DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL MICROBIOLOGY

TITLE

"MUCORMYCOSIS IN COVID-19 POSITIVE PATIENTS IN LUCKNOW-A SYSYEMATIC REVIEW"

Submitted By,

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INTEGRAL INSTITUTE OF MEDICAL SCIENCE AND RESEARCH

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DISSERTATION

SUBMITTED TO: -INTEGRAL UNIVERSITY

In partial fulfilment of the need for the award of degree of

Master of Science

In

Medical Microbiology

BY: ASHISH KUMAR SHUKLA

Enrolment No: -1900103595

UNDER THE GUIDANCE OF

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DECLARATION OF CANDIDATE

I hereby declare that this dissertation entitled **"Mucormycosis in covid-19 positive patients-A systemic review"** is bonafide and genuine research work carried out by me under the guidance of **Dr. Tasneem Siddiqui** Assistant Professor, Department of Microbiology, Integral Institute of Medical Sciences and Research, Lucknow.

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ENDORSEMENT BY THE HOD

This is to certify that the dissertation entitled **"mucormycosis in Covid-19 positive patients - A systemic review"** is a bonafide and genuine research work carriedout by **Ashish Kumar Shukla** under the guidance of **Dr. Tasneem Siddiqui** Assistant Professor, Department of Microbiology, IIMS&R, Lucknow in partial fulfilment of requirement for the degree of Master of Science in Medical Microbiology. The research methods and procedure described have been done by the candidate and the results have been observed by the guides periodically.

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The research methods and procedures described are done by the candidate and results are observedby the guide periodically.

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INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2022/57

CERTIFICATE

This is to certify that research work entitled **"A systemic review of Mucormycosis in Covid-19 positive patients"** submitted by **Ashish Kumar Shukla, Dr.Tasneem Siddiqui, Dr.Ausaf Ahmad** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **19 May 2022**.

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(Jt.Member Secretary)
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(Member Secretary)
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DATE: 15-07-2022 **ASHISH KUMAR SHUKLA**

DEDICATED TO MY FAMILY & FRIENDS

LIST OF ABBREVIATIONS

ROCM	-	Rhino-orbital cerebral mucormycosis
CT	-	Computed Tomography
MRI	-	Magnetic Resonance Imaging
DM	-	Diabetes Mellitus
DKA	-	Diabetic Ketoacidosis
HPE	-	Histo pathological Examination
KOH	-	Potassium Hydroxide
SDA	-	Sabouraud Dextrose Agar
LAM	-	Liposomal Amphotericin-B
PCR	-	Polymerase Chain Reaction
LPCB	-	Lacto phenol cotton blue stain

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CONTENT

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome corona virus-2 infection (SARS-CoV-2). It is a beta coronavirus with a single strand of RNA and is a member of the coronaviridae family. SARS-CoV-2 has infected over 230 million individuals and killed more than 4.8 million people worldwide since its discovery in Wuhan, China, in November 2019.

The typical symptoms of this virus are cough, fever, and dyspnea. Extrapulmonary signs can also include serious neurologic complications such altered awareness, dizziness, and cerebrovascular events as well as changes in taste, olfactory alterations, erythematous rashes, and urticaria.

The virus causes lower respiratory tract infections that result in acute respiratory distress syndrome and finally ground-glass opacity of the lungs, just like SARS-CoV and MERS-CoV. Patients with COVID-19 exhibit a reduction in CD-4+ and CD-8+ T cell counts as a result of the acute inflammatory response and diffuse alveolar injury, making them vulnerable to a variety of infections, especially fungal infections.

A class of moulds known as mucoromycetes are the source of the deadly fungal infection known as mucormycosis (also known as zygomycosis). Rhizopus spp., Mucor spp., Rhizomucor spp., Syncephalastrum spp., Cunninghamella bertholletia, Apophysomyces spp., and Lichtheimia (formerly Absidia) spp. are the main fungi that cause mucormycosis. The most prevalent form, Rhizopus oryzae, accounts for about

60% of mucormycosis cases in humans and accounts for 90% of the Rhino-orbital-cerebral (ROCM) form.

Particularly in soil and in organic debris that is decomposing, such as leaves, compost piles, or rotten wood By coming into contact with environmental fungal spores, it is spread. According to the organs involved, mucormycosis may have varied clinical symptoms. There has been an increase in mucormycosis cases among COVID-19 patients worldwide, particularly in India.

Patients who were hospitalised for 10 days or longer or who required mechanical ventilation upon admission to the ICU were more likely to develop fungal co-infections. Globally, mucormycosis affects anywhere between 0.005 and 1.7 million people. While its frequency in the Indian population is 0.14 per 1000, which is around 80 times more.

This is because the affected patient's hospitable environment encourages the growth of the spores. These include hypoxia, high ferritin levels brought on by inflammation, high glucose levels brought on by diabetes or steroids-induced hyperglycemia, acidic medium produced by metabolic acidosis or diabetic ketoacidosis, decreased activity and count of white blood cells, along with a number of underlying conditions that encourage the germination of spores and result in the disastrous picture of rhino cerebral mucormycosis co-infection with COVID-19. Fever, blindness, abnormal eyeball protrusion, bleeding from the nose, paralysis or weakness of the facial muscles as a result of trigeminal nerve invasion are some of the symptoms. If rhino-sinus mucormycosis is not appropriately treated, it might result in cavernous sinus thrombosis.

The septum and nasal turbinate may turn reddish-black.

While long term use of corticosteroids have often been associated with several opportunistic fungal infection including aspergillosis and mucormycosis, even a short course of corticosteroids has recently been reported to link with mucormycosis especially in people with DM. A cumulative prednisone dose of greater than 600 mg or a total methyl prednisone dose of 2–7 g given during the month before, predisposes immunocompromised people to mucormycosis . There are few case reports of mucormycosis resulting from even a short course (5–14 days) of steroid therapy, especially in people with DM . Surprisingly, 46% of the patients had received corticosteroids within the month before the diagnosis of mucormycosis in the European Confederation of Medical Mycology .

AIM & OBJECTIVES

AIM

- The aim of the study is to analyze the mucormycosis associated Covid-19 positive patients .

OBJECTIVES

- To study the demographic data, risk factors of Covid 19 associated mucormycosis patients .
- To study the diagnostic challenges & treatment modalities in mucormycosis in covid-19 positive patients .

Review of Literature

W. Jeong et al. examined 851 cases from 2000 to 2017 in their work The epidemiology and clinical symptoms of mucormycosis: a systematic review and meta-analysis of case report, published in 2018. 31% of respondents were from Asia, 28% from North and South America, and 34% were from Europe.

The most prevalent cutaneous underlying disease was diabetes, which accounted for 40% of cases, 20% of which had diabetic ketoacidosis. 14 percent underwent solid organ transplants, and 42% had haematological malignancies. In 33 percent of cases, corticosteroid usage was present. 22 percent had cutaneous mucormycosis, 34 percent had rhino-orbital-cerebral mucormycosis, and 22 percent had pulmonary mucormycosis. ROCM was found more frequently in diabetics (51%) than in non-diabetics (23 percent). 12 percent likely had the condition, while 88% had it confirmed. 83 percent of cases involved histopathology, whereas 69 percent involved culture (of which 79 percent grew). The most prevalent species was Rhizopus.

