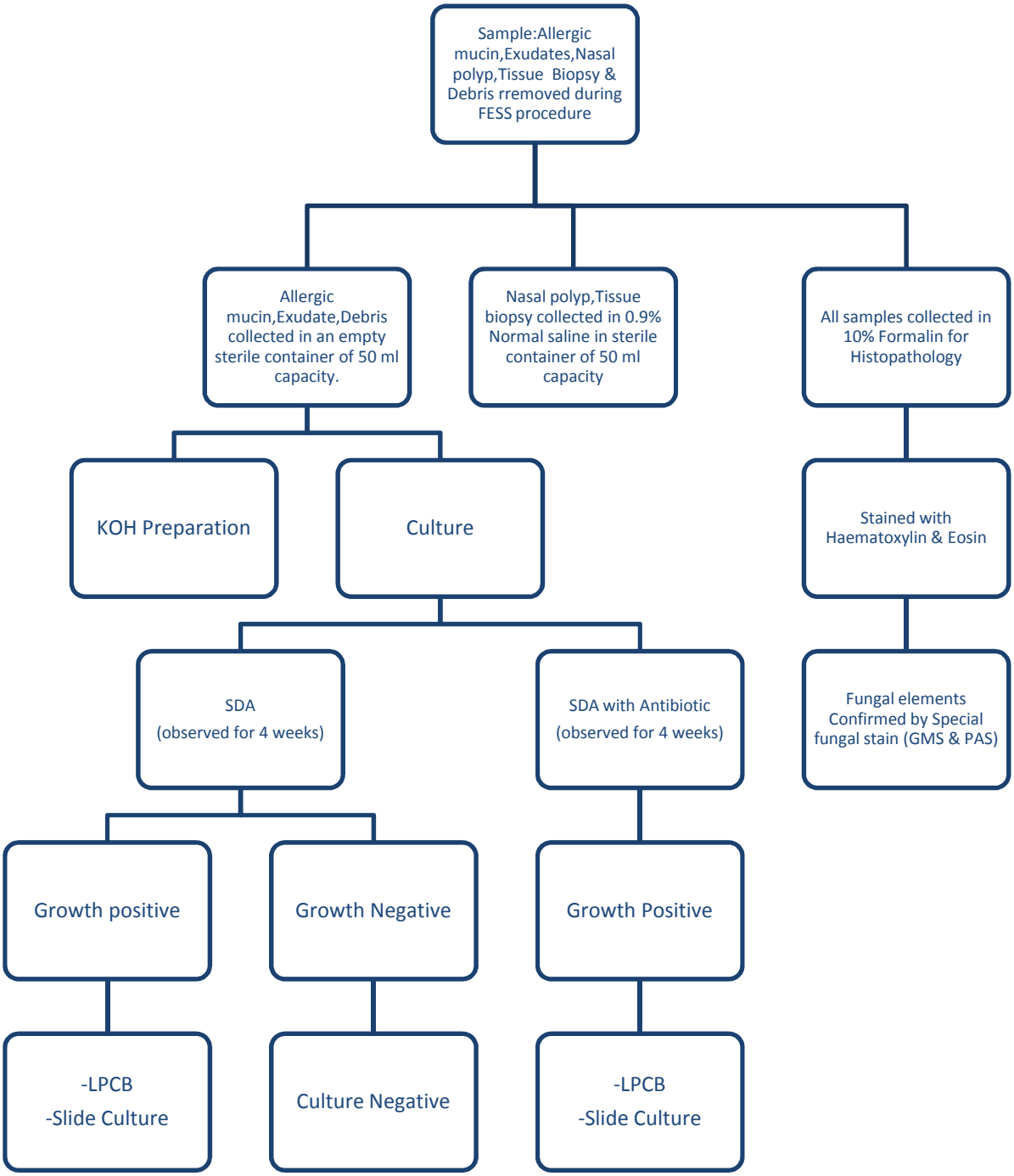
Microscopic Examination of Culture:

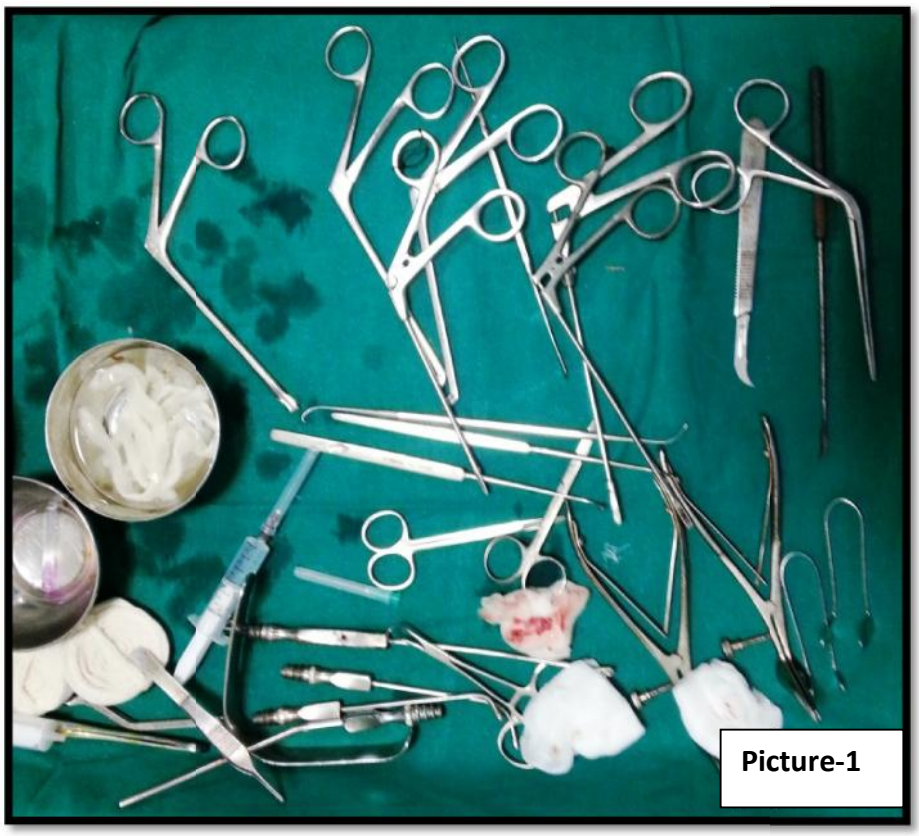
a) **Tease Mount**: For preparing a mount, a portion of fungal growth was removed with a sterile spud and was teased on a clean glass slide in a drop of LPCB stain using 2 sterile teasing needles. A cover slip was placed and examined under the microscope.

Slide culture: A microscopic slide was placed on a bent glass rod at the bottom of a sterile petri dish along with 1-2 cover slips and a filter paper. Petri dishes were closed with their lid, wrapped with craft paper and sterilized using hot air oven at 160°C for

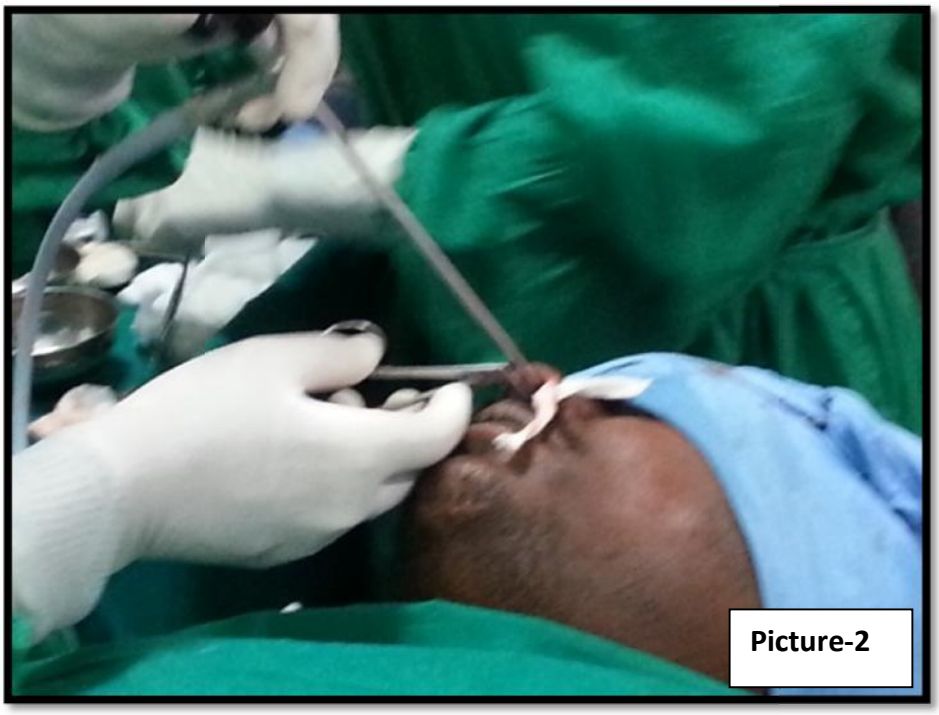
1 hour. Block of 1x1cm of Sabourauds Dextrose agar with a depth of 4mm was cut using sterile scalpel blade on a template. The block was transferred to the surface of the sterile glass slide. The fungal strain to be identified was inoculated at four sides of the agar block with the sterile spud. The inoculated block was covered with sterile cover slip and incubated at 25^0C. A little sterile distilled water was added on the filter paper to avoid drying of agar. When growth appears, a drop of LPCB was placed on a slide and cover slip from block was placed over it. Likewise drop of stain was placed on glass slide of the slide culture after removing agar block; fresh cover slip was applied over it and examined under the microscope.

Tissue received for histopathology examination was fixed in 10% formalin, processed & paraffin sections of 3-5μ thickness were cut and stained with Haematoxylin & eosin stain. The sections were studied under light microscope for identification of fungal elements and classification of fungal sinusitis. The presence of fungal elements were confirmed with special fungal stains (Gomori Methanamine silver & Periodic Acid Schiff).

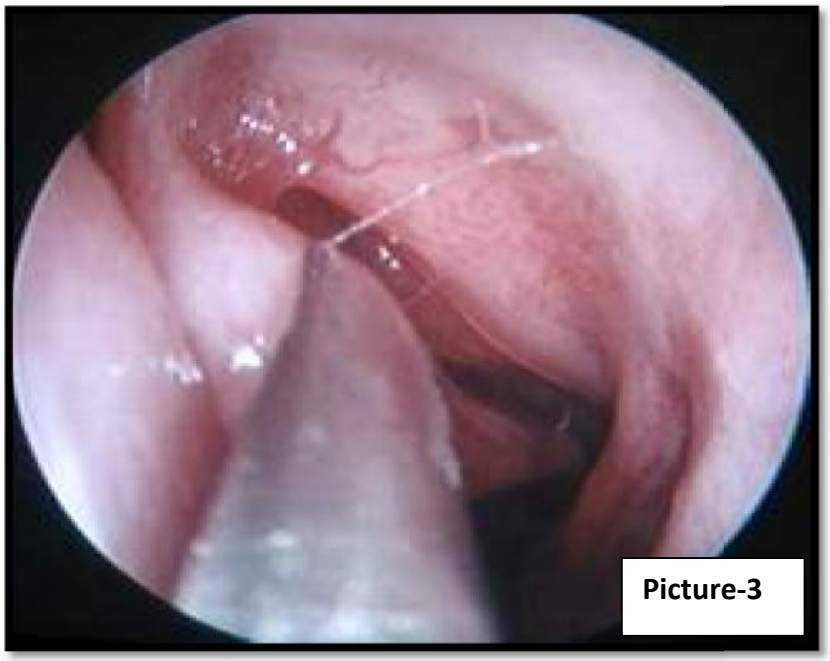

Sample:Allergic mucin,Exudates,Nasal polyp,Tissue Biopsy & Debris rremoved during FESS procedure
Allergic mucin,Exudate,Debris collected in an empty sterile container of 50 ml capacity.
Nasal polyp,Tissue biopsy collected in 0.9% Normal saline in sterile container of 50 ml capacity
All samples collected in 10% Formalin for Histopathology
KOH Preparation
Culture
Stained with Haematoxylin & Eosin
SDA (observed for 4 weeks)
SDA with Antibiotic (observed for 4 weeks)
Fungal elements Confirmed by Special fungal stain (GMS & PAS)
Growth positive
Growth Negative
Growth Positive
-LPCB -Slide Culture
Culture Negative
-LPCB -Slide Culture



