International Journal Dental and Medical Sciences Research
Volume 5, Issue 2, Mar - Apr 2023 pp 1035-1041 www.ijdmsrjournal.com ISSN: 2582-6018

To study clinico-mycological profile and technique to diagnose fungal infections in intensive care unit patients

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Date of Submission: 25-04-2023 Date of Acceptance: 05-05-2023

ABSTRACT: Invasive fungal infections (IFI) kill around 1.5 million people every year making it one of the deadliest problems among ICU patients. The last two decades have seen a prominent rise in incidences of IFIs worldwide and the population cohorts in India shows overall IFI incidence rate from 3% to 20%[5].
IFIs are diagnosed using the EORTC/MSG criteria, which take into account a variety of host variables, clinical traits, radiological findings, and mycological results[8]. Studies on the diagnosis of IFIs in intensive care unit patients has been conducted globally. However, research on the immunological profile and aetiology of invasive fungal infections from India is lacking. To identify the clinico-mycological and immunological characteristics of IFIs in ICU patients, early and improve targeted treatment for the same, this study is conducted. This report incorporates different techniques and instrumentation used for identification of IFIs alongside patient data took between 16th January to 26th March 2023. The common symptoms experienced by ICU patients suffering from IFIs is analysed.
KEYWORDS: Clinico-mycological profile, fungal infections, Intensive care unit

I. INTRODUCTION

Invasive fungal infections are becoming more common in severely ill patients and are linked to higher morbidity and mortality rates. Candida species, particularly Candida albicans, are responsible for most of them. Candidaemia, disseminated candidiasis with significant organ involvement, and persistent disseminated candidiasis are all examples of invasive candidiasis. Rare pathogenic fungi like Aspergillus species, Zygomycetes, Fusarium species, and Scedosporium have all evolved over the past few decades. The key to a favorable result is prompt diagnosis and appropriate care.

Based on the outcome of a multicenter EPICII study from 2007, which included more than 1200 ICUs in 72 countries, 20% of the disease-causing microbes isolated from patients were fungi[2]. Most of the pathogens were from Candida species followed by Aspergillus species.

In this paper, the diagnostic techniques used for determining fungal infections in the samples of ICU patients are studied. Invasive aspergillosis is thoroughly reviewed.

Candida Infection

This infection is caused by Candida species. There are many different Candida species, and they are a typical component of human microbiota. Only a tiny portion of the recognized species are known to have damaging effects on people. A very broad range of diseases are caused by Candida spp.[3,4]. The infections due to invasive Candida species include Candidaemia which could have endothalmitis and in haematological patients, chronic infection of hepatosplenic candidiasis can occur[5].Both endogenous (such as mucocutaneous colonization or gastrointestinal flora) and exogenous (such as infected infusates or healthcare employees' skin) sources of Candida infection may result in local outbreaks [6].

Yeasts (Candida and Cryptococcus), moulds (Aspergillus species, Scedosporiumprolificans, Fusarium species, Mucor species, Rhizopus species, and Absidia species), and dimorphic fungi, such as (Histoplasma capsulatum, Coccidioides immitis, Paracoccidioides spp., Blastomyces dermatitidis, Sporothrix spp. and Penicillium marneffii). Though infrequently, it has also been observed that yeasts such Saccharomyces, Trichosporon, and Malassezia can result in IFIs.

Just a few categories of anti-fungal medicines are accessible in edible and injectable forms, despite the fact that the anti-fungal medications utilised in clinical applications seem to be varied and many. In addition, the demand for novel therapies for Candida infections is being exacerbated by the growth and evolution of antifungal resistance based on multiple

mechanisms. Novel anti-fungal Products combination medicines, and the creation of new bioactive chemicals may all be helpful in this regard for an improved treatment result. Three drugs especially show real potential for the management of invasive candidiasis and are now being studied in Phase 2or3.

Aspergillus Fumigatus

A saprophytic fungus called Aspergillus fumigatus is crucial for recycling nitrogen and carbon from the environment[7,8,9]. It thrives and develops naturally in the soil, which is its ecological niche. While this type of fungus isn't particularly the most widespread fungus on our planet, it is one of the most common to have airborne conidia present on them[10,11,12]. It produces thousands of conidia per conidial head and sporulates extensively. The conidia ejected into the environment have a diameter of 2 to 3 micrometer which is capable of reaching the alveoli of our lungs[13,14].