According to Chakrabarti et al, antemortem diagnoses were made in 81 percent of the 75 cases that were reported from July 2006 to December 2007. The most prevalent variety, rhino-orbito-cerebral mucormycosis, affected 48% of people. Uncontrolled diabetes mellitus (58%) and diabetic ketoacidosis (38%), which were both important underlying conditions in ROCM, were both prevalent. 33 years old on average, with a 2.6:1 male to female ratio.

Ophthalmoplegia (75%) and proptosis (72%) were the most frequent appearances in the orbits, followed by visual loss (61%). Fever was rare in 44% of cases. Depending on the degree of involvement, ROCM was categorised into three clinical stages: stage I had symptoms that were restricted to the sino-nasal region, stage II had sino-orbital illness, and stage III had intracranial extension from the sino-nasal disease. The mortality rate was 45% overall. 11 out of 13 patients that were treated without surgical debridement had a high mortality rate (85%).

General Characteristics of Mucorales :

The hyphae of the Mucorales order (Zygomycetes) are typically large, ribbon-like, variable in diameter, and occasionally septate. The septa may not be visible in some preparations, hence this group has occasionally been labelled as aseptate. Observing distinctive sac-like fruiting structures (sporangia) that produce internally spherical, yellow or brown spores helps to specifically identify these organisms (sporangiospores). Each sporangium develops at the end of a structure that supports it (sporangiophore). The sporangium breaks down during maturation, releasing sporangiospores into the surrounding space. Stolons, which attach at contact areas where root like structures (rhizoids) may develop and anchor the organism to the agar surface, are the typical means by which sporangiophores are attached to one another.

PATHOGENESIS :

There are spores of the fungus that cause mucormycosis everywhere. Transmission can happen through inhalation, injection, or very rarely spore ingestion. Spores grow into a mycelial form with broad aseptate hyphae that are angioinvasive in nature, which causes the infection to spread.

Medically important members of the order Mucorales share many features with other filamentous fungi such as portals of the host for infection (airway s and disrupted muco cutaneous barriers),the main lines of innate host defences (phagocytes, specific ligands in fungals pores such as pathogen-associated molecular patterns (PAMPs) and immune cells such as Toll-like receptors (TLRs), as well as histopathological and clinical features. Hyperglycemia common feature in mucormycosis patients can cause excessive glycosylation of proteins such as ferritin and transferrin. This in addition to low pH strongly impairs their ability to chelate iron. Low serum pH affects both the phagocytic effect of macrophages and the chemotactic and oxidative burst of neutrophils.

Other reason may be poor recognition, reduce uptake of low cytokines response to fungi in diabetic patients. One hallmark of mucormycosis is angioinvasion, and the ability of a fungal pathogen to invade host cells is adaptative virulence factor.

Recently, the glucose- regulated protein 78 (GRP78) has been identified to enable adherence and invasion of the pathogen into the endothelial cell via an endocytotic mechanism.

Their on acquisition is another virulence factor. The genes for the enzymes that create hydroxamate siderophores are absent in R. oryzae. It largely depends on rhizoferrin, which is less effective, reductive iron assimilation by (iron permease, a cell membrane protein), and perhaps iron acquisition by hemeoxygenase -mediated.

The Aspects of Disease :

Patients with immune deterioration are most susceptible, especially those with

uncontrolled diabetes mellitus and transplant recipients receiving prolonged corticosteroid, antibiotic, or cytotoxic treatment. The pathogens that cause mucormycosis (a Mucorales infection) have a significant susceptibility for vascular invasion and rapidly induce tissue necrosis and thrombosis. The rhinocerebral type, in which the nasal mucosa, palate, sinuses, orbit, face, and brain are all involved, is one of the most typical manifestations each has severe necrosis and vascular invasion. Mucormycoses can also exhibit perineural invasion, which has the potential to spread retroorbitally (invasion into the brain). Other infection types affect the lungs and gastrointestinal system, and some patient develop disseminated infections. In particular to infected subcutaneous tissue in patients who have had surgery, the Mucorales have also caused localized skin infections in immunocompetent individuals with severe burns. Infection can also be carried on by damage and contaminated with spores.

PREDISPOSING FACTORS :

Uncontrolled diabetes mellitus, haematological malignancy, solid organ and bone marrow transplantation, immune compromised status, neutropenia, chronic steroid use, trauma, metabolic acidosis, previous fungal prophylaxis, extensive antibiotic therapy, aluminium and iron overload, deferoxamine therapy, illicit intravenous drug use, malnutrition, neo-natal prematurity, nosocomial spread, etc. are among the risk factors present. In the majority of studies, uncontrolled diabetes mellitus was the most prevalent risk factor. Mucormycosis may be the initial sign of diabetes mellitus in approximately 40% of patients.

CLINICAL MANIFESTATIONS:

Agents of mucormycosis are angioinvasive in nature. Mucormycosis has six types of clinical presentations:-

Rhino cerebral mucromycosis: It occurs commonly in patients with Diabetic ketoacidosis. It is the most common form; starts in eye and facial pain, may progress to cause orbital cellulits, proptosis and vison loss common manifestations of mucormycosis. Infection is initially localized to the nasal turbinates and paranasal sinuses following inhalation of sporangiospores but rapidly progresses to the orbit (sino-orbital) or brain (rhinocerebral), Sinus pain, congestion, headache, mouth or facial pain, otologic symptoms, and hyposmia and anosmia are common.

- **Cutaneous mucormycosis**: is typically the results of direct spore inoculation or exposure

to skin already compromised by burn or trauma. Cutaneous mucormycosis typically begins as erythema and induration of the skin at a puncture site and progresses to necrosis with a black eschar. Cutaneous infections can progress rapidly to involve the deep fascia and muscle layers. Necrotizing fasciitis has been reported in patients with cutaneous mucromycosis and is related to a **very** poor prognosis.

- **Pulmonary mucormycosis:** Patients with persistent neutropenia, those who have received hematopoietic cell or solid organ transplants, and those who have undergone deferoxamine therapy are at the highest risk of developing pulmonary mucormycosis. When pulmonary mucormycosis affects immune-competent hosts, it might manifest as a slowly developing pneumonia with pulmonary aneurysms and pseudoaneurysms, bronchial obstruction, or single nodules.

- **Disseminated mucormycosis**: Brain is the most common site of disseminated, but can affect any organ.

IMMUNODEFICENCY :

Innate immunity is the first-line defense against all pathogens, but patients with defect so innate immunity tend to be particularly susceptible to recurrent bacterial abscesses and to local and disseminated fungal infection. Humoral immunity is predominantly involved in neutralization of soluble viral particles and the killing of extracellular bacteria. Cell-mediated immunity is important in the defense against protozoa, fungi, viruses,

Mycobacteria, and other intracellular bacteria. The risk factors include long term antibiotic usage, in dwelling catheters, nasal intubations, immune suppressant drugs,

steroids, metabolic abnormalities (diabetic ketoacidosis), prolonged hospitalization, diabetes mellitus, prolonged neutropenia, sinus disease. Over-colonization of the sinuses by fungi and mucosal modification by microbia, allergic or virus can lead to opportunistic and lethal infections.

Preventive measures:-

(1) Decreasing exposure to pathogenic fungi and

(2) The use of prophylactic antifungal agents

(3) Surveillance

LABORATORY DIAGNOSIS :-

Specimen Collection:-

Nasal discharge or scrapings, sinus aspirate, or a tissue specimen from a vascularized tissue should be collected for the diagnosis of rhinocerebral infections. Sputum and bronchoalveolar lavage fluids are examples of respiratory samples. Specimens for the microbiology and histology laboratories should be collected separately.