Picture-1: Armamentarium for FESS

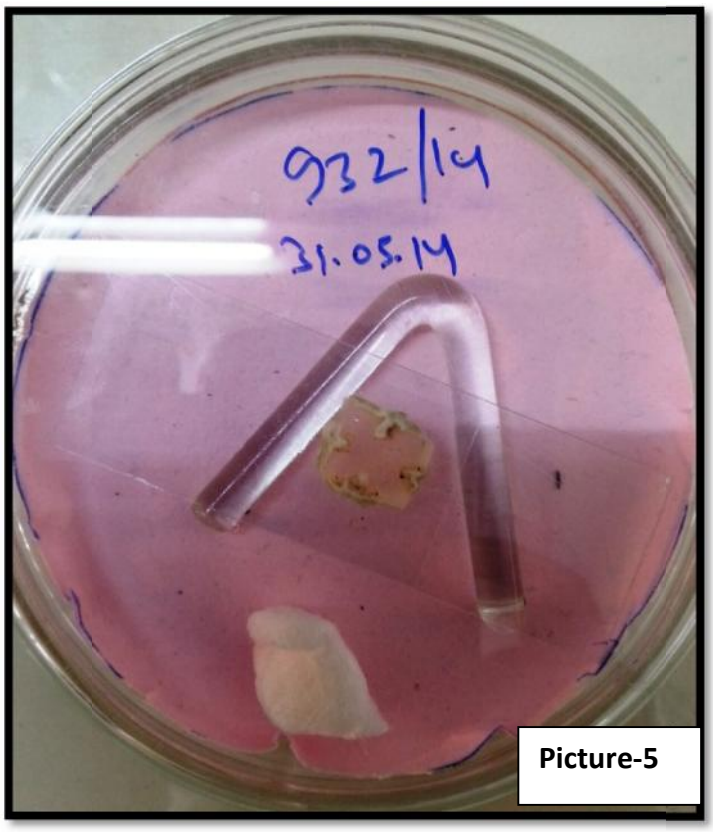


Picture-2: Patient undergoing FESS procedure



Picture-3: Endoscopic picture of Sinus Ostium

Picture-4: Sterile 50 ml container used for sample collection

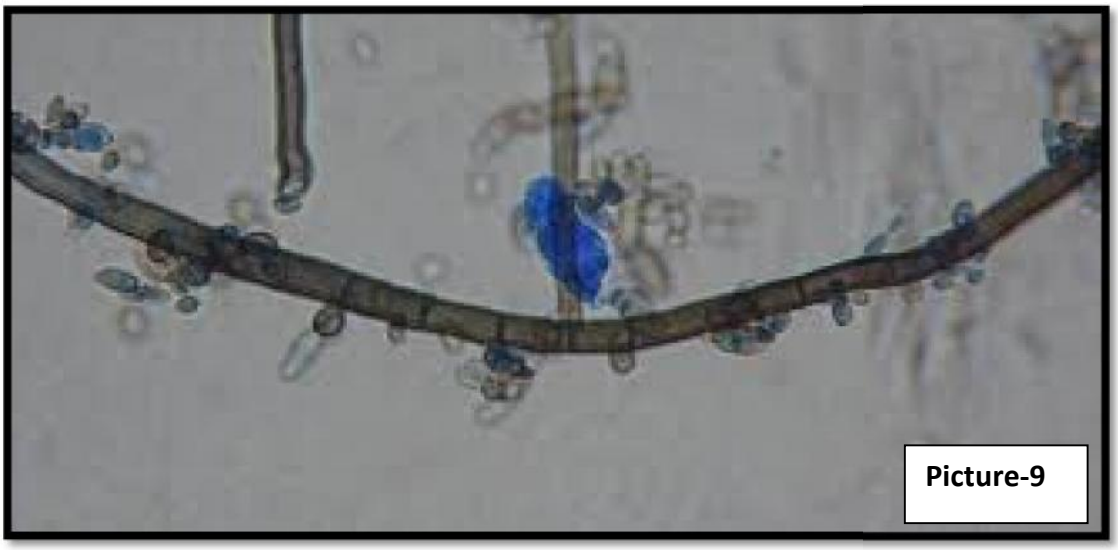


Picture-5: Slide culture set with SDA block

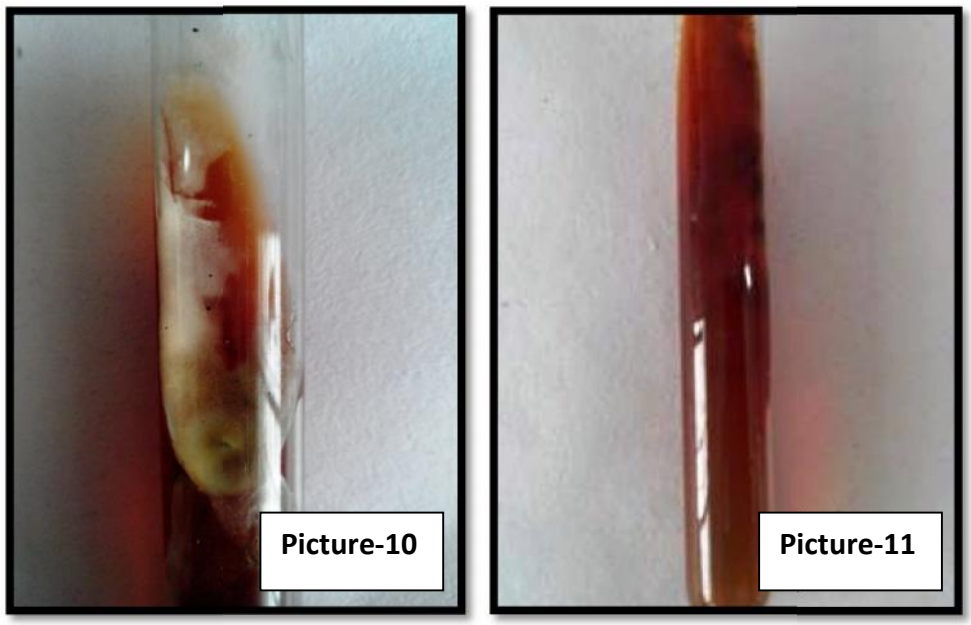
Picture-6: Microscopic picture of KOH positive sample

Picture-7:SDA slope Obverse side

Picture-8:SDA slope Reverse side

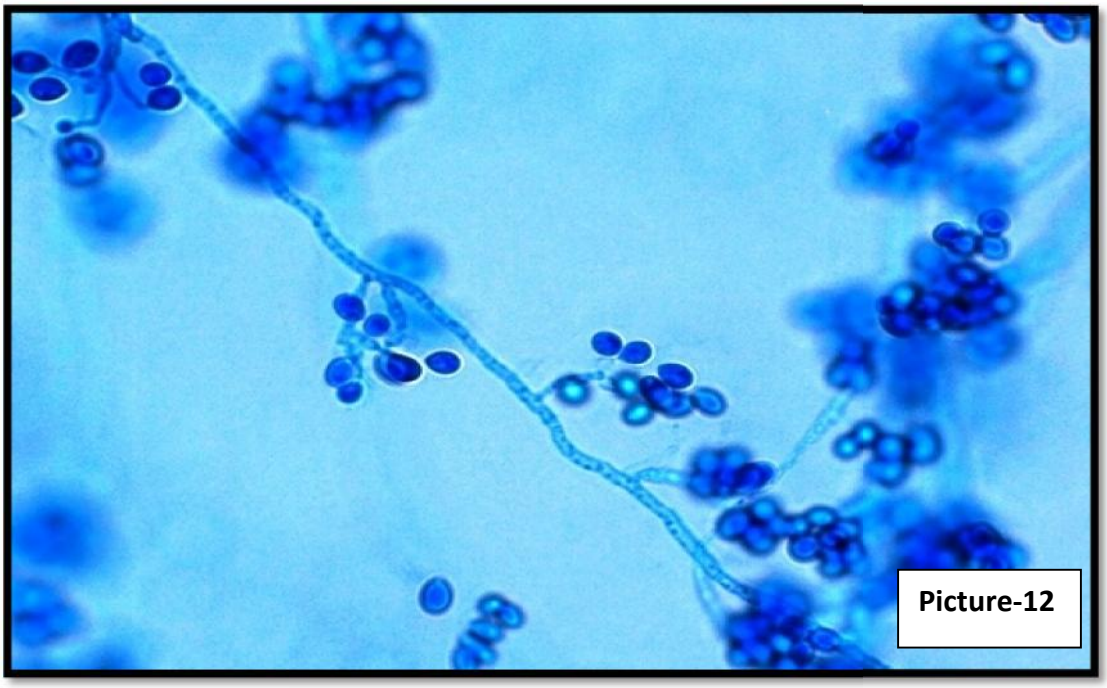


Picture 9: Photomicrograph showing LPCB picture of *Hormonema dematioides*



Picture-10: SDA slope Obverse side

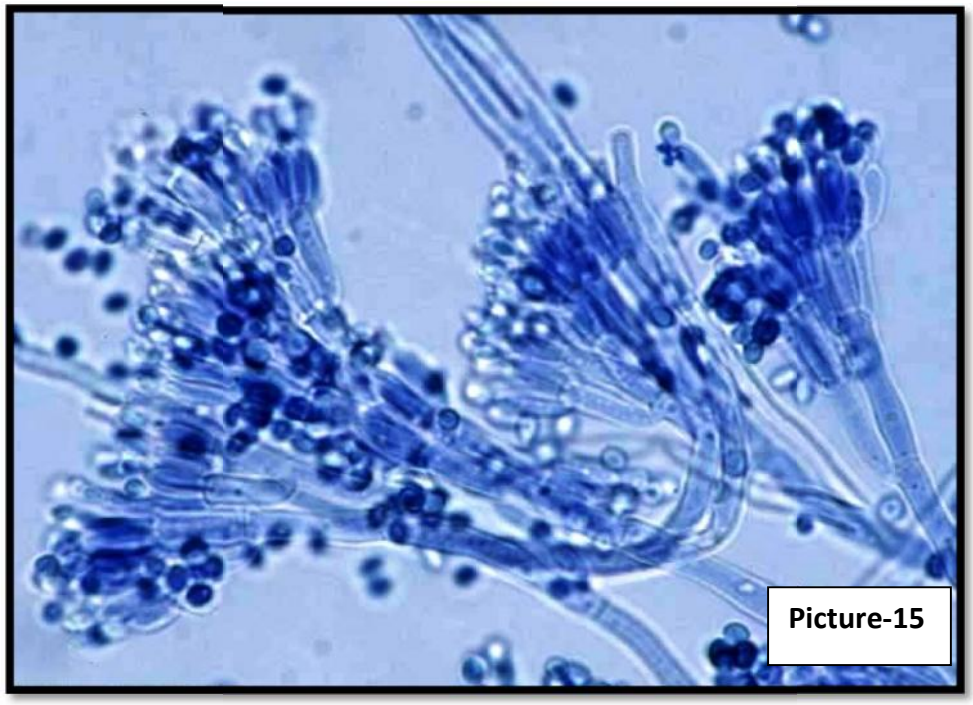
Picture-11:SDA slope Reverse side



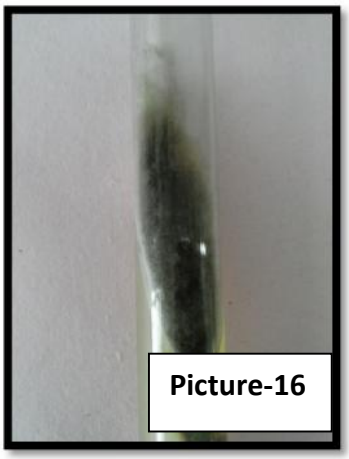
Picture-12: Photomicrograph showing LPCB picture of *Scedosporium prolificans*