Immunocompetent people rarely experience any negative effects from inhaling conidia because the conidia are cleared by innate immune systems very well. As a result, A. fumigatus was thought to be a weak microbe that caused allergic diseases like farmer's lung, a situation seen in people who are constantly subjected to aspergilloma or conidia, an overgrowth of the fungal infection on the surface of previously established pores in the respiratory system of patients who had successfully undergone tuberculosis treatment [15,16,17].Now, due to the rise of immunosuppressed patients and the harshness of current immunosuppressive medicines, the scenario has significantly changed in recent years [18,19,20]. A. fumigatus has overtaken other airborne fungal pathogens in recent years, becoming the most common one to inflict severe and frequently deadly invasive infections on immunosuppressed hosts in developed regions of the world[21,22,23]. In the past 30 years, invasive aspergillosis (IA) cases have increased by a huge factor. IA is thought to affect 10 to 25% of all leukaemia patients, with a fatality rate of 80 to 90% even when cured [24,25,26]

Immunosuppression and Invasive Fungal diseases

The infection most usually affects immunosuppressed hosts with protracted neutropenia or hematopoietic organ transplants in the lower respiratory tract (LRT)[27]. Fever that is resistant to at least three days of proper antibiotic treatment, fever that returns after at least forty-eight hours of defervescence while still taking antibiotics, pleuritic chest ache or pain, shortness of breath, blood in cough (hemoptysis), or rapidly deteriorating breathing impairment despite proper antibiotic therapy and ventilator assistance are all signs or symptoms that indicate invasive pulmonary aspergillosis (IPA) [28]. Yet, since these indications are not exclusive to IPA, this infection could fail to be properly diagnosed. In order to distinguish between individuals with "confirmed," "probable," and "potential" IPA, the Euro-Organisation for the Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) has created a set of clinical guidelines[28].

Systemic illnesses brought on by fungi like Candida and species of Aspergillus have emerged during the past twenty years as a significant cause of illness, particularly in individuals who are critically ill or immunosuppressed. The likelihood of nosocomial fungal infection has risen as a result of improvements in medical technology and life support systems, which are now an important contributor of morbidity and mortality in the ICU [29]. While Candida albicans is the most common cause of candidiasis, non-C. albicans Candida species, including C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei, have recently been identified [30]. There are just a few antifungal medications that can be used to treat systemic mycoses. These include echinocandins (anidulafungin and caspofungin), triazoles (fluconazole and voriconazole), and amphotericin B (conventional and liposomal).

The usage of antibiotics before the infection, the existence of a central vein or tunnelled vasculature catheter, surgical operations, separation of Candida at different anatomical site, along with a low neutrophil count are Some of the important indicators of risk for Candida infection [31]. Invasive aspergillosis in the intensive care unit is at danger from mechanical ventilation and persistent lung conditions managed by corticosteroids [32]. Serious fungal infections do, however, frequently arise in ICU patients who have recently experienced aseptic instances, and sepsis-induced immunoparalysis is a significant component contributing to impaired host defences. In this situation, invasive candidiasis and the amount of time spent in the ICU have both been linked to the extent of the illness in ICU patients [32].

Individuals who report with ICU-acquired immunodeficiency exhibit substantial impairment in both the innate and adaptive arms of defence mechanisms. Due to their failure to effectively treat initial infections, these individuals are also more

susceptible to contracting nosocomial/fungal illnesses. As a result, boosting the patient's defence mechanism turns into a positive treatment approach.

They should be thoroughly and extensively examined in randomised clinical studies. However, so as to avoid making the same errors twice, it is crucial for the upcoming clinical trials to thoroughly evaluate patients' immune systems in attempt to determine customised immunotherapy.

II. METHODOLOGY

Major lab experimentation

KOH mount

This method aids in identification of fungal infections when there are ambiguous results of clinical exams. In broad terms, Potassium hydroxide mount is utilized for Bronchoalveolar Lavage samples, skin or sputum and tracheal samples, nail and tissue samples. A drop of 20% KOH is added during the operation to the sample that is stored on a clean glass slide. The slide is prepared for observation under a microscope once any more bumps have been eliminated.