Transport, and Processing:-

Formalin, a preservative used in histologic processes, inhibits fungal growth. Sterile containers should be used to transport specimens. Tissue (biopsy specimens) should be moistened in the container with a few drops of sterile saline. Specimens should be transported to the laboratory and processed within 2 hours of collection. Mucorales are extremely sensitive to changes in the environment.

Stains :-

A mucormycosis may be diagnosed rapidly by examining tissue specimens or exudate from infected lesions in a calcofluor white and potassium hydroxide preparation.

Molecular Methods:-

Nucleic acid testing may be performed on formalin-fixed, paraffin-embedded, fresh or frozen tissue samples.

Polymerase chain reaction (PCR) amplification of the internal transcribed spacer, as well as semi nested PCR of the 18S ribosomal ribonucleic acid (RNA)/deoxyribonucleic acid (DNA) sequence, has been used to confirm identification in samples that have been identified as histopathology positive. A real-time PCR assay has also been developed that amplifies the cytochrome b gene. Nucleic acid purification from formalin-fixed and paraffin-embedded. The technique uses synthetic oligonucleotides specific to the 5.8S and 18S ribosomal ribonucleic acid (rRNA) of the fungi. PCR amplification of fungal genes from the serum of high-risk patient populations has demonstrated the potential for early diagnosis of mucormycosis before the demonstration of tissue pathology

Growth media containing high concentrations of carbohydrates inhibits the production of asexual fruiting bodies that Mucormycosis. A orbital involvement in a cancer patient. B, Necrotic eschar on the hard palate of a cancer patient with rhinocerebral mucormycosis are required for the proper identification of the Mucorales species. It is therefore recommended that media such as potato dextrose, 2% malt, and cherry decoction (acidic) agars be used for cultivation. Growth and development of the mycelium in the Mucorales occurs within 24 to 48 hours. Cultures should be incubated at 27°C to 30°C.

Colonies characteristically produce a fluffy, white to gray or brown hyphal growth that resembles cotton candy and that diffusely covers the surface of the agar within 24 to 96 hours.

The hyphae can grow very fast and may lift the lid of the agar plate (also known as a "lid lifter"). The hyphae appear to be coarse, tube rapidly fills with loose, grayish hyphae dotted with brown or black sporangia.

Approach to Identification:-

Mucorales are characterized by the production of branched, non septate, wide mycelia (10-20 mm). Sexual reproduction occurs by the formation of a thick walled zygospore.

Asexual reproduction occurs by the formation of sporangiospores in sac-like structures termed sporangiophores.

The central axis of the sporangia (multispored structure) is termed the **columella (singular)** and a swelling of the sporangiophore below the columellae (plural) is termed the **apophysis.** Some species also produce rhizoids that hold the sporangiophore within the soil or growth substrate. The rhizoids are then connected to a branching root, or stolon.

Serologic Testing:-

Serology is not useful for diagnosing mucormycosis.

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry:-

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) has been evaluated for the identification of Mucorales. This technique has demonstrated a high correlation with sequencing methods for the identification of fungal

isolates (97%), indicating a potential for use as a routine identification method in the clinical laboratory.

ANTIFUNGAL SUSCEPTIBILITY TEST

The microbroth dilution method was used to determine the in vitro antifungal sensitivity.

Potato dextrose agar (PDA); cultures that had been grown for seven days were used to make inoculum solutions by adding sterile saline solution and softly scraping the surface of mature colonies with a sterile cotton swab. After transferring the homogeneous conidial suspensions into sterile tubes, the supernatants were spectro photometrically adjusted at 530 nm wavelength to an optical density (OD) that varied from 0.15 to 0.17.The non germinated conidia that made up the inoculum suspensions were diluted 1:50 in RPMI 1640 medium. Micro dilution plates were incubated at 35◦C and examined visually after 24 hr and 48 hr. As a quality check, Candida parapsilosis (ATCC 22019) was employed. the minimum inhibitory concentration (MIC)after24and48hours,respectively.

TREATMENT:-

Optimal management of mucormycosis is achieved by coordinated and interdisciplinary efforts by various sectors. as a result of its high mortality, even the slightest clinical suspicion should warrant initiation of antifungal therapy. The management primarily involves **a** mixture of surgical debridement and antifungal therapy. Amphotericin B is that the first-line management of the condition and might contribute significantly to patient outcomes. This was established in an exceedingly retrospective analysis of 70 patients with mucormycosis who had delayed amphotericin B treatment (initiation of treatment 6 days after diagnosis), which culminated during a nearly twofold increase in death 12 weeks post-diagnosis. Treatment with amphotericin-B is suggested till evident clinical improvement is observed, which usually takes **some** weeks. A lipid formulation of IV amphotericin B is generally used instead of amphotericin B deoxycholate, which may be a cheaper and more toxic alternative . Isavuconazole may be a recently approved drug within the u. s. and Europe for mucormycosis and has the potential of becoming the mainstay treatment for invasive fungal infections. Multicentric clinical studies have also found that there's no difference in mortality rates between patients treated with Amphotericin B or isovuconazole. A significantly improved survival rate has also been reported in DKA mice infected with Rhizopus spp. and combination therapy with caspofungin plus Amphotericin B Lipid Complex (ABLC) as compared to monotherapy. Liposomal Amphotericin B plus either micafungin or anidulafungin has resulted in better outcomes in disseminated mucormycosis also. If LFAB-echinocandin treatment for mucormycosis is taken into

account within the current scenario, it should be administered at doses approved by the US Food and Drug Administration (FDA).

SURGICAL DEBRIDEMENT:-

Debridement of the afflicted location during surgery has been demonstrated to considerably reduce mortality. To remove the damaged tissue, an endoscopic sinus approach should be used under MRI/CT guidance. Exenteration of the orbits and vigorous debridement of the paranasal sinuses should be used to treat rapid invasion of the orbits (72 hours). Step-down therapy should be administered after continuing the patient's IV amphotericin B. Triazoles should be used to treat cases that are resistant. When an individual's immune system is significantly suppressed, attempts should be done to restore it before treating it with antifungal therapy.

METHODOLOGY

AREA OF STUDY:- Mycology

TYPE OF STUDY: -Systematic- Review

RESEARCH OF DESIGN:-Qualitative and Quantitative.

DATA TYPE: - Data for this meta-analysis were collected from following sources.

a) Data from various publications in indexed journals.

b) Data from recent editions of textbooks.

c) Online data from various literature reviews.

d) Data from websites of CDC, NCDC, WHO,

TIME FRAME: - All the studies in indexed journal from year 2005 to 2021.

SEARCH STRATEGY: - This systematic-review followed the PRISMA guidelines. Articles were searched on PubMed, Google scholar, Mid line , and Scopus using terms related to Mucormycosis, SARS COv-2, Rhino-orbital-cerebral mucormycosis.

INCLUSION CRITERIA: - Article titles and abstracts were screened to include relevant articles.