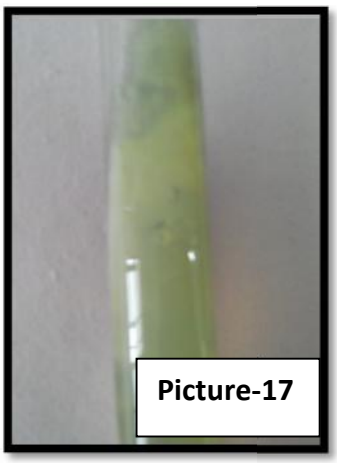
Picture-13: SDA slope Obverse side

Picture-14:SDA slope Reverse side



Picture-15: Photomicrograph showing LPCB picture of *Penicillium expansum*

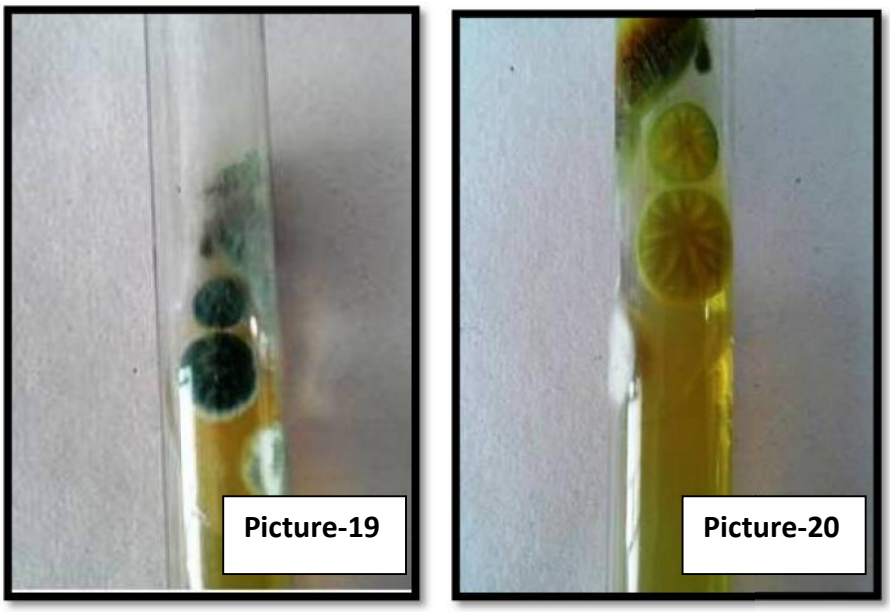




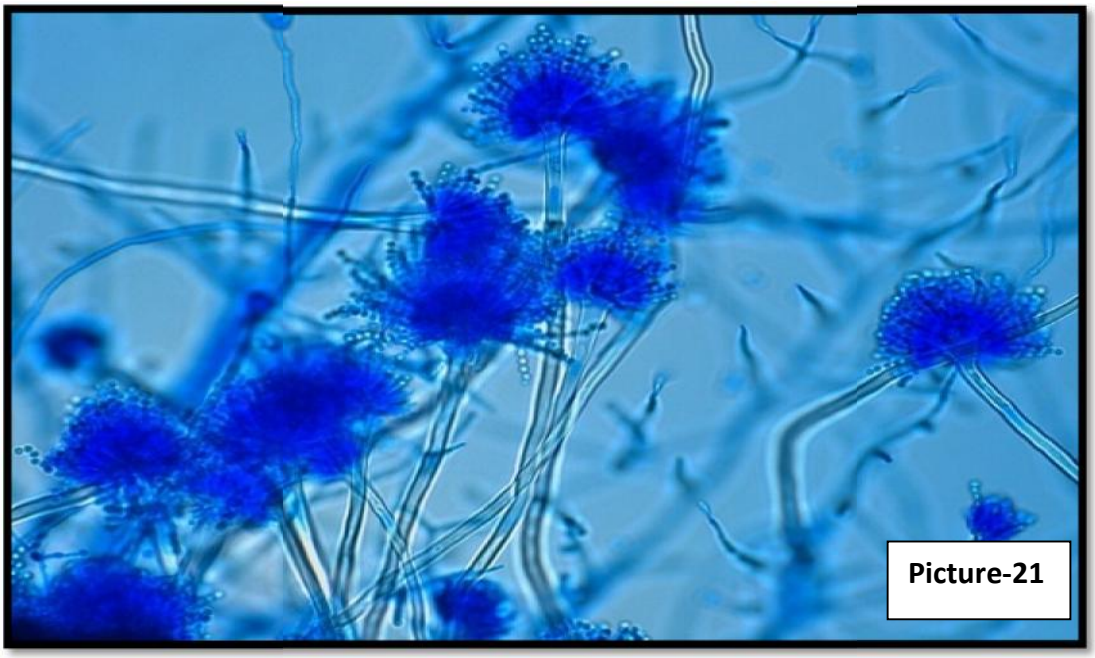
Picture-16: SDA slope Obverse side

Picture-17:SDA slope Reverse side

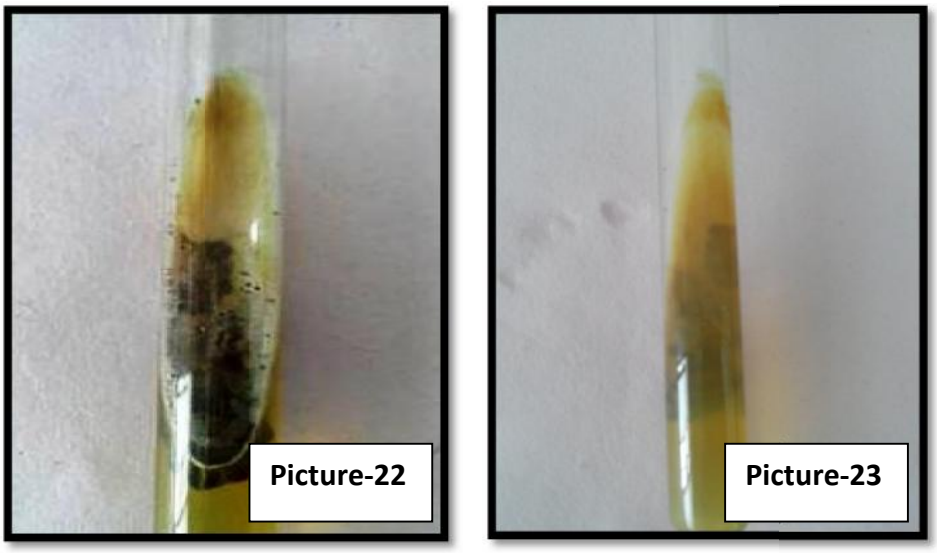
Picture-18: Photomicrograph showing LPCB picture of *Aspergillus niger*



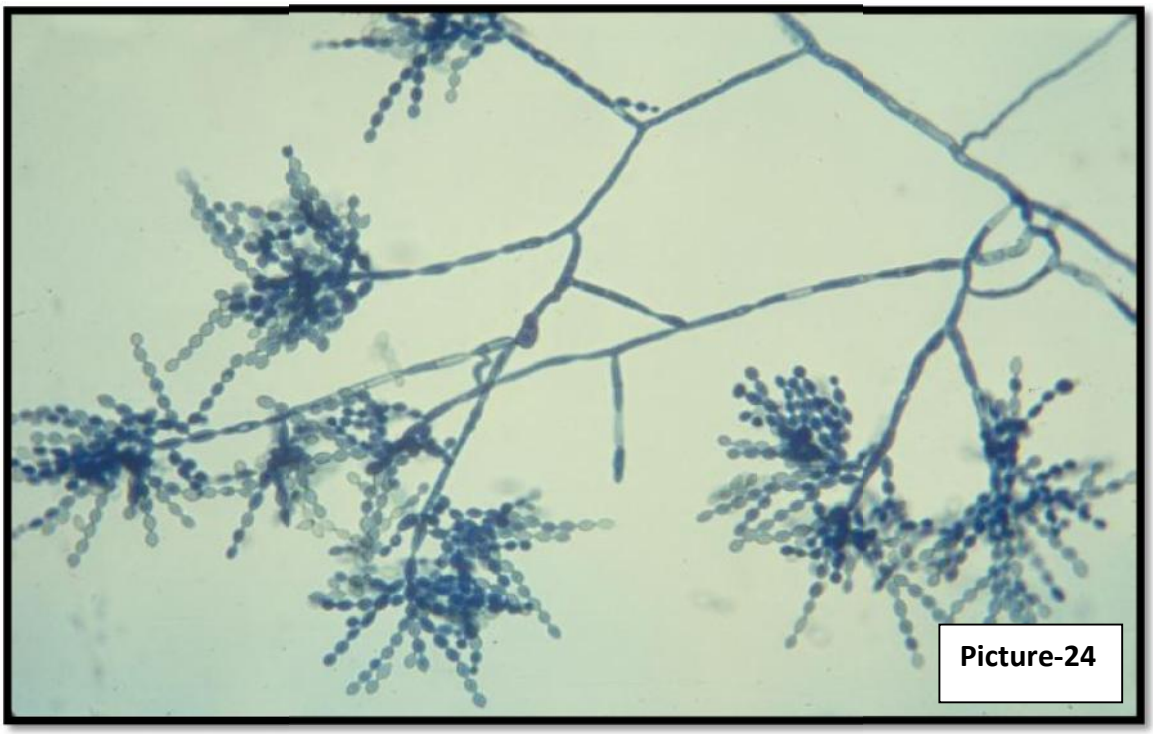
Picture-19: SDA slope Obverse side **Picture-20**:SDA slope Reverse side