Gram staining

Gram staining is used if yeast like growth is observed after wet mount/ KOH mount examination followed by SDA incubation (at 25°C and 37°C).Hans Christian Gram, a Danish professor of bacteriology, employed it for the first time in 1882, mainly to detect bacteria that cause pneumonia. The very first step in the gramme staining procedure is the preliminary staining of the slide with crystal violet dye. The next step, generally referred to as fixing the dye, includes using iodine to combine with crystal violet to prevent the color from being easily erased. As is customary for scientific words, the eponym (Gramme) while not the usual noun (stain) arecapitalised since the word Gramme staining originates after Hans Christian Gram's identity. According to the formatting guideline (if any) rules the text is following, the beginning letters of gram-positive as well as gram-negative, which are descriptive adjectives, can be both uppercase G or lowercase g. The US Centres for Disease Control and Prevention along with additional style guidelines like the AMA type both employ lowercase style.A dictionary might employ both lowercase and uppercase letters. Throughout numerous academic papers and documents, it is also usual to use uppercase Gramme positive or Gramme negative. Each publication can decide to apply the home design to the post-print edition when papers get sent to them.

The dye is subsequently eliminated using a decolorizer, which is frequently an ethanol and acetone-based solvent. The fundamental idea behind gram stain is that the crystal violet dye can be retained by the bacterial cell wall after being exposed to solvent. Gram-negative microbes have more lipid, while gramme-positive organisms have more peptidoglycan.

Clinical symptoms of infection have not shown to be sufficient for a quick determination of the presence of bacteria, which is necessary for the successful treatment of infected wounds. Traditional culture-based approaches are limiting and time-consuming for microbial identification. Furthermore, it has been estimated that only ten percent of bacteria may be properly cultivated in a lab setting, indicating that culture alone is not a sensitive enough method. Thus, a fast and repeatable approach for identifying microorganisms in wounds that are infected would help with immediate and effective therapy. Modern techniques utilising histology and molecular-based technologies have proved helpful given the limits of conventional culture.Hematoxylin and eosin (H&E) stains have recently been recommended above Gramme stains for the identification of biofilms and bacteria in organs. The infection state of a wound can be partially revealed by existing patterns of inflammatory revealed by H&E staining, but specific bacteria are difficult to identify with the staining of H&E alone. The most popular method for identifying bacterial smears in vitro is the Gramme stain, which can distinguish between Gramme positive and Gramme negative microorganisms. Yet, adjustments of this approach are required inside sections of tissue, which result in the preferred detection of various species (e.g., Goodpasture method, Brown-Brenn staining, Brown-Hopps, Steiner).