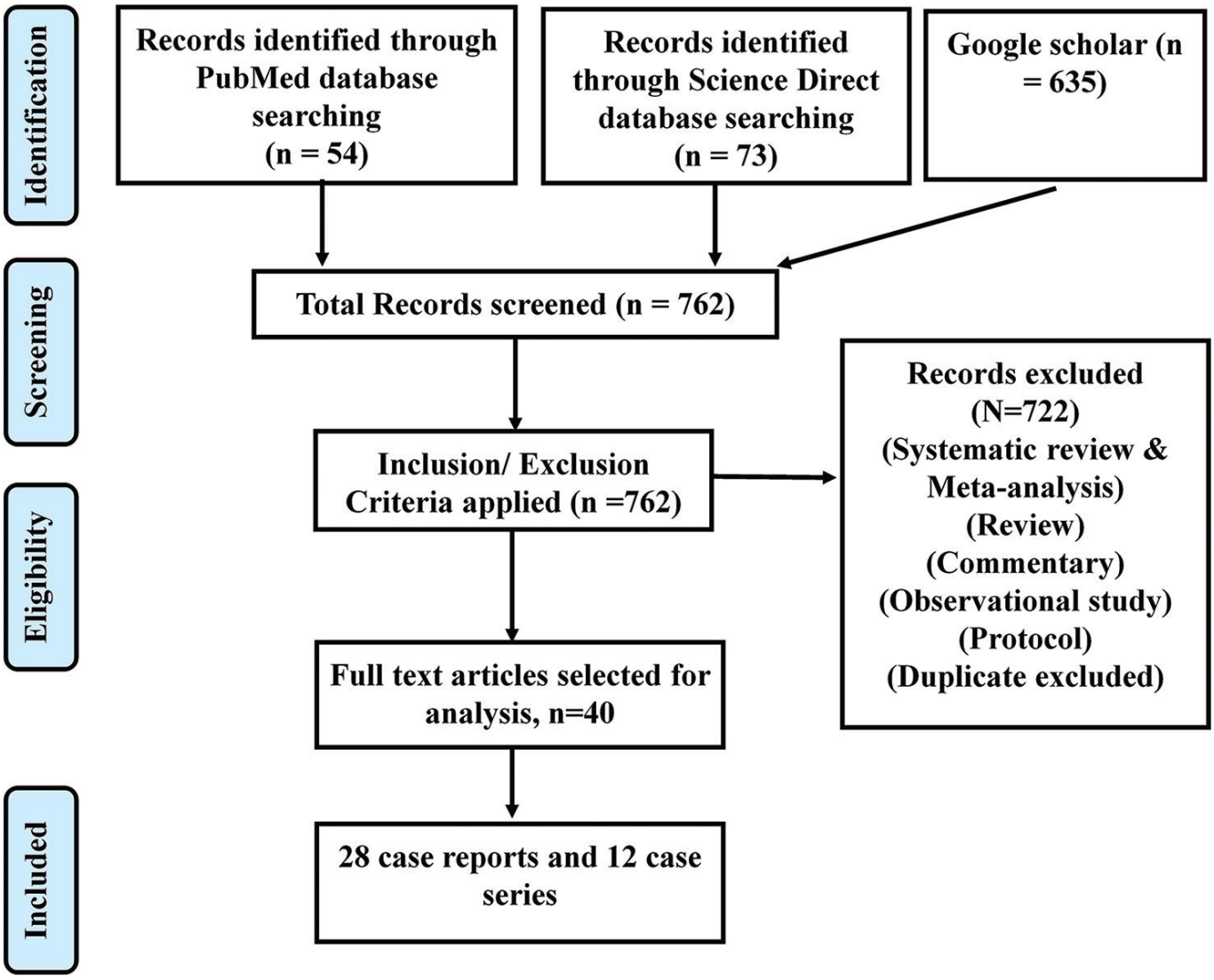
Mucormycosis infection in India, types of mucormycosis , risk factor of mucormycosis , Epidemiology of mucormycosis, treatment modalities of mucormycosis in covid-19 positive patients.

EXCLUSION CRITERIA: - Article titles and abstracts were screened by researchers independently to exclude irrelevant articles .

Study Selection Process:

This systematic review was carried out along the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines statement. An extensive literature search was conducted using PUBMED/MEDLINE and Google Scholar from inception to June 2021. 40 articles were found to report the original cases and series which were included for the synthesis of this review In this study, only case reports and case series were included as they were the only available literature when the incidence of mucormycosis in COVID-19 patients were at the peak.

The population in this study included the cases of mucormycosis irrespective of their COVID-19 status.Microsoft Excel v. 16 was used to record the demographic and clinical data of the patient and evaluated for various conclusions.


Identification
Records identified through PubMed database searching (n = 54)
Records identified through Science Direct database searching (n = 73)
Google scholar (n = 635)
Screening
Total Records screened (n = 762)
Eligibility
Inclusion/ Exclusion Criteria applied (n =762)
Records excluded (N=722)
(Systematic review & Meta-analysis)
(Review)
(Commentary)
(Observational study)
(Protocol)
(Duplicate excluded)
Full text articles selected for analysis, n=40
Included
28 case reports and 12 case series

OBSERVATION & RESULT

Table 1: Summary of case reports[13-40]

Author	Country	Sample Size	Age, range sex	COVID Status	Comorbidities				Steroid received	Location of Mucormycosis	Isolate confirmation	Outcomes
					DM							
Garg et al.	India	1	55/M	Positive	+	+	+	+	Pulmonary	+		Alive
Mehta et al.	India	1	60/M	Positive	+	-	-	+	Rhino-orbital	NM		Death
Maini et al.	India	1	38/M	Positive	-	-	-	+	Rhino-orbital	+		Alive
Saldanha et al.	India	1	32/F	Positive	+	-	-	NM	Rhino-orbital	NM		Alive
Revannavar et al.	India	1	NM/F	Positive	+	-	-	NM	Rhino-orbito-cerebral	+		Alive
Sai Krishna et al.	India	2	34/M	Positive	+	+	-	NM	Maxilla	NM		Alive
Rao et al.	India	1	66/M	Positive	+	-	-	+	Rhino-orbital	NM		NM
Meshram et al.	India	2	47/M	Positive	+	+	+	+	Rhino-orbital	NM		Death
Alekseyev et al.	USA	1	41/M	Positive	+	-	-	+	Rhino-cerebral	NM		Alive

Khan et al.	USA	1	44/F	Positive	+	-	-	+	Pulmonary	+	Death
Mekonnen et al.	USA	1	60/M	Positive	+	+	+	-	Rhino-orbital	+	Death
Kanwar et al.	USA	1	56/M	Positive	-	-	+	+	Pulmonary	+	Death
Khatri et al.	USA	1	60/M	Positive	+	+	+	+	Chest Wall	+	Death
Placik et al.	USA	1	49/M	Positive	-	-	-	+	Pulmonary	+	Death
Werthman-	USA	1	33/F	Positive	-	+	-	-	Rhino-orbito-cerebral	NM	Death
Ehrenreich et al.											
Johnson et al.	USA	1	79/M	Positive	+	+	+	+	Pulmonary	+	Alive
Ahmadikia et al.	Iran	1	44/F	Positive	+	-	-	+	Sinus	+	Alive
Karimi-Galougahi et al.	Iran	1	61/F	Positive	-	-	-	+	Rhino-orbital	NM	NM
Veisi et al.	Iran	1	40/F	Positive	-	-	-	+	Rhino-orbito-cerebral	NM	Death
Bellanger et al.	France	1	55/M	Positive	-	-	-	NM	Pulmonary	+	Death
Monte Junior et al.	Brasil	1	86/M	Positive	-	+	-	+	Gastrointestinal	NM	Death
Pasero et al.	Italy	1	66/M	Positive	-	+	+	-	Pulmonary	+	Death
Sargin et al.	Turkey	1	56/F	Positive	-	-	+	+	Rhino-cerebral	NM	Death
Krishna et al.	UK	1	22/M	Positive	-	-	+	+	Pulmonary, Brain	NM	Death
Pericardium											