Picture-21: Photomicrograph showing LPCB picture of *Aspergillus flavus*

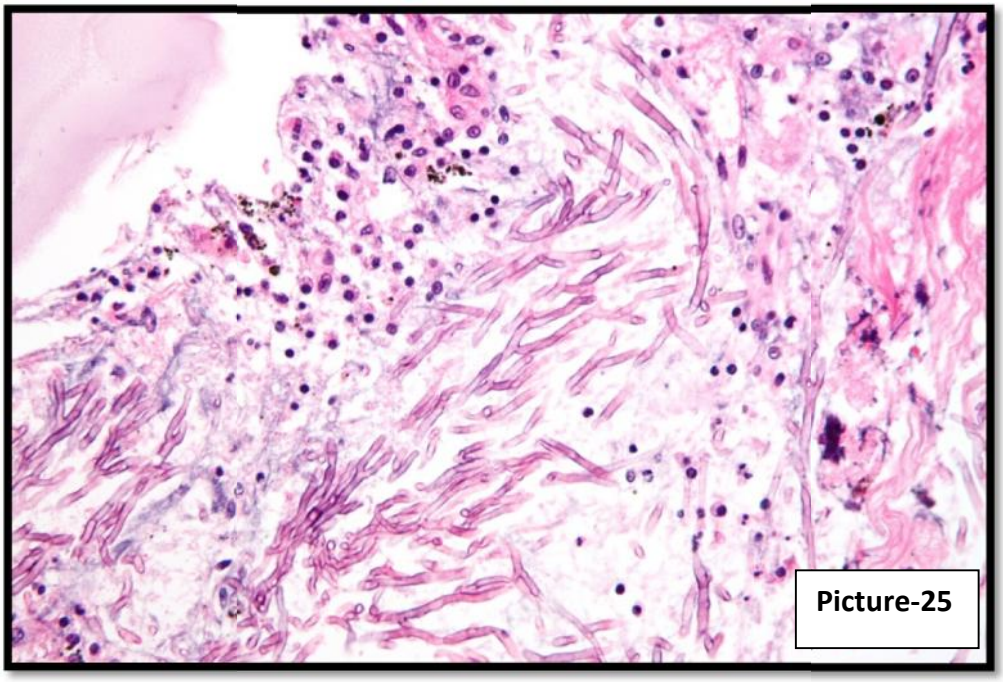


Picture-22: SDA slope Obverse side **Picture-23**:SDA slope Reverse side

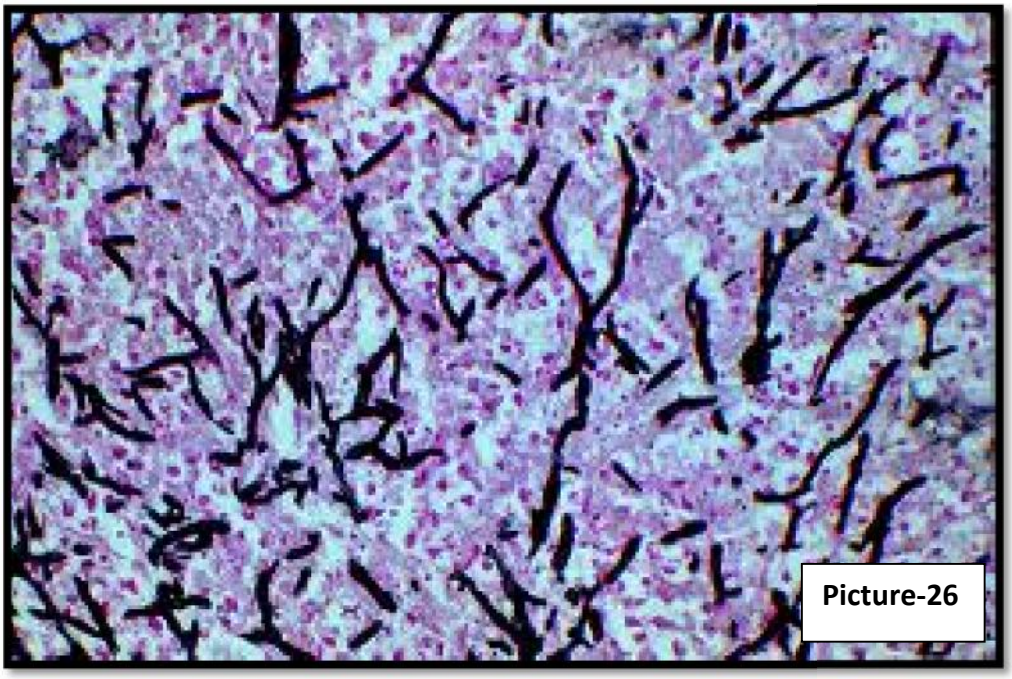


Picture-24: Photomicrograph showing LPCB picture of *Cladosporium carionii*

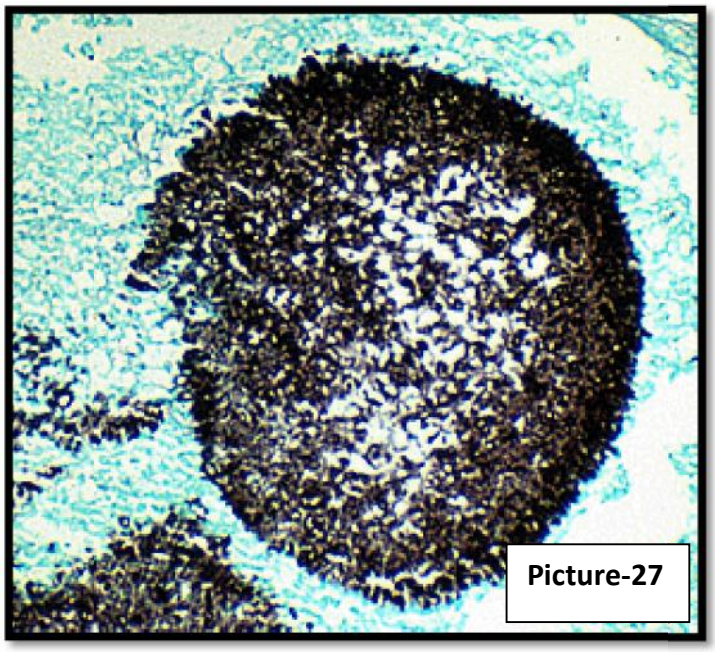
Histopathology pictures



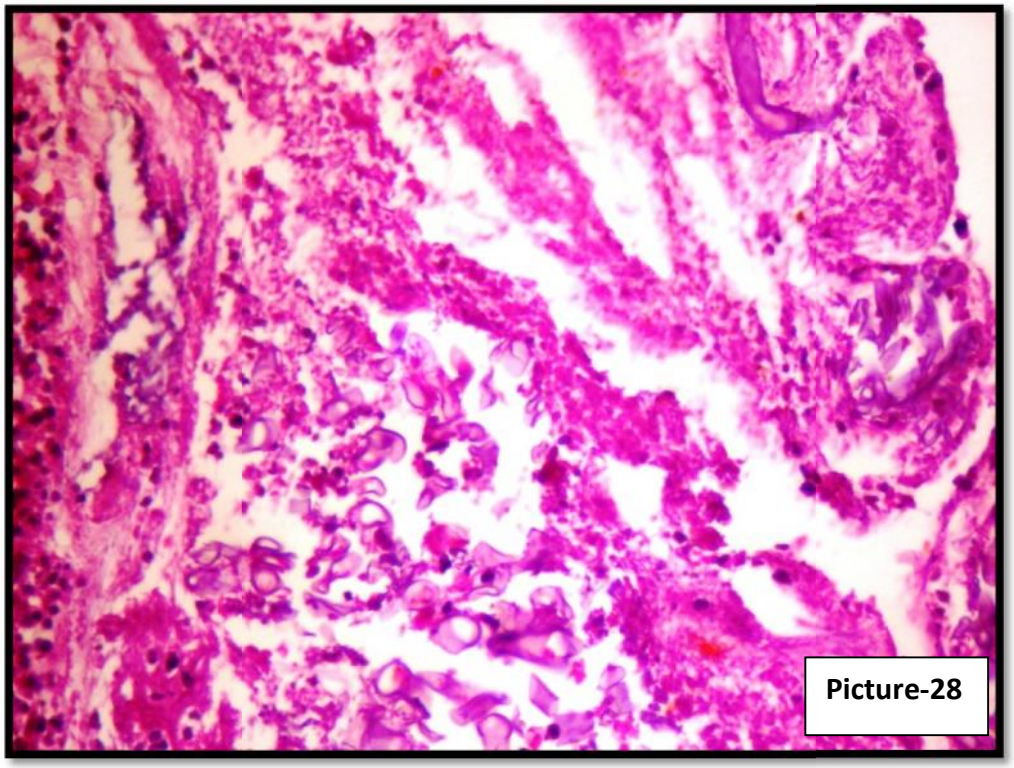
Picture 25: Photomicrograph showing fungal hyphae with surrounding reaction(H&E stain;400x)



Picture 26: Photomicrograph showing *Aspergillus* hyphae(GMS stain;400x)



Picture 27: Photomicrograph showing Fungal Ball(GMS stain;400x)



Picture 28: Photomicrograph showing *Mucor* hyphae (H & E stain; 400x)

RESULT

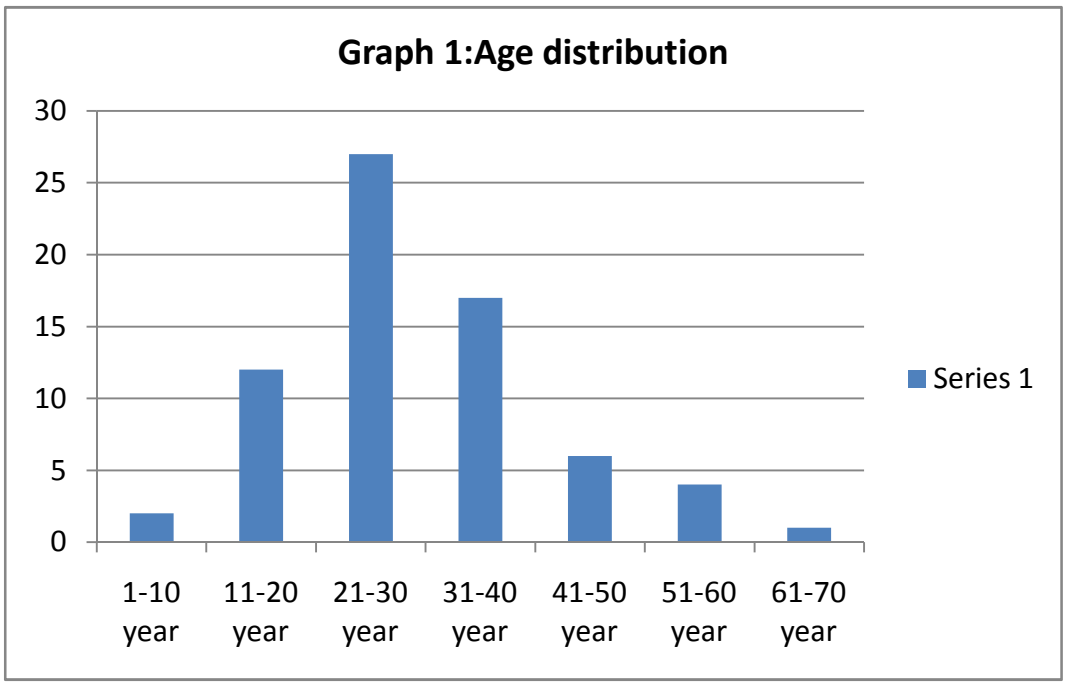
The present study of fungal sinusitis was carried out in the Department of Microbiology, KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre Belgaum, over a Period of one year from January 2013 to December 2013. All clinically diagnosed cases of chronic rhinosinusitis in all age groups and of both sexes, attending Otorhinolaryngology OPD & undergoing Functional Endoscopic Sinus Surgery were taken for this study Written informed consent was taken from all the patients prior to surgery.

Data was included in a predesigned proforma. It included patient's identification number, name, age, sex, patient's history, Clinical presentation, Clinical assessment, Microbiological diagnosis and Histopathological correlation.

Aspergillus flavus(13) was the most common isolate, while *Hormonema.dematioides, Penicillium expansum* & *Scedosporium prolificans* (1 each) were the other isolates.

Table 1: Age distribution of cases is given below:

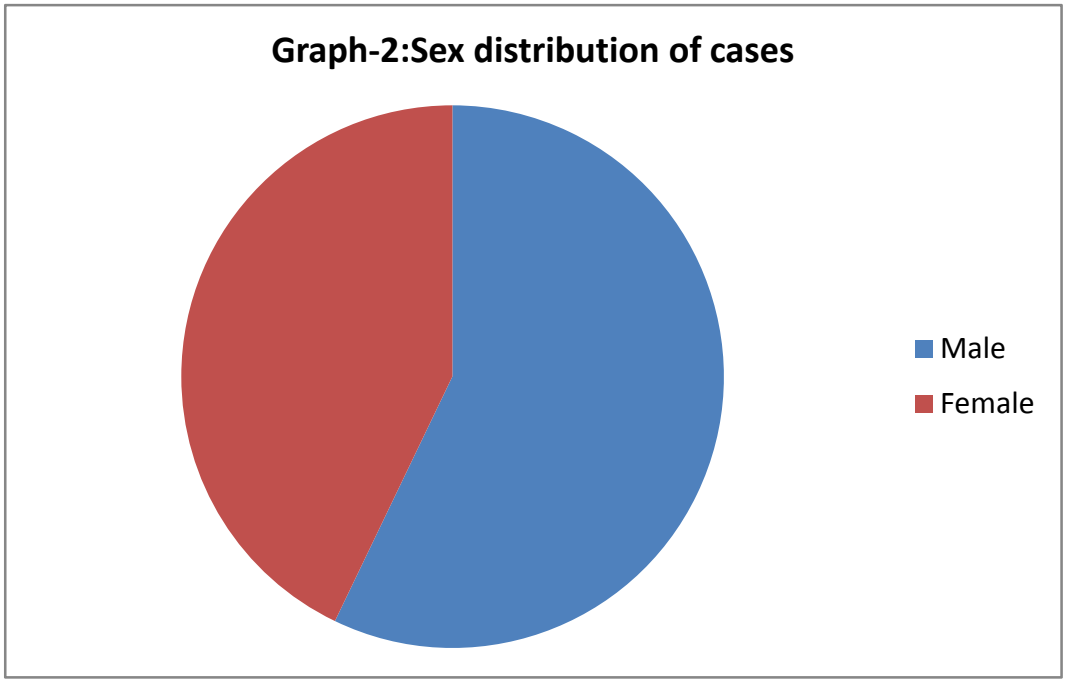
Age range	Cases
1-10 year	2
11-20 year	12
21-30 year	28
31-40 year	17
41-50 year	6
51-60 year	4
61-70 year	1
Total	70



Commonest age group found to be affected is 21-30 years (40%). Least age group affected is 61-70 year(1.42%).