PLATELIA™ Aspergillus Ag assay

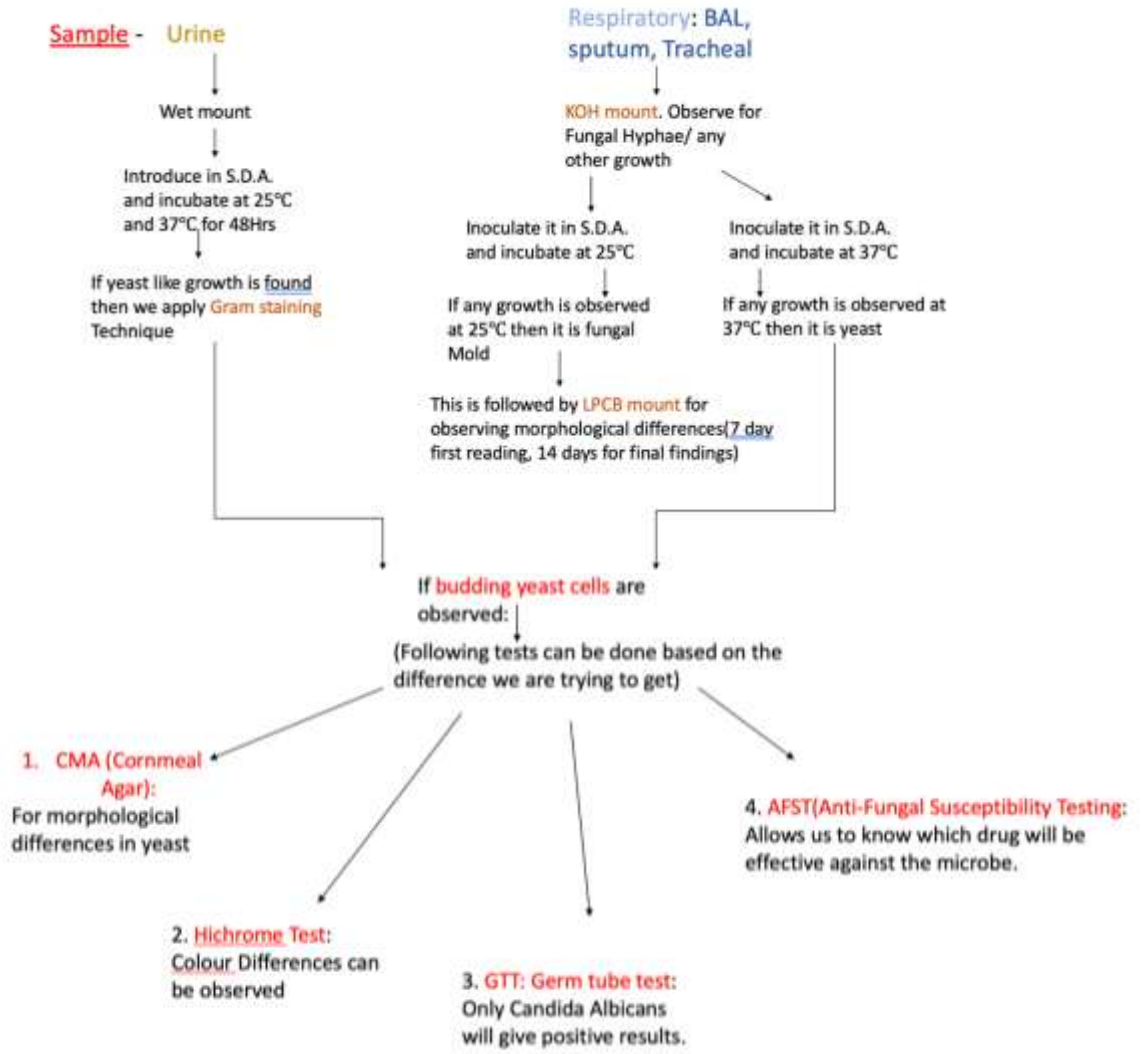
This sandwich enzyme-linked immuno-sorbent test is used to identify the antigen galactomannan in blood and BAL fluid specimens from children and adults. Along with other methods including histological analysis of biopsy samples, microbiological culture, magnetic resonance imaging, CT scan, etc., this test aids in the diagnosis of invasive aspergillosis (IA). It has been beneficial for determining IA to find galactomannan Ag in bronchoalveolar lavage (BAL) [33,34,35]. The general principle based on the guidelines set by BIORAD kit is as follows: Rat EBA-2 monoclonal antibodies, which have been characterized in earlier investigations and are directed against Aspergillus galactomanna.

International Journal Dental and Medical Sciences Research
Volume 5, Issue 2, Mar - Apr 2023 pp 1035-1041 www.ijdmsrjournal.com ISSN: 2582-6018

Monoclonal antibodies are employed to coat the microplate wells, bind the antigen, and detect the protein bound to the sensitised microplate. monoclonal protien peroxidase-linked serve as the conjugation reagent. Serum or BAL fluid samples are heated in the presence of EDTA to dissolve immune complexes and precipitate proteins that may impede the test. The chemical and the samples after treatment are then inserted into the holes coated with monoclonal antibodies and given time to incubate. A complex of monoclonal antibodies, galactomannan, and monoclonal antibodies/peroxidase formed when galactomannan antigen is available. In order toremove any leftover substance, the strips of paper are cleansed. The Chromogen TMB liquid is then added, and this will cause the complexes that are attached to the pores to respond and create a blue colour reaction. The addition of acid stops the enzyme function, turning the blue colour to yellow. A spectrophotometer tuned to 450 and 620/630 nm wavelengths is used to measure the absorption (optical density) of samples and standards

Workflow

General lab workflow for Urine and respiratory samples is as follows:



SDA: Saborauds Dextrose Agar
LPCB: Lactophenol Cotton Blue

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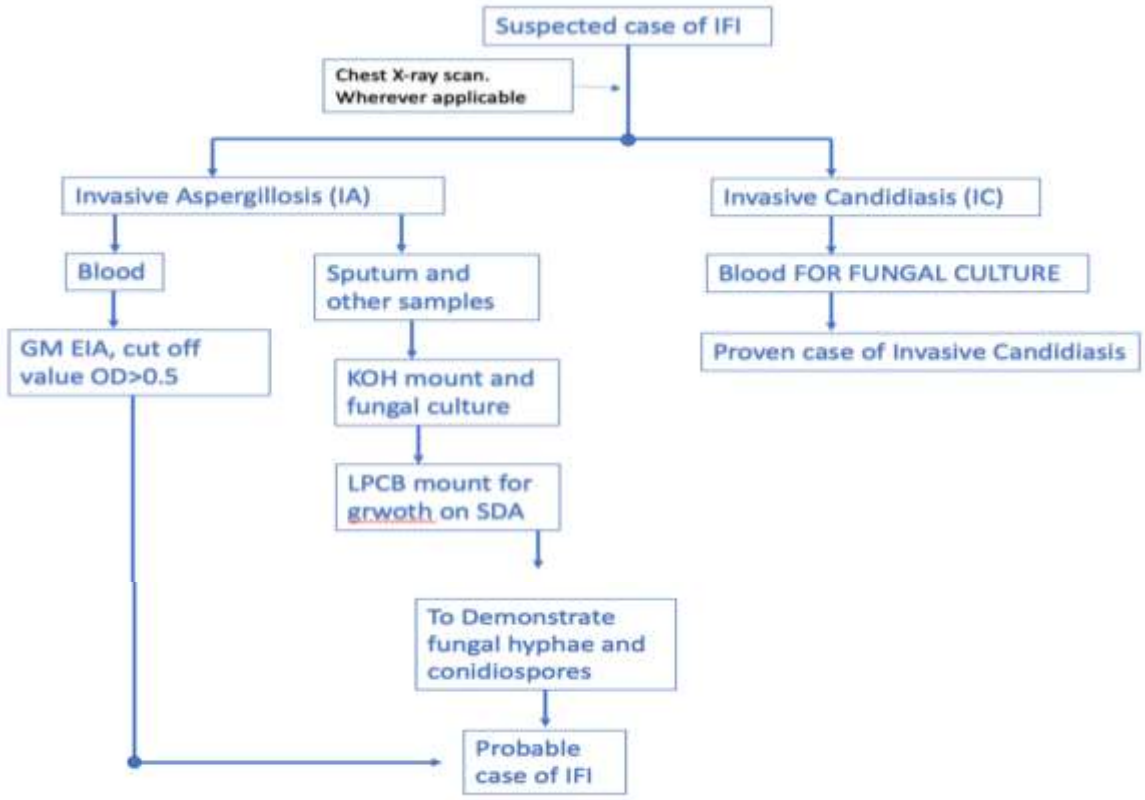

(Patient data was collected from ICU-1,2,3, R-ICU,P-ICU,SSB-ICU at VMMC & Safdarjung Hospital from 10.01.2023 to 26.03.2023 for analysis of Invasive Fungal infections in different types of samples.)

III. OBSERVATION AND RESULT

Total no. of sample for GM and fungal Culture –	73
Females -	15 (20.54%)
Males –	22 (30.13%)
Paediatric–	36 (49.31%)
Total no. of samples for GM –	50 (68.49%)
Total probable case of Aspergillosis –	24 (32.87%)
Total no. of Blood samples for fungal culture –	23 (31.50%)
Total Proven cases of Invasive Candidiasis –	17 (23.28%)

Comorbidities:

1.	Shortness of Breath –	24 (32.87%)
2.	Fever –	23 (31.50%)
3.	Cough –	22 (30.13%)
4.	Neutropenia –	19 (26.02%)
5.	Pneumonia –	17 (23.28%)
6.	Smoking –	09 (12.32%)
7.	Thrombocytopenia –	06 (08.21%)
8.	Hemoptysis –	04 (05.47%)

IV. CONCLUSION

It is concluded from this report that Invasive fungal infection is most common in pediatric patients probably due to their underdeveloped Immune system. They are most vulnerable group of patients and require maximum care as multiple caregivers are involved in their treatment, so importance is to be given to aseptic methods in order to avoid any infection.

Reduced number of neutrophil counts was common to observe in patients with IFI (26.02%), which was further associated with symptoms like shortness of breath (32.87%), fever (31.50%) and cough (30.13%). Pneumonia (23.28%) was also common to observe in pediatric patients, while relatively less common in adults. Smoking habits may have contributed to Significant worsening of the symptoms but did not directly contribute to the appearance Invasive fungal infection.

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