Brian et al.	UK	1	22/M	Positive	-	-	+	NM	Pulmonary, Brain	NM	Death
Kidney											
Zurl et al.	Austria	1	53/M	Positive	-	-	+	+	Pulmonary	+	Death
Waizel-Haiat et al.	Mexico	1	24/F	Positive	-	-	+	NM	Rhino-orbital	+	Death
Arana et al.	Spain	2	62/M	Positive	+	-	+	+	Maxillary Sinus	+	Death
48/M	Positive		-	-	+		-	Musculoskeletal		+	Death

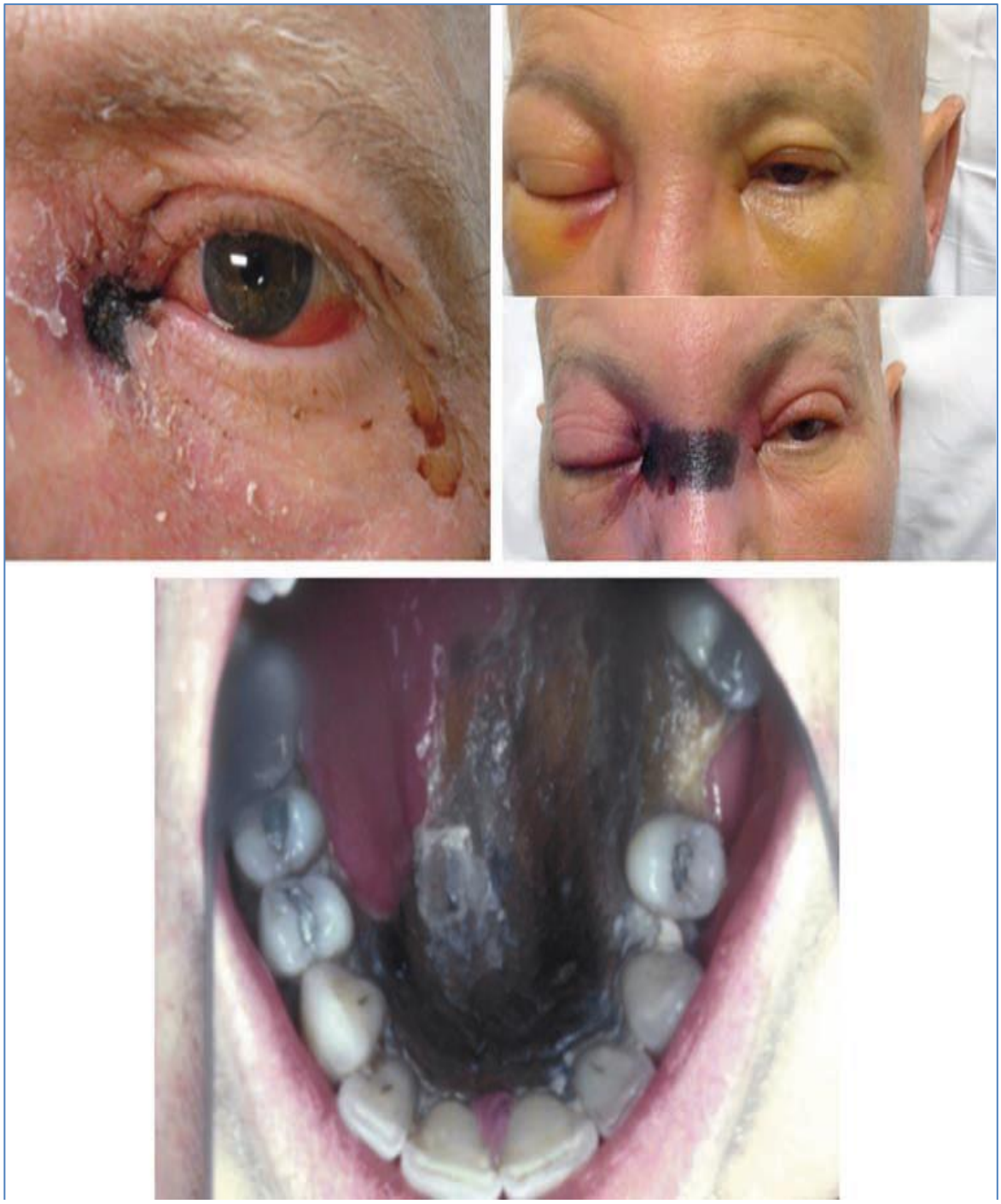


FIGURE NO. – 1 (A TO C) MUCORMYCOSIS. A, ORBITAL INVOLVEMENT IN A CANCER PATIENT. B, NECROTIC ESCHAR ON THE HARD PALATE OF A CANCER PATIENT WITH RHINOCEREBRAL MUCORMYCOSIS. C, CHRONIC NONHEALING ULCER AFTER TRAUMATIC INOCULATION.