Table-2: Sex Distribution

	Male	Female	Total	M:F ratio
No. of Cases	40	30	70	1.3:1
Percentage	57.14	42.86	100	

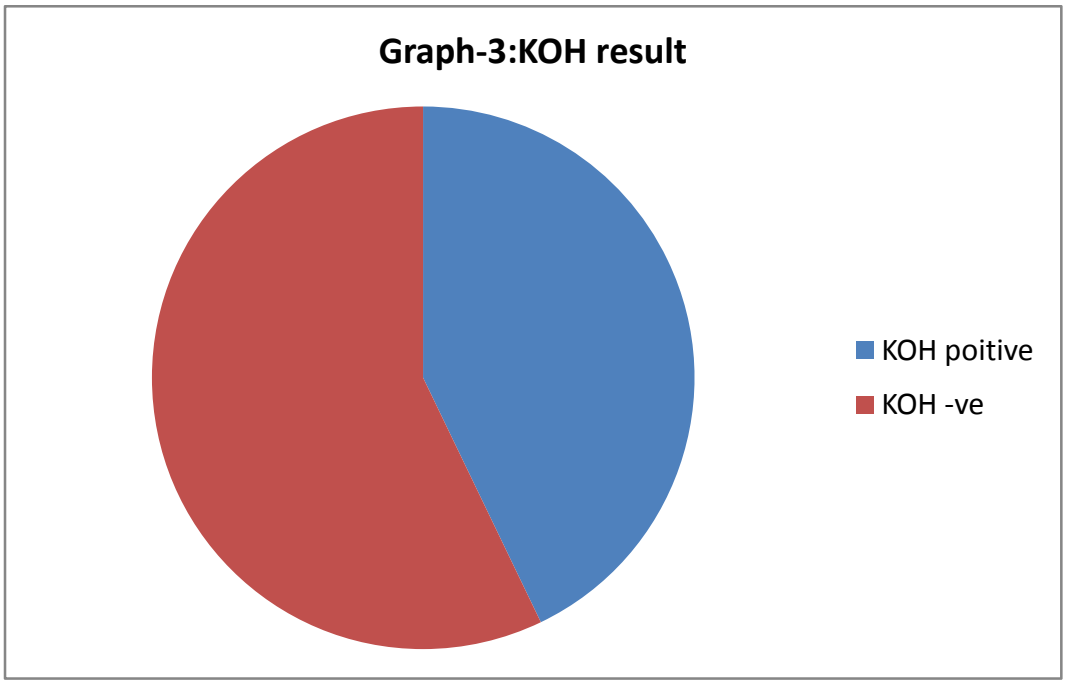


There were 40 male and 30 female patients. Total male:female ratio is 1.3:1

Details of samples processed

Table-3: Direct Examination (KOH)

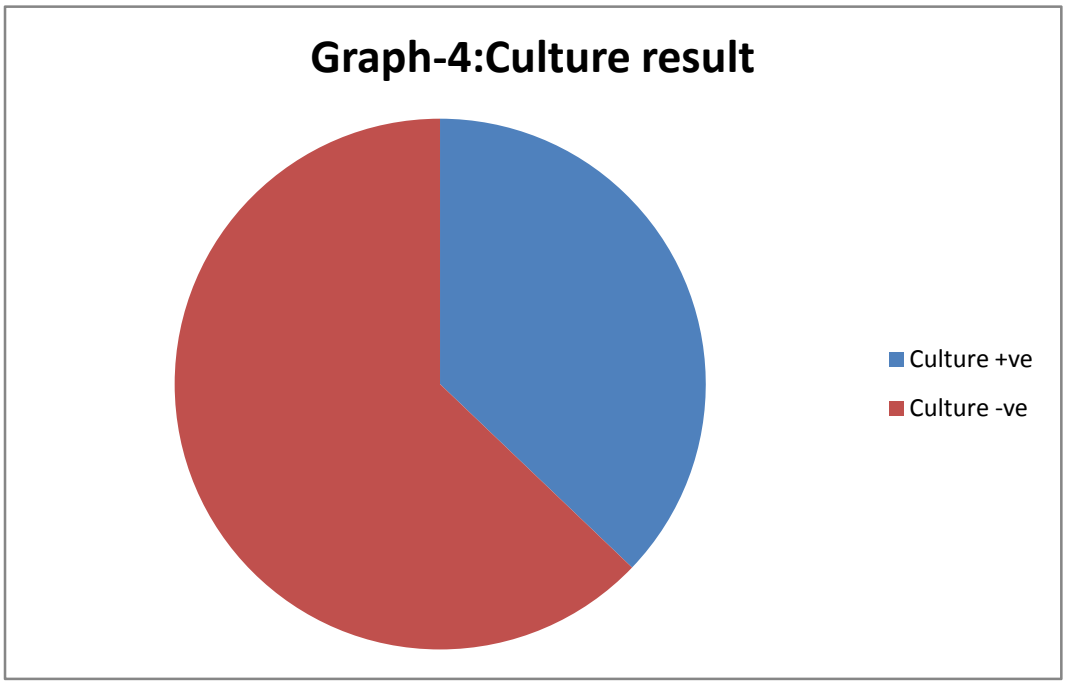
KOH status	No. of samples
KOH positive	30(42.85%)
KOH negative	40(57.14%)
Total	70



Thus, KOH preparation result in present study is 42.85%, while 57.14% of samples were KOH negative.

Table-4 Culture result of Clinical Samples

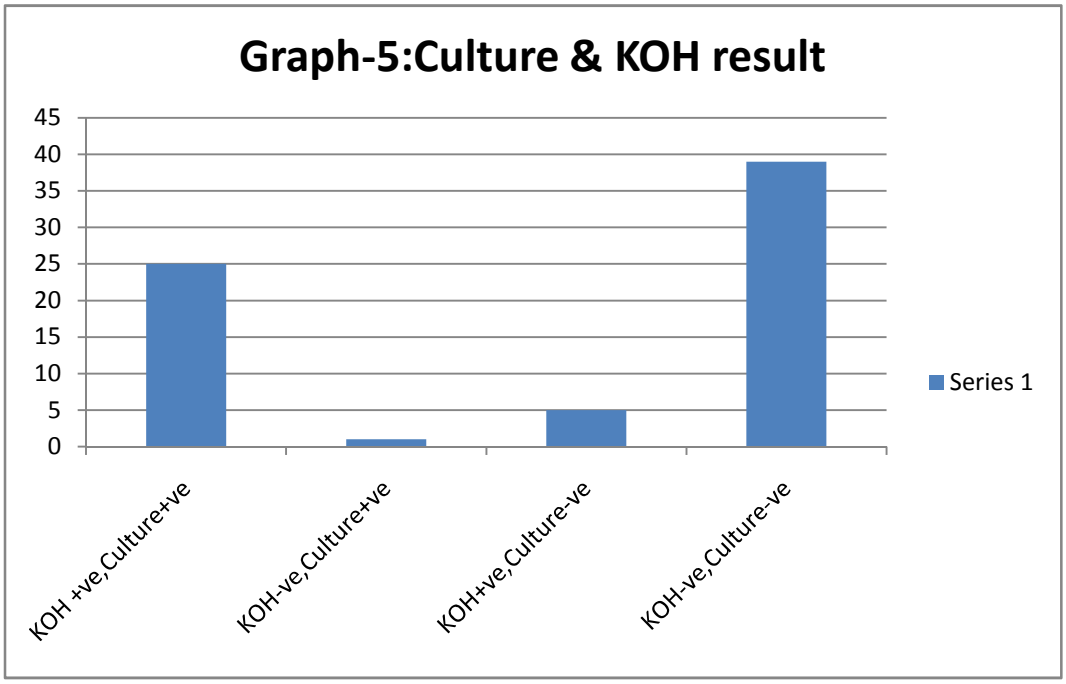
Culture status	No. of samples
Culture positive	26(37.14%)
Culture Negative	44(62.8%)
Total	70(100%)



Out of 70 samples, 26 were culture positive and 44 were culture negative. The culture positivity among KOH positive samples was 25/30(83.3%), Sensitivity of test is 83.3%, while 1 of KOH negative samples was culture positive, thus specificity was 98%.

Table-5: Correlation of KOH & Culture result of Clinical samples

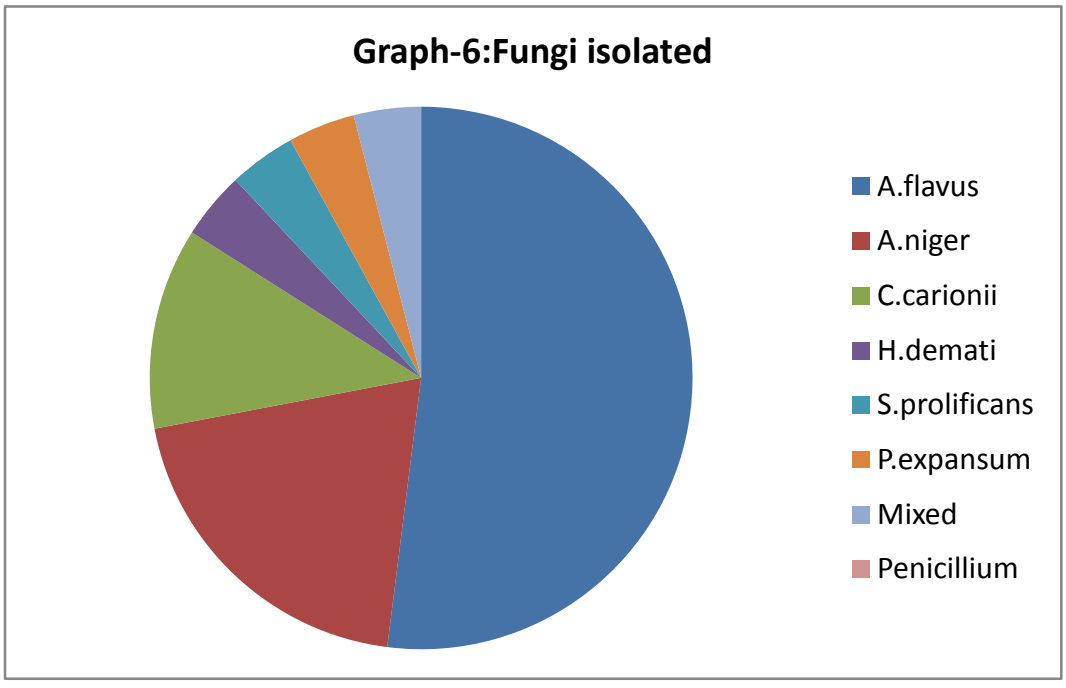
Culture & KOH status	No. of samples
KOH+ve,Culture +ve	25(35.17%)
KOH-ve,Culture +ve	1(1.42%)
KOH +ve,Culture –ve	5(7.14%)
KOH –ve,Culture –ve	39(55.7%)
Total	70(100%)



Out of 70 samples, 25 were both KOH & culture positive, 39 samples were both KOH & culture negative,5 samples were KOH positive but culture negative. One of sample was KOH negative & culture positive.