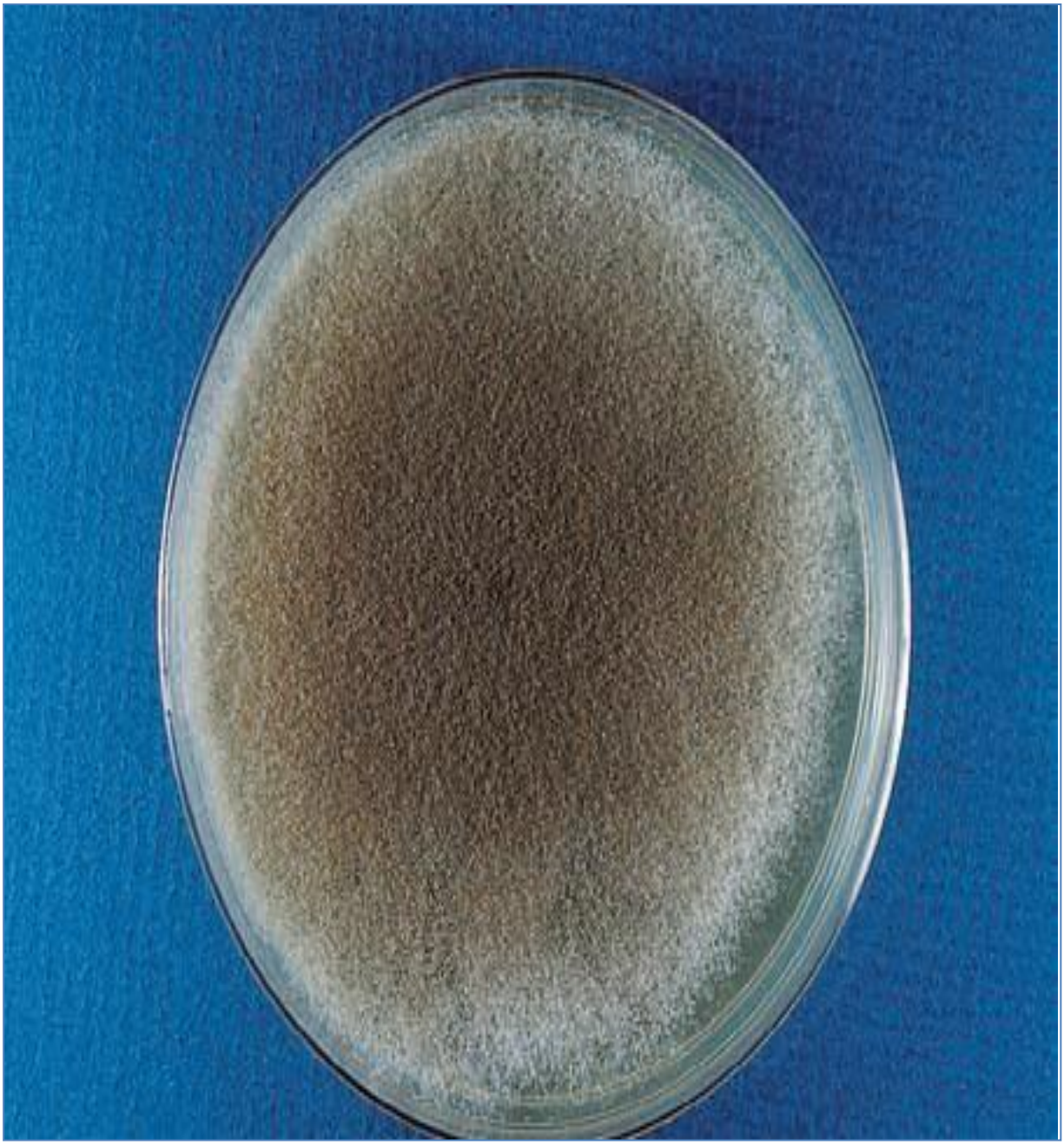


FIGURE NO. – 2 rhizopus

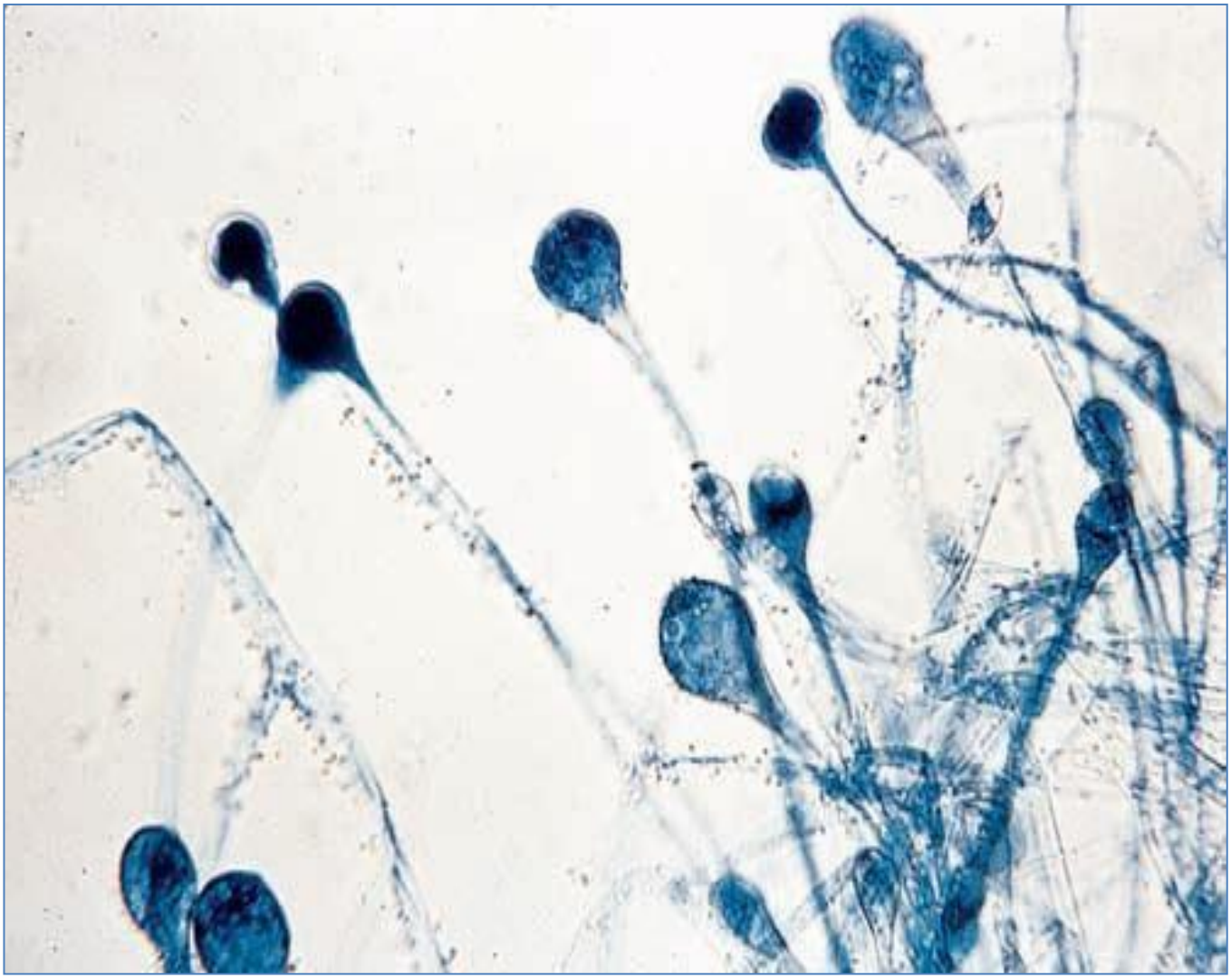


Figure No-3 : *Mucor* spp. showing numerous sporangia without rhizoid

Figure No-4 : Lactophenol cotton blue mount showing rhizoids and sporangium of *Rhizopus arrhizus* (100 × magnification)