Table-6: Fungi isolated from samples

S.no.	Isolate	Number of cases
1.	*Aspergillus flavus*	13(50%)
2	*Aspergillus niger*	5(19.2%)
3.	*Cladosporium carionii*	3(11.5%)
4	*Hormonema dematioides*	1(3.8%)
5	*Scedosporium prolificans*	1(3.8%)
6	*Penicillium expansum*	1(3.8%)
7	Mixed*(Cladosporium spp.& Penicillium spp.)*	1(3.8%)
8	*Penicillium* spp	1(3.8%)
	Total	26(100%)



The commonest isolate is *Aspergillus flavus*,other isolates are *A.niger, C.carionii, H.dematioides, S.prolificans, P.expansum.*

Table-7:Growth of isolates in SDA tubes

S.no	Isolate	SDA without antibiotic	SDA with antibiotic
1	*A.flavus(*13)	5	8
2	*A.niger (5)*	5	-
3	*Cladosporium carionii(3)*	3	-
4	*Hormonema dematioides(1)*	1	-
5	*Scedosporium prolifians(1)*	1	-
6	*Penicillium expansum(1)*	1	-
7	Mixed(1)	1	-
8	*Penicillium spp(1)*	-	1
	Total(26)	17	9

Out of 70 samples,*A.flavus* was most common isolate(13) with 5/13 isolate showing growth in SDA with & without antibiotic.*A.niger* was next common isolate (5), all of them showing growth in SDA without antibiotic only. *C.carionii* (3), *H.dematioides*(1), *S.prolificans*(1), *P.expansum*(1) were the other isolates.

Table-8:Histopathological classification of culture positive samples

Culture positive	Allergic fungal sinusitis	Fungal ball/Mycetoma	Invasive fungal sinusitis
26	**25(96.15%)**	**1(3.84%)**	**0**

One isolate showing fungal ball grew *Aspergillus flavus* on culture.

One isolate showed evidence of Mucormycosis on histopathology, was KOH negative and Culture negative. Thus majority of samples in present study 25(96%) were of Allergic fungal sinusitis type, while 1(4%) was of Fungal ball/Mycetoma type.

Table-9:Distribution of fungal isolates among various histopathological types of fungal rhinosinusitis.

Isolate	AFRS	FB	IFS	Total	Percentage(n=26)
A.flavus	12	1	0	13	50
A.niger	5	0	0	5	19.2
C.carionii	3	0	0	3	11.5
H.dematioides	1	0	0	1	3.8
P.expansum	1	0	0	1	3.8
S.prolificans	1	0	0	1	3.8
Mixed	1	0	0	0	3.8
Penicillium sp	1	0	0	0	3.8
Total	25	1	0	26	100

Prevalence of fungal sinusitis based on Culture positive status in present study is 35.17%.

Out of 26 isolates, 25 were of Allergic fungal rhinosinusitis, one *A.flavus* isolate was of mycetoma type of fungal siolates.

Table-10:Comparison between Direct Smear(KOH),Culture & Histology

	Histology +ve	Histology -ve
Smear – Culture +	1	0
Smear – Culture -	0	39
Smear + Culture -	0	5
Smear + Culture +	4	21
Total n=70	5	65

Out of 70 samples, 5 showed evidence of fungal rhinosinusitis on histology,1 of them was smear(KOH) negative and culture positive, other 4 were both smear and culture positive.

DISCUSSION

Fungal rhinosinusitis, once considered a rare disorder, is now being recognized and reported with increasing frequency worldwide.

The current study, on the basis of Clinical, Histopathological and Microbiological findings, reported 26 cases of fungal rhinosinusitis among 70 suspected cases of chronic rhinosinusitis over a period of 1 year.

Clinically, patients presented with symptoms of chronic sinusitis, *viz* Nose block, headache, facial pain, loss of appetite, weakness and myalgia.

Our study reports, out of 70 CRS cases 26 cases were of FRS. Thus we report a Prevalence of 37.14% of fungal sinusitis.

In our present study, 70 cases of chronic rhinosinusitis were reported with age range of 6-63 years, most patients were in age range of 21-30 years (40%). Male to female ratio is 1.3:1. A total of 55 out of 70 patients (78.5%) were from rural background. The duration of symptoms ranged from 3 week to 6 months. This correlated well with studies done by Michael *et al*[1], Ragini *et al* [3], Klossek *et a*l[25], Das *et al* [66],Prateek *et al*[68] & Giri *et al*[79].

Michael *et al*[1] showed in their study, patients with mean age of 45.7 years ranging from 11 to 79 years with male to female ratio of 0.8:1. Most of the patients were from rural areas than from urban areas.

Ragini *et* al[3] reported patients with mean age of 40 years. There was female predominance (6/10 cases). The duration of symptoms ranged from 1-60 month with mean duration of 14.5 months.

As per study done by Klossek *et a*l[25], most patients were between 30-59 year old. Female predominance was seen. Majority of patients were from rural area.

Das *et al* [66] reported age of patients ranged from 2-81 years (mean 31 years), with male:female ratio of 1.8:1. *Prateek et al [68]* showed the age of patients with fungal rhinosinusitis ranged from 22-63 years with 42.86% of patients in 3rd decade of life followed by 4th decade (28.57%) with mean age being 39 years and male to female ratio being 1.33:1. Sixty-two percent of patients belonged to urban area and 38% of patients belonged to rural areas with 71.43% of patients coming from low socio-economic background.

Giri *et al* [79] shows the age range was from 26 to 84 years with mean of 54 years. Male:Female ratio was 1.5:1.The duration of symptoms ranged from 2 week to 8 months.

Thus our study correlates well with all the above studies with preponderance to younger age, females & rural background. This could be because of hot humid climate, poor hygiene, increased outdoor activities, inhalation of spores and immunosuppression.

In our study, 30 out of 70 samples(42.85%) were KOH positive, 40 out of 70 samples(57.14%) were KOH negative. This correlated well with studies by Ragini *et al*[3], Klossek *et al* [25] and Das *et al*[66.]

Ragini *et al*[3] proved 7 out of 10 patients were KOH positive (70%) and 3 were KOH negative (30%). Klossek *et al* [25] shows 78 out of 102 samples were KOH positive (72%) and 24 out of 102 samples (28%) were KOH negative. Das *et al*[66] shown

evidence that 164 out of 222 samples(73.8%) were KOH positive and 58 out of 222 samples(26.12%) were KOH negative.

Our study shows, 26 out of 70 samples (37.14%) were culture positive, 44 out of 70 samples (62.8%) were culture negative. Ragini *et al*[3] proved, 10 out of 47 patients (21.2%) were culture positive and 37 out of 47 (78.8%) were culture negative. Klossek *et al* [25] showed, 33 out of 109 patients (30%) were culture positive while 76 out of 109 samples (69.7%) were culture negative. Das *et al*[66], showed that, 137 out of 222 samples(61.7%) were culture positive while 85 out of 222 samples (38.28%) were culture negative.

Prateek *et al*[68] proved 21 out of 100 cases (21%) were culture positive while 79 out of 100 cases (79%) were culture negative.

Giri *et al*[79] proved,15 out of 60 cases (25%) were culture positive while 45 out of 60 cases(75%) were culture negative.

Our study shows ,out of 70 samples, 25 (35.17%) were KOH and culture positive, 39 samples (55.7%) were both KOH & culture negative, 5 samples (7.14%) were KOH positive but culture negative. One of sample (1.42%) was KOH negative & culture positive. Failure of the fungus to grow on fungal culture is common, with only 23% to 50% of cultures resulting in fungal growth[22]. Study by Ferrerio et al reported that of 22 cases which were KOH positive, 17 showed no growth(77%)[20].

deShazo's review showed no fungal growth occurred 50% of time[2]. Similarly in Klossek's study, only 31% of cases had positive cultures[25]. This difficulty in getting fungi to grow may be attributed to culture techniques, such as overhomogenization of

the specimen or lack of viability of the fungi over a prolonged time course. In our study also, 5 of samples (7.14%) were KOH positive but culture negative.

In present study, *Aspergillus flavus* (50%) is the most common isolate. *Aspergillus niger* (19.2%) is next common isolate. This correlated with studies by Michael *et al*[1], Ragini *et al* [3], Klossek *et al*[25], Das *et al* [66], Prateek *et al*[68] & Giri *et al*[79]. Michael *et al*[1] showed, *A. flavus* (79.6%) was the most common isolate. *A.niger* (3%), *A.fumigatus* (10.5%), *A.terreus* (0.7%) are other isolates. 2.25% of isolate showed mixed growth. Our study also shows one(3.8%) mixed growth.

Ragini *et al* [3]showed on culture, *Aspergillus flavus* (30%), *Aspergillus fumigatus* (20%), *Fusarium* (10%), *Rhizopus* (10%), *Bipolaris* (10%) and *Candida albicans* (20%). Klossek *et al* [25], from South America shows *A.fumigatus* as only identified fungus.

Das *et al* [66] shows *Aspergillus Flavus* as most common isolate. Other types of fungi grown on culture were *A. niger*, *A. fumigatus* (three cases) and *Bipolaris* (one case). Prateek *et al*[68], from northern India shows, *Aspergillus flavus* (57.14%) being the most common fungal isolate followed by *Aspergillus fumigatus* (14.29%).

Study by Giri *et al*[79], *A.flavus* was most common isolate (60%). Thus various studies show that *A.fumigatus* is the most common fungus associated with AFRS in western countries and second most common fungus in some parts of northern India.While *A.flavus* is the most common fungus in Indian continent. Our study, from South India shows *A.flavus* as the commonest etiological agent associated with AFRS.

Other rare isolates in our study are, *Cladosporium carionii* (11.5%), *Hormonema dematioides* (3.8%), *Scedosporium prolificans* (3.8%), *Penicillium*

expansum (3.8%). Giri *et al* [79]reported unusual fungal isolates, i.e. *Acremonium* sp., *Scedosporium apiospermum*, *Cladosporium cladosporioides* and *Lasiodiplodia theobromae*.

Reports of unusual causes of FRS have been on the rise from India as well as other countries. Baradkar *et al*. have reported a fatal case of rhino-orbito-cerebral infection caused by *Saksenaea vasiformis* in an immunocompetent individual[74]. In a case report by Swain *et al*., *Schizophyllum commune* was reported to cause sinusitis in an immunocompetent individual[75]. Our study also included immunocompetent patients in younger age group (84.2%) from whom unusual fungi were isolated.

Another case reported from South India by Premamalini *et al* also found *S. commune* to be a causative agent of FRS[77]. Janagond *et al* reported a case of rhinosinusitis caused by *Trichosporon inkin* from South India[76]. Shivaprakash *et al* reported a case of AFRS caused by *Neosartorya hiratsukae* for the first time from India[78].

Histopathological study with special fungal stains were done on all 70 CRS cases, but only 26 culture positive samples were classified. Out of 26 cases, 25 (96.15%) were of Allergic fungal sinusitis type, while 1 (3.84%) was of Fungal ball/Mycetoma type. None of Fungal sinusitis cases were of Invasive type. This correlated well with studies done by Michael *et al*[1], Das *et al* [66], Prateek *et al*[68] & Giri *et al*[79].

Michael *et al*[1] reported, 133 out of 211(63%) had allergic fungal sinusitis, 72(34%) had Invasive fungal sinusitis, 6 (3%) had fungal granuloma. Study by Das *et al* [66], 130 (58.5%)were of AFRS,8(3.6%) were Mycetoma,80(36%) were IFS,4(1.8%) showed mixed reaction, out of 222 cases.

Study by Prateek *et al*[68] , 12 out of 21(57.14%) were Allergic fungal sinusitis,2 out of 21(9.5%) were of Fungal ball & 7 out of 21(33.3%) were of Invasive fungal sinusitis. Giri *et al*[79] reported, 2 out of 15 (13.3%)were of Allergic fungal sinusitis,8 out of 15(53.3%) were Fungal ball, 5 out 15(33.3.%) were of Invasive fungal sinusitis.

One of the sample, in our study showed evidence of *Mucor* on histopathology, but grew *Aspergillus* on culture. The diagnosis of *Mucor* was based on the absence of septations present in the hyphae[22].Ferrerio suggested these cases were actually caused by Aspergillus species in which the hyphal septations were not appreciated on histopathology[20].

Two of patients which were HIV seropositive and Diabetic respectively, grew *A.flavus* on culture.

CONCLUSION

Fungal sinusitis is very common & often underdiagnosed cause of chronic rhinosinusitis. It is very common in our country with several contributing factors like hot humid climate, poor hygiene, increased outdoor activities, inhalation of spores and immunosuppression.

Accurate and early diagnosis of fungal sinusitis helps in preventing progression to Invasive fungal sinusitis, which is associated with high morbidity and mortality, and unnecessary administration of antibiotics to the patient. Thus, early diagnosis helps in better patient care & management.

Prevalence of fungal sinusitis based on culture positivity in present study is 37.14%.

Most predominant fungus isolated is *Aspergillus flavus* followed by, *A.niger, Cladosporium carionii, Penicillium sp, Hormonema dematioides, Scedosporium prolificans,* species isolated from clinical samples.

Histopathologically, they were of Allergic Fungal sinusitis & Fungal ball/mycetoma type. None of isolates were of Invasive Fungal sinusitis type.

SUMMARY

The present study was conducted in the Department of Microbiology, J.N. medical college, Belgaum for a period of one year from January 2013 to December 2013.

The samples were collected from Otorhinolaryngology OPD and patients undergoing FESS at KLE Dr. Prabhakar Kore Charitable hospital & medical research Centre, Belgaum.

- The study was conducted over a period of one year from January 2013 to December 2013.
- Patients who are not clinically diagnosed as chronic rhinosinusitis but undergoing FESS were not included in the study.
- The present study included 70 clinically diagnosed cases of chronic rhinosinusitis, undergoing FESS.
- Commonest age group affected were between 21-30 years.
- Males were more commonly affected than females with male to female ratio 1.3:1.
- *A.flavus* (13) is the commonest isolate followed by *A.niger* (5), *C.carionii* (3).
- Histopatholoically, out of 26 culture positive samples, 25 were of Allergic fungal sinusitis type and one mycetoma/fungal ball type.
- None of isolates were of Invasive type.

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ANNEXURE-I:

CONSENT FOR PARTICIPATION RESEARCH

TITLE: "Isolation, identification and speciation of fungi associated with chronic rhinosinusitis from specimens collected during Functional Endoscopic Sinus Surgery"

Study Investigator **Dr. _____**

Post Graduate Student,

Department of Microbiology,

Jawaharlal Nehru Medical College,

KLE University, Belgaum – 590 010

Guide **Dr. _____**

The purpose of research is to Isolate and identify Fungal species causing rhinosinusitis.

You are requested to participate which will help to provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

PURPOSE OF THE STUDY:

The study will help in better treatment of your disease & will help prevent recurrence of the same.

PROCEDURE INVOLVED:

FESS Samples from clinically diagnosed cases of chronic rhinosinusitis will be collected to isolate and identify fungi causing sinusitis.

RISKS AND BENEFITS: There are no risks/minimal risks involved.

PRIVACY AND CONFIDENTIALITY:

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except in emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed, in a conference no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not have to pay/offer any gifts for participating in the research. You will not be reimbursed for expenses.

Queries & Contact Details:

In case you have any questions related to the study, you can contact Dr. _____ (Post graduate student, mobile no._____) or Dr_____ (Professor, mobile no._____)

In case you have any questions about your rights as a participant, you can contact Dr (Mrs) _____, Professor and Head of Department and Chairman, J.N. Medical College Institutional Ethical Committee for Human Subjects Research, Ph _____. at J.N. Medical College, Belgaum.

CONSENT STATEMENT

I undersigned _____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participants Name _____signature_____

Parents Name (if participant is less than 18 years of

age)_____signature_____

Witness Name _____signature_____

Experimenters Name _____signature_____

Date:

Place:

ANNEXURE-II:

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

Name of student:

Name	:	Sex	:
Age	:	IP/ O.P. No	:
DOA	:	LAB. NO	:
Occupation	:		
Address	:	Date of sample collection:	

Presenting complaints:

History of presenting illness:

- History of Presence of Predisposing factors ie Diabetes, Steroid intake, Transplant, immunosuppressive drug intake etc.
- Environmental factors: Type of house, Climate, Plantations.

Past history:

- History of similar episode in the past.

Family History:

- History of atopy/allergic disease in family.

Personal history:

- Habits/ Sleep/ Appetite

General Physical Examination:

- General build/nutrition, BMI
- Orientation to time, place& person
- Presence of Pallor/Icterus/Cyanosis/Clubbing/Koilonychia/Lymphadenopathy

Local examination:

Systemic Examination :

- Respiratory system
- CVS
- Per Abdomen
- CNS

Laboratory Investigations:

Haematological findings: RBC, WBC, ESR, Platelet count

Laboratory findings:

1. Specimen :
2. Direct microscopy:

	Fungal elements Present	Fungal elements Absent
KOH		

Gram stain for yeast identification.

1. Culture :SDA and SDA with chloramphenicol & cycloheximide at 25^0C & 37ºC for 4 weeks,2 slopes on each.

Slopes are examined every 24 hours in 1st week and twice weekly subsequently.

Macroscopic morphology	1st week	2nd week	3rd week	4th week
Wooly				
Velvety				
granular				
Waxy				
Pigment production				

4.slide culture

Final Identification:

Follow up: Treatment given

Improved/Not improved/ Recurrence

ANNEXURE-III:

PREPARATION OF MEDIA

Sabouraud's Dextrose Agar: [55,57]

Glucose – 4 % (40gm)

Peptone -1% (10gm)

Agar -2% (20gm)

Distilled water – 1000ml

pH – 5.6

Autoclave the above mentioned ingredients at 121^0C for 15 minutes. Dispense in tubes and allow it to cool.

Sabouraud's Dextrose Agar with Antibiotics:[55,58]

Composition of Sabouraud's Dextrose Agar (Emmons Modification)

Dextrose:20gm

Peptone: 10g

Aga :20g

Distilled Water: 1000ml

Final pH : 6.9

The ingredients were dissolved by boiling to it 0.05mg/ml of Chlormphenicol was added and 0.5 mg /ml of Cycloheximide after autoclaving at 121° for 15 minutes, Dispense in Tubes and allow to Cool in slanted position.

POTASSIUM HYDROXIDE MOUNTS [55,59]

It is prepared from the following ingredients

Potassium hydroxide -10 gms/20 gms

Glycerol -10ml

Distilled water- 80ml

To a solution of 10%/20% KOH, 10% Glycerol is added to prevent drying. Mix ingredients and store at room temperature.

LACTOPHENOL COTTON BLUE STAIN [55,59]

The lacto phenol cotton blue (LPCB) is used to study the morphological features of the fungal isolates. It is of two types:

- Plain LPCB

It contains the following ingredients:

Melted phenol -20ml

Lactic acid -20ml

Glycerol-40ml

Cotton blue -0.05 gm

Distilled water- 20ml

Mix all the reagents properly and dissolve 0.05 g of cotton blue stain in the distilled water before mixing with the remaining reagents. The phenol acts as disinfectant, Lactic Acid preserves the morphology of the fungi and glycerol is hygroscopic agent which prevents drying. The cotton blue stains the outer wall of the fungus. Tease out of a fragment of the culture on a glass slide in a drop of LPCB using

two teasing needles. Put of a cover slip and examine under the microscope. If the plane LPCB is used the edges of the cover slip can be sealed with nail polish to keep it for longer period of time.

Haematoxylin & eosin stain:[60,61]

Procedure:

1. Deparaffinise the sections.
2. Bring sections to distilled water.
3. Stain nuclei with the alum haematoxylin for 2 minutes.
4. Rinse in running tap water.
5. Differentiate with 0.3% acid alcohol. The correct end-point is when, after blueing up, the background is almost colourless.
6. Rinse in running tap water.
7. Stain with eosin 2 mins.
8. Dehydrate, clear and mount.

Results:

Collagen-pale pink

Muscle-deep pink

Acidophilic cytoplasm-red

Basophilic cytoplasm-purple

Nuclei-blue

Erythrocytes-cherry red

GMS - METHENAMINE SILVER - GROCOTT'S, MODIFIED STAIN

REAGENTS:[61]

10% Chromic Acid:

Chromium trioxide 10.0 gm

Distilled water 100.0 ml

Mix, solution is stable for 6 months.

1% Sodium Metabisulphate:

Sodium metabisulfite 5.0 gm

Distilled water 500.0 ml

Mix, solution is stable for 6 months.

5% Borax:

Sodium borate 5.0 gm

Distilled water 100.0 ml

Solution is stable for 3 months.

Methenamine Solution:

3% Methenamine (hexamethylenetetramine) 100.0 ml

0. 5% Silver nitrate

Silver nitrate 0.5gm

Distilled water 100.0 ml

Store in amber colored bottle away from light

0.5% Gold Chloride:

Gold chloride 0.5 gm

Distilled water 100.0 ml

Mix, store in acid cleaned bottle,

refrigerate.Stable for 1 year.

1% Light Green:

Light green SF yellow. 0.2 gm

Distilled water 100.0 ml

Glacial acetic acid 0.2 ml

Mix.Stable for 6 months.

PROCEDURE:

1. Deparaffinize the section.

2. 10% Chromic acid, allow to stand for 10 minutes.

3. Wash in tap water.

4. 1% Sodium metabisulphate, 1 minute.

5. Wash in distilled water.

6. Working methanamine silver nitrate solution at 45°C, till colour changes to brownish black.

7. Rinse in distilled water.

8. 0.5% Gold chloride, 5 minute.

9. Wash in distilled water.

10. 5% Sodium thiosulphate, 5 minutes.

11. Wash in tap water.

12. Working Light green solution, 5 minute.

13. Rinse in distilled water.

14. Dehydrate, clear, and coverslip.

RESULTS:

Fungi black

Background green

Periodic Acid Schiff (PAS) Stain [61]

Solutions and Reagents:

1% Periodic Acid Solution:

Periodic acid -1 g

Distilled water -100 ml

Schiff Reagent:

Basic Fuschin 1gm

Potassium metabisulphate-2 gm

HCl (10%)-20ml/10ml

D/W- 200 ml

Activated Charcoal - 1 gm

200 ml of D/W is taken in 500 ml flask, keep it for boiling. When boiling point is reached add 1 gm of basic fuschin & boil for 2 min.Remove it from flame. The solution is filtered & allowed to cool to 50°C.

Add 2 gm potassium metabisulphate. Its cooled at room temperature & add 2% 20 ml HCl.

Keep it overnight in dark. Next day 1 gm of activated charcoal is added & shaken for 1-2 minute. The solution is filtered using Whitmann's filter paper. Store at 0-5°C.

Procedure:

1. Deparaffinize the section.
2. Treat with periodic acid solution for 10 minutes.
3. Rinse in distilled water for 2 minutes.
4. Place in Schiff reagent for 15 minutes.
5. Wash in running tap water till section turn pink color.
6. Counterstain in Mayer's hematoxylin for 1 minute**.**
7. Blueing(keep in hot water bath)
8. Dehydrate in alcohol. Clear & mount.