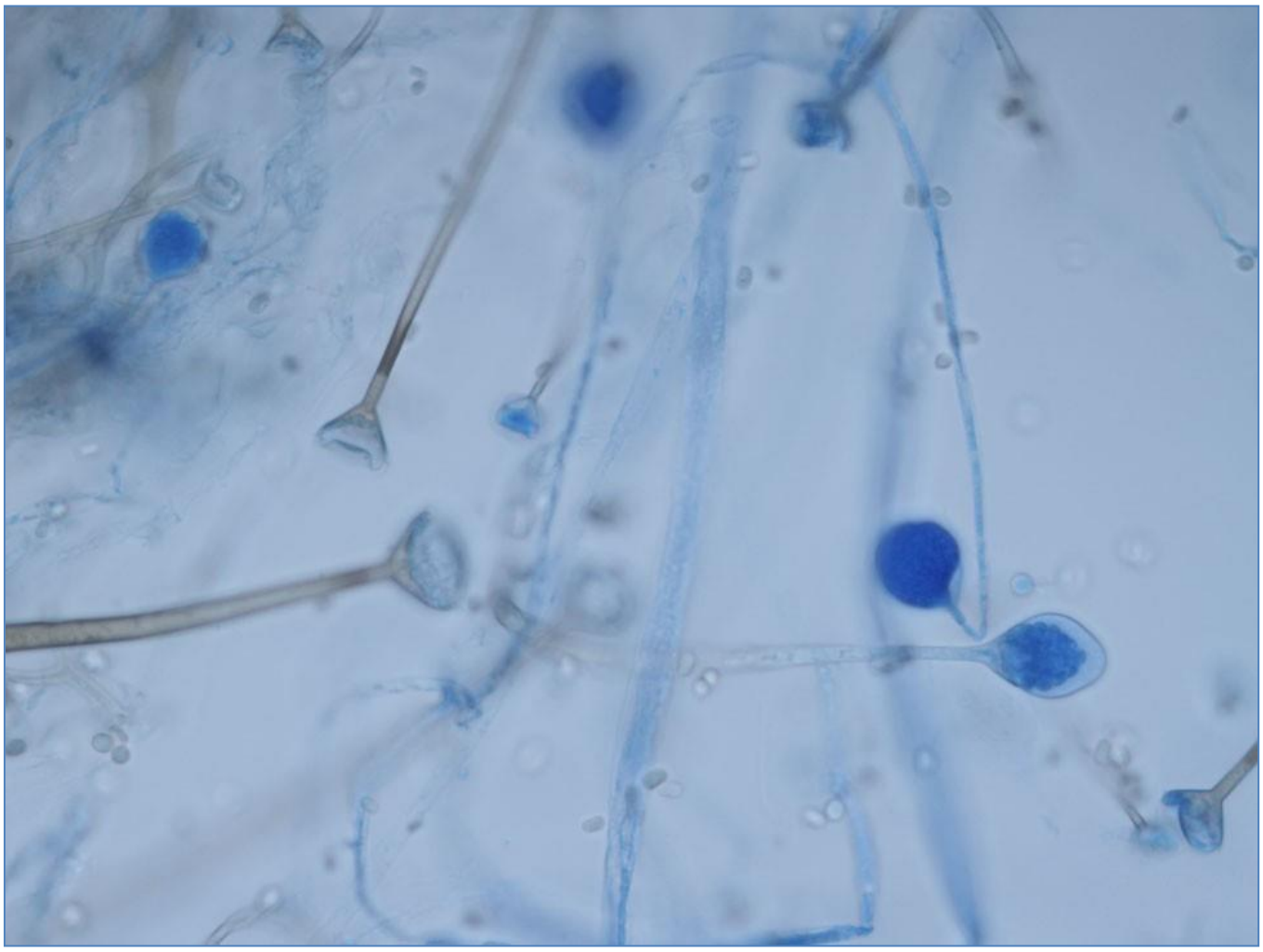


Figure No-5 : Lactophenol cotton blue mount showing champagne glass shaped apophysis of *Apophysomyces variabilis* (400 × magnification).

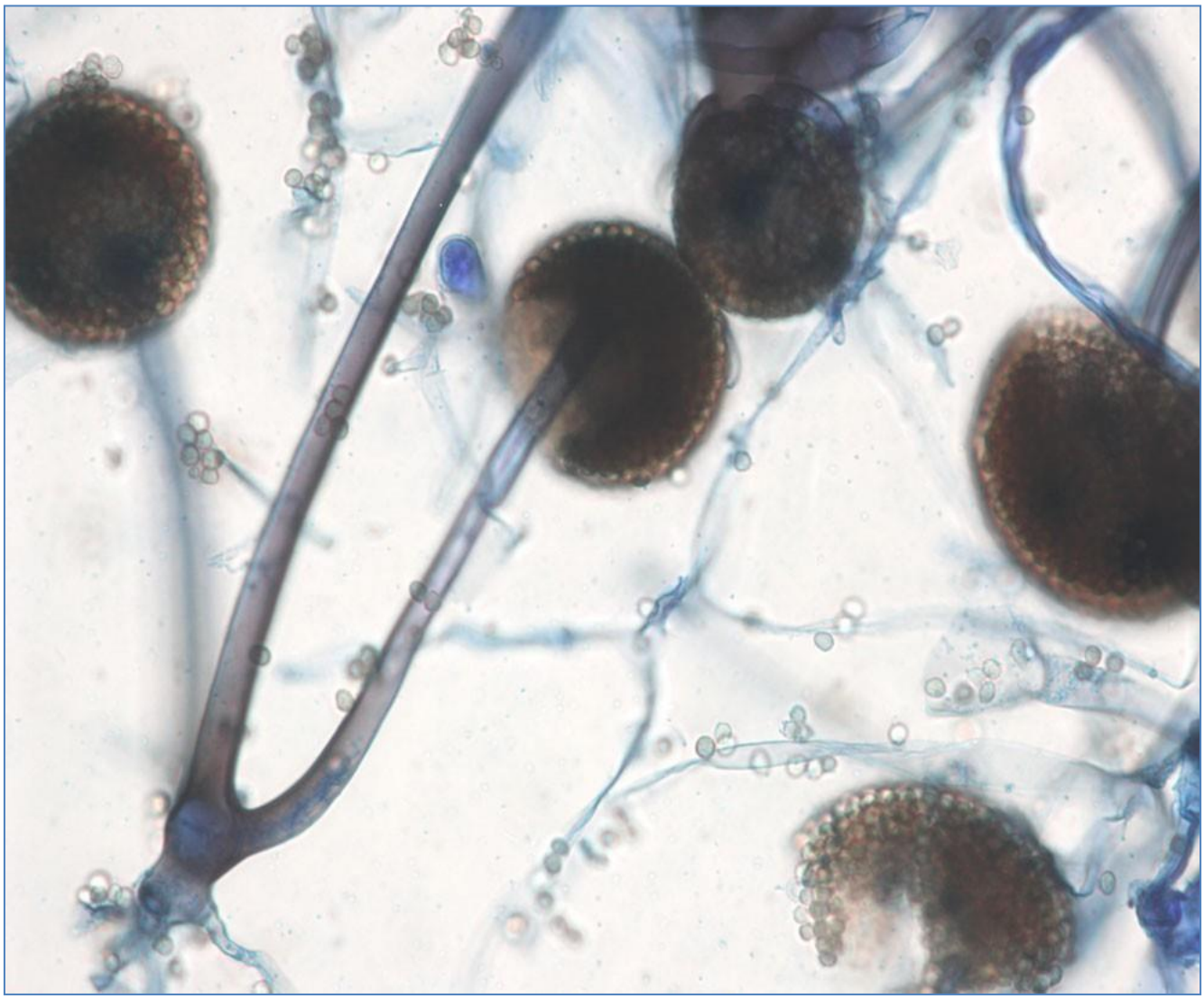


Figure No 6: Lactophenol cotton blue mount showing rhizoids and sporangium of *Rhizopus microsporus* (400 × magnification);