Results:

Glycogen, mucin and active carbohydrate- Magenta/pink

Fungi -red/purple

Nuclei-blue

ANNEXURE-IV

KEY TO MASTER CHART

I.P. No	-	Inpatient number
S.No.	-	Serial number
HPR	-	Histopathology Requisition number
KOH	-	Potassium hydroxide
NFPI	-	No Fungal Pathogen Isolated

S.no	IP no	Date of sample collection	Date of admission	Age	Sex	Occupation	Lab no	HPR no	KOH	Culture	Growth on SDA with antibiotic	Growth on SDA without antibiotic	HPR
1	485967	2.08.2012	30.07.2012	22	M	Farmer	My 804/12	3635/12	-ve	NFPI	-	-	Inflammatory polyp,-ve for fungus
2	513702	2.02.2013	31.01.2013	42	M	Farmer	My-86/13	795/13	-ve	NFPI	-	-	Inflammatory polp,-ve for fungi
3	523625	19.04.2013	16.04.13	36	M	Farmer	My-233/13	2721/13	-ve	NFPI	-	-	Allergic poly,-ve for fungi
4	526563	20.04.13	17.04.13	45	F	Housewife	My-237/13	2711/13	-ve	NFPI	-	-	Allergic polyp,-ve for fungi
5	526981	20.04.13	17.04.13	60	F	Housewife	My-249/13	2742/13	-ve	NFPI	-	-	Fibrinosuppurative inflammatory polyp,-ve for fungi
6	531804	22.05.13	20.05.13	17	F	Student	My-286/13	3432/13	-ve	NFPI	-	-	Inflammatory polyp,-ve for fungi
7	531437	23.05.13	20.05.13	6	F	Student	My-293/13	3466/13	-ve	NFPI	-	-	Acute on chronic inflammatory polyp,-ve for fungi
8	533228	30.05.13	28.05.13	23	M	Mechanic	My-307/13	3645/13	+ve	*Cladosporium carionii* isolated	-	+	Allergic polyp.-ve for fungi
9	534616	08.06.13	06.06.13	6	M	Student	My-312/13	3863/13	+ve	*Cladosporium carionii* isolated	-	+	Inflammatory polyp,-ve for fungi
10	533592	10.06.13	7.06.13	19	M	Farmer	My-318/13	3928/13	+ve	*Aspergillus flavus* isolated	+	+	Allergic polyp.-ve for fungi
11	544903	12.07.13	10.07.13	35	F	Housewife	My-355/13	4845/13	+ve	*Cladosporium carionii* isolated	-	+	Allergic polyp,-ve for fungus
12	542745	19.07.13	16.07.13	38	M	Farmer	My-356/13	5066/13	-ve	NFPI	-	-	Inflammatory lesion,-ve for fungi
13	543455	24.07.13	22.07.13	21	M	Student	My-360/13	5193/13	-ve	NFPI	-	-	Inflammatory lesion,-ve for fungi
14	544384	29.07.l3	27.07.13	24	M	Farmer	My-364/13	5328/13	-ve	NFPI	-	-	Inflammatory lesion,-ve for fungi
15	544255	29.07.13	27.07.13	34	M	Farmer	My-365/13	5327/13	-ve	NFPI	-	-	Inflammatory lesion,-ve for fungi
16	583962	10.03.14	7.03.14	37	F	Housewife	My-439/14	1888/14	+ve	*Aspergillus flavus* isolated	+	+	Allergic polyp,-ve for fungi
17	592109	23.04.14	20.04.14	15	M	Student	My-850/14	2972/14	-ve	*NFPI*	-	-	Nasal tissue with chronic inflammation,-ve for fungi
18	591698	23.04.14	20.04.14	23	F	Housewife	My-851/14	2973/14	-ve	*NFPI*	-	-	Nasal tissue with chronic inflammation,-ve for fungi
19	594781	07.05.14	05.05.14	14	M	Student	My-931/14	3317/14	+ve	*Penicillium expansum isolated*	-	+	Hyperplastic nasal tissue, congested,-ve for fungi
20	594777	07.05.14	5.05.14	35	M	Mechanic	My-934/14	3316/14	-ve	*NFPI*	-	-	Congested fibrocollagenous nasal tissue,-ve for fungi
21	594708	07.05.14	05.05.14	21	M	Farmer	My-932/14	3315/14	+ve	*Aspergillus flavus isolated*	+	+	Fibrocollagenous nasal tissue with focal clacification,-ve for fungi
22	594761	07.05.14	05.05.14	15	M	Student	My-935/14	3313/14	-ve	*NFPI*	-	-	Hyperplastic nasal tissue with focal area lined by squamous epithelium,-ve for fungi
23	594705	07.05.14	05.05.14	24	M	Farmer	My-933/14	3314/14	`+ve	*NFPI*	-	-	Linear bit of nasal tissue,-ve for fungi
24	595343	09.05.14	7.05.14	14	F	Student	My-956/14	3401/14	+ve	*NFPI*	-	-	Hyperplastic nasal tissue,congestion & haemorrhage,-ve for fungi
25	595342	09.05.14	07.05.14	12	F	Student	My-955/14	3402/14	+ve	*Scedosporium prolificans* isolated.	-	+	Hyperplastic nasal tissue,congestion & haemoorhage,-ve for fungi

26	595444	10.05.14	8.05.14	28	M	Farmer	My-973/14	3435/14	+ve	*Hormonema dematioides* isolated	-	+	Bony tissue,-ve for fungi
27	602866	16.06.14	14.06.14	28	M	Farmer	My-1092/14	4429/14	+ve	*Aspergillus flavus* isolated.	+	+	Allergic polyp,-ve for fungi
28	606078	05.07.14	03.07.14	35	F	Housewife	My-1320/14	5294/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungi
29	606710	5.07.14	2.07.14	21	M	Farmer	My-1321/14	4940/14	+ve	*Aspergillus flavus* isolated	-	+	Nasal tissue,-ve for fungi
30	606795	5.07.14	3.07.14	13	M	Student	My-1322/14	4939/14	+ve	*Aspergillus flavus* isolated	-	+	Nasal tissue,-ve for fungi
31	606706	5.07.14	3.07.14	24	F	Housewife	My-1323/14	4941/14	+ve	*NFPI*	-	-	Nasal tissue,-ve for fungi
32	607319	7.07.14	5.07.14	27	M	Mechanic	My-1330/14	5016/14	-ve	*NFPI*	-	-	Nasal tissue,-ve for fungi
33	607453	8.07.14	6.07.14	30	M	Mechanic	My-1341/14	5039/14	+ve	*Aspergillus niger* isolated	-	+	Nasal tissue,-ve for fungi
34	607419	8.07.14	6.07.14	26	M	Farmer	My-1342/14	5038/14	+ve	*Aspergillus niger* isolated	-	+	Nasal tissue,-ve for fungi
35	607477	8.07.14	6.07.14	30	F	Housewife	My-1343/14	5037/14	-ve	*NFPI*	-	-	Nasal tissue,-ve for fungus
36	607911	9.07.14	7.07.14	23	F	Housewife	My-1354/14	5070/14	+ve	*Aspergillus niger* isolated	-	+	Nasal tissue,-ve for fungus
37	608109	10.07.14	8.07.14	30	F	Housewife	My-1370/14	5098/14	-ve	*NFPI*	-	-	Nasal tissue,-ve for fungus
38	608823	14.07.14	12.07.14	20	M	Farmer	My-1387/14	5204/14	-ve	*NFPI*	-	-	Inflammatory lesion,-ve for fungus
39	609221	16.07.14	14.07.14	35	F	Housewife	My-1419/14	5264/14	-ve	*NFPI*	-	-	Nasal tissue,s/o chronic sinusitis,-ve for fungi
40	608780	17.07.14	15.07.14	48	F	Housewife	My-1427/14	5293/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
41	609732	18.07.14	16.07.14	35	M	Mechanic	My-1438/14	5346/14	-ve	*NFPI*	-	-	Allergic polyp,-ve for fungus
42	609353	18.07.14	16.07.14	55	M	Farmer	My-1439/14	5345/14	-ve	*NFPI*	-	-	s/o chronic sinusitis,-ve for fungus
43	609729	18.07.14	16.07.14	28	M	Farmer	My-1440/14	5344/14	-ve	*NFPI*	-	-	s/o chronic sinusitis,-ve for fungus
44	609730	18.07.14	16.07.14	38	F	Housewife	My-1441/14	5347/14	-ve	*NFPI*	-	-	s/o Chronic sinusitis,-ve for fungus
45	609961	19.07.14	17.07.14	40	F	Housewife	My-1459/14	5397/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
46	610133	21 7.14	19.07.14	16	F	Student	My-1460/14	5395/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
47	610107	21.07.14	19.07.14	27	F	Housewife	My-1461/14	5396/14	-ve	*NFPI*	-	-	s/o chronic sinusitis,-ve for fungus
48	610676	23.07.14	21.07.14	28	M	Mechanic	My-1475/14	5468/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
49	611528	28.07.14	26.07.14	12	M	Student	My-1499/14	5591/14	-ve	*NFPI*	-	-	Nasal tissue,-ve for fungus
50	611966	30.07.14	28.07.14	38	F	Housewife	My-1544/14	5622/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus

51	611947	30.07.14	28.07.14	27	M	Mechanic	My-1545/14	5621/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
52	612170	31.07.14	29.07.14	29	M	Farmer	My-1546/14	5653/14	+ve	*Aspergillus flavus* isolated.	-	+	Inflammatory polyp,-ve for fungus
53	612341	2.08.14	31.07.14	28	M	Farmer	My-1569/14	5722/14	+ve	*Aspergillus flavus* isolated.	+	+	Allergic polyp,-ve for fungus
54	611492	2.08.14	31.07.14	12	M	Student	My-1570/14	5721/14	-ve	*NFPI*	-	-	s/o inflammatory polyp,-ve for fungus
55	612643	4.08.14	2.08.14	35	F	Housewife	My-1571/14	5757/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
56	613241	6.08.14	4.08.14	16	F	Student	1581/14	5820/14	-ve	*NFPI*	-	-	Allergic polyp,-ve for fungus
57	613476	7.08.14	5.08.14	44	F	Housewife	My-1582/14	5950/14	+ve	*Aspergillus niger isolated*	-	+	s/o chronic sinusitis.Fungal hyphae present(hyphae & spores)
58	613781	8.08.14	6.08.14	59	M	Farmer	My-1605/14	5952/14	+ve	*Cladosporium & Penicillium sp* isolated.	-	+	Inflammatory polyp,-ve for fungus
59	612670	8.08.14	6.08.14	45	F	Housewife	My-1606/14	5951/14	+ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
60	612939	12.08.14	10.08.14	60	F	Housewife	My-1624/14	5984/14	+ve	*Aspergillus flavus* isolated.	+	+	Inflammatory polyp,**Fungal hyphae present** (?contaminant)
61	614630	13.08.14	11.08.14	35	F	Housewife	My-1637/14	6021/14	-ve	*NFPI*	-	-	Nasal tissue,-ve for fungus.
62	614643	13.08.14	11.08.14	28	M	Farmer	My-1638/14	6020/14	+ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
63	614636	13.08.14	11.08.14	28	M	Farmer	My-1639/14	6019/14	+ve	*Aspergillus flavus* isolated.	-	+	Inflammatory polyp,-ve for fungus
64	614658	13.08.14	11.08.14	25	F	Housewife	My-1640/14	6018/14	+ve	*Aspergillus flavus* isolated.	+	+	**Fungal ball containing branching hyphae**
65	615342	18.08.14	16.08.14	45	F	Housewife	My-1688/14	6116/14	+ve	*Aspergillus flavus* isolated.	-	+	s/o chronic sinusitis.**Fungal elements present.**
66	615602	18.08.14	16.08.14	40	F	Housewife	My-1689/14	6117/14	-ve	*NFPI*	-	-	Nasal mucosa-presence of only amorphous material,-ve for fungus
67	616010	20.08.14	18.08.114	35	M	Farmer	My-1709/14	6185/14	-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
68	616658	22.08.14	21.08.14	35	M	Mechanic	My-1731/14		-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
69	616499	22.08.24	20.08.14	35	M	Farmer	My-1732/14		-ve	*NFPI*	-	-	Inflammatory polyp,-ve for fungus
70	614561	5.09.14	3.09.14	63	M	Farmer	My-1855/14	6627/14	-ve	*Penicillium* spp *isolated.*	+	+	Large areas of infarction containing f**ungal hyphae resembling mucormycosis,** s/o fungal rhinosinusitis.