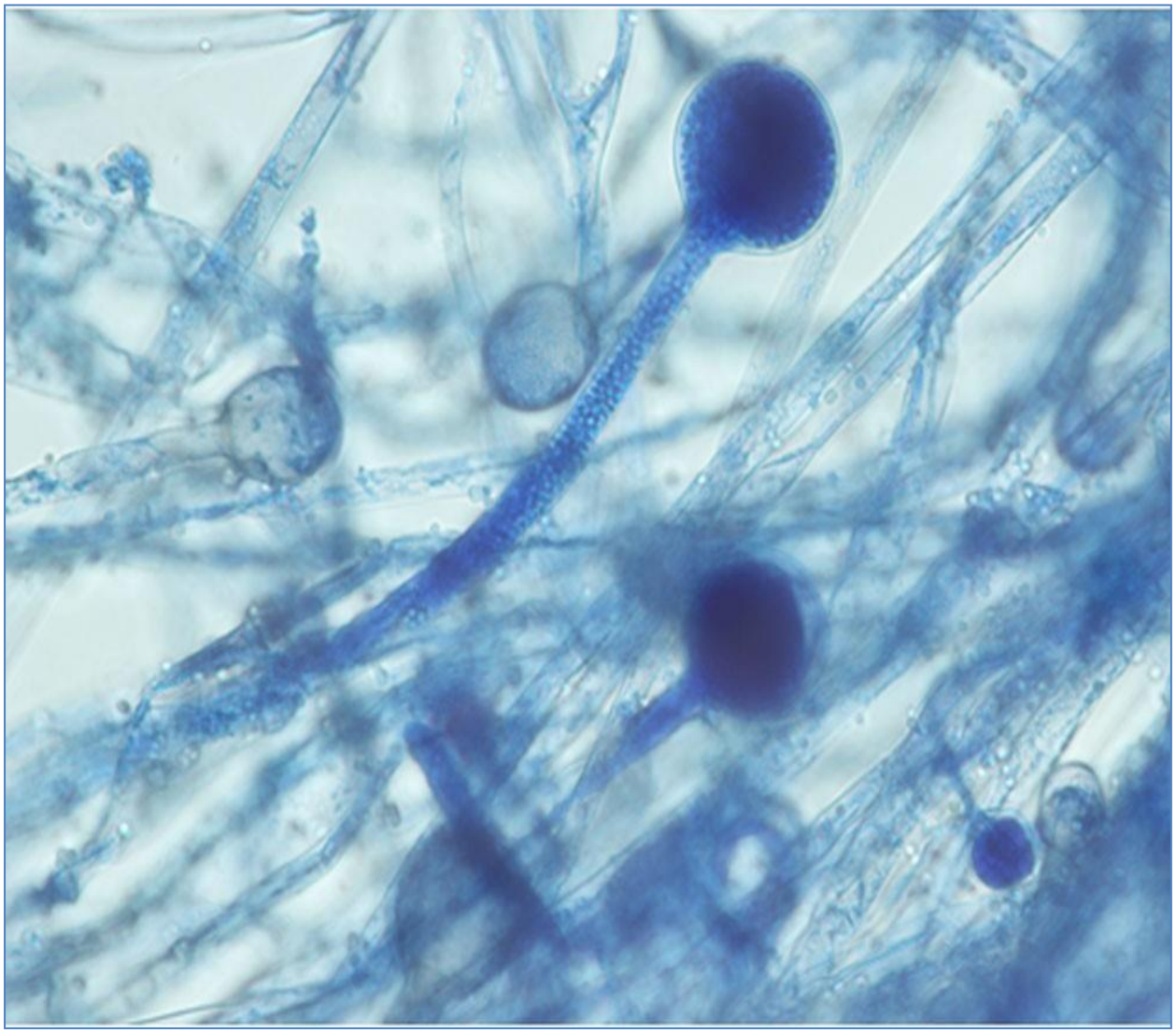


Figure No-7 : Lactose phenol cotton blue mount showing pyriform to globose columellae and irregularly branched sporangiophores of *Rhizomucor pusillus* (400 × magnification).

DISCUSSION

Mucormycosis was originally identified as a disease-causing agent in humans in 1885. Invasive fungal infections have dramatically increased in frequency during the past 20 years, partly as a result of a rise in the size of the population at risk. Mucormycosis is not the exception in this time of rising incidence. Acute necrotizing fungal infection with a fulminant outcome typically results from angioinvasion.

In industrialised nations, the prevalence of mucormycosis has increased among those undergoing hematopoietic stem cell transplants and those with haematological malignancies. An increase in invasive mucormycosis cases may be associated with an increase in the number of diabetics in emerging and tropical countries. Therefore, uncontrolled diabetes mellitus is a risk factor for the development of mucormycetes in these patients. Diabetes mellitus (56%) continues to be the most common risk factor, followed by intramuscular injection (10%), incision and drainage (5%) deferoxamine therapy (2%) and trauma (2%) and in 24 percent of cases, no risk factor was found. When fasting blood glucose levels were >140 mg/dl in this study, uncontrolled diabetes mellitus was regarded, and 56 percent of patients had it compared to 50 percent of patients who had it under control.

Bicarbonate concentration 10 mmol/l and low serum pH 7.2. In line with prior research, this analysis found that 30.7% of type 2 diabetes patients developed ketoacidosis; Chakrabarti et al. reported diabetic ketoacidosis in 27.3 and 20.7% of patients, respectively, in 2006 and 2009.

Mucormycosis, formerly thought to be always community acquired, is now recognized as also being a nosocomial infection. It has been associated with various procedures or devices used in hospitals, including antifungal prophylaxis, bandages or medication patches, intravenous catheters, and even tongue depressors. In one study 9% of patients had acquired nosocomial infection either at the site of the ECG leads or the adhesive tapes or from contaminated intra muscular injections or from air in the hospital environment.

15.4% patients acquired nosocomial infection from intramuscular injections or during the incision and drainage procedure of abscess. All of these patients presented with cutaneous mucormycosis and isolates obtained from five patients were Apophysomyces variabilis and Apophysomyces elegans from one patient.

For diagnosis of mucormycosis, CT scan and magnetic resonance imaging (MRI) may help especially when multiple infarcts are visible. These may also help in delineating the extension of the lesion. The imaging techniques also help to collect samples from the site of the lesion in deep tissue for establishing diagnosis.

CT scan was done in 24 cases of rhino-orbital-cerebral cases, and it helped in delineating the extent of lesion. Fourteen cases of sinusitis and six cases of rhino-orbital and four cases of rhino-orbital-cerebral mucormycosis were identified with the help of these imaging techniques.

The best management of mucormycosis is presumed to be aggressive surgical debridement combined with medical treatment and control of predisposing factors.

Amphotericin B is the first line drug of choice in most cases. In recent years posaconazole as a substitute for amphotericin B, especially as salvage therapy, has gained popularity. The ischemic necrosis as a result of mucormycetes mediated angioinvasion is likely important mechanism by which the fungus survives therapy.

Surgery is considered necessary due to the massive amount of tissue necrosis occurring during disease process that may prevent entry of antifungal agents in adequate concentrations. Additionally surgery is supposed to minimize the fungal load in the tissue.

CONCLUSION

- Diabetes mellitus (79.1%), chronic hypertension (30%), and renal disease/failure (13.6%), were the most common medical comorbidities, while steroids (64.5%) were the most frequently prescribed medication for COVID-19, followed by Remedisivir (18.2%), antibiotics (12.7%) and Tocilizumab (5.5%).

- Mucormycosis, which indicates a bad prognosis, is a developing issue in people with COVID-19 as well as the healed instances. Priority must be given to extra medical intervention due to the disease's multisystem involvement and quick progression.

- Diabetes, steroids and Remedsivir were not associated with an increased risk of mortality, thus confirming that the use of steroids to manage severe and critical COVID-19 patients should not be discontinued. Lung involvement, bilateral manifestation, and *Rhizopus spp.* isolation were associated with increased mortality risk, this confirming that proactive screening should be followed for critically ill patients.

- Finally surgical managements and antifungal therapy, such as amphotericin B and Posaconazole, were associated with a decreased mortality risk, thus suggesting their effectiveness.

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