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Original Research Article

CLINICOPATHOLOGICAL STUDY OF FUNGAL INFECTIONS AT A TERTIARY CARE HOSPITAL IN MANGALORE: A FOUR-YEAR RETROSPECTIVE HISTOPATHOLOGICAL ANALYSIS

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Abstract

Background: Fungal infections represent an increasing cause of morbidity and mortality worldwide, particularly among immunocompromised individuals. Histopathological examination remains an important diagnostic modality for the identification of fungal organisms and assessment of tissue response.

Aim: To evaluate the clinicopathological spectrum of fungal infections diagnosed histopathologically in a tertiary care hospital in Mangalore and to analyze their demographic distribution, organ involvement, and diagnostic characteristics.

Materials and Methods: A retrospective descriptive study was conducted over a four-year period (2010–2013) in the Department of Pathology of a tertiary care teaching hospital. All histopathological specimens demonstrating fungal elements on routine and special stains were included. Demographic data, clinical presentation, organ involvement, predisposing factors, and histopathological findings were analyzed. Sections were stained using Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Gomori Methenamine Silver (GMS) stains wherever indicated.

Results: Sixty histopathologically confirmed fungal infections were identified, accounting for 0.31% of all histopathological specimens received during the study period. Males constituted 61.7% (n=37) of cases. The highest incidence was observed in the 41–50 years age group (26.7%). Skin and subcutaneous tissue were the most commonly affected sites (33.3%), followed by the respiratory tract (21.7%) and sinonasal region (18.3%). Aspergillosis (30.0%) and candidiasis (25.0%) were the predominant fungal infections. Histopathological examination successfully identified fungal organisms in all cases, while clinical suspicion was documented in only 65% of patients.

Conclusion: Histopathology remains a rapid, reliable, and cost-effective diagnostic tool for fungal infections. A significant proportion of cases were incidentally diagnosed, highlighting the importance of maintaining a high index of suspicion and routinely employing special stains in suspicious lesions.

Keywords: Fungal infections; Histopathology; Aspergillosis; Candidiasis; PAS stain; Gomori Methenamine Silver stain; Opportunistic infections.

Introduction

Fungal infections have emerged as a major public health challenge worldwide. The increasing prevalence of immunosuppressive conditions, diabetes mellitus, malignancies, organ transplantation, prolonged antibiotic use, and HIV infection has contributed significantly to the rising burden of fungal diseases [1]. Although superficial fungal infections are common, invasive fungal infections are associated with considerable morbidity and mortality [2-3].

Culture remains the gold standard for definitive diagnosis; however, it is time-consuming and may delay treatment initiation [4]. Histopathological examination offers the advantage of rapid diagnosis, identification of tissue invasion, and assessment of host response [5]. Special stains such as PAS and GMS enhance fungal visualization and facilitate species identification [6].

In developing countries, histopathology often serves as the primary diagnostic modality due to limited availability of advanced molecular diagnostic techniques.

Understanding the clinicopathological spectrum of fungal infections is therefore essential for improving diagnostic accuracy and patient outcomes.

Aim: To study the clinicopathological profile of fungal infections diagnosed histopathologically at a tertiary care hospital in Mangalore.

Objectives

1. To determine the age and sex distribution of fungal infections.
2. To identify the common anatomical sites involved.
3. To evaluate the spectrum of fungal organisms encountered.
4. To assess histopathological patterns and diagnostic utility of special stains.
5. To correlate clinical suspicion with histopathological diagnosis.

Materials and Methods

Study Design and Setting: This retrospective descriptive observational study was conducted in a tertiary care teaching hospital in Tamilnadu, India. The study aimed to evaluate the clinicopathological spectrum of fungal infections diagnosed through histopathological examination over a four-year period. The study was carried out by reviewing archived histopathology records and tissue specimens received between January 2010 and December 2013. As a retrospective analysis of previously collected specimens and patient records, no direct patient intervention was involved.

Study Population: The study population comprised all patients whose biopsy or surgical specimens demonstrated fungal elements on histopathological examination during the study period. Both clinically suspected fungal infections and cases in which fungal organisms were detected incidentally during routine microscopic examination were included. Histopathologically confirmed fungal infections from various organ systems were analyzed to determine their demographic characteristics, anatomical distribution, and pathological features.

Inclusion Criteria: All tissue specimens showing definitive histopathological evidence of fungal infection were included in the study. Cases were considered

eligible when fungal organisms were identified on routine Hematoxylin and Eosin (H&E) staining and/or confirmed by special fungal stains such as Periodic Acid-Schiff (PAS) and Gomori Methenamine Silver (GMS). Biopsy specimens, excision specimens, and surgically resected tissues with adequate material for microscopic evaluation were included irrespective of the clinical suspicion of fungal infection. Both immunocompetent and immunocompromised patients were considered eligible for inclusion.

Exclusion Criteria: Specimens with inadequate tissue for histopathological assessment, autolyzed samples, poorly preserved tissue, and cases in which fungal morphology could not be conclusively established were excluded from the study. Duplicate specimens obtained from the same lesion and repeat biopsies from previously diagnosed cases were also excluded to avoid duplication of data and ensure the accuracy of analysis.

Sample Size: A total of 60 histopathologically confirmed cases of fungal infection were identified and included in the study. These cases represented all eligible fungal infections diagnosed in the department during the four-year study period and constituted approximately 0.31% of all histopathological specimens received during the study duration.

Data Collection: Clinical and pathological information was obtained retrospectively from pathology requisition forms, laboratory records, histopathology reports, and hospital case records. The collected data included demographic variables such as age and sex, clinical presentation, provisional clinical diagnosis, anatomical site of involvement, predisposing medical conditions, and relevant radiological or laboratory findings when available. Histopathological findings including tissue reaction patterns, fungal morphology, extent of tissue invasion, and results of special stains were also recorded systematically using a structured data collection format.

Histopathological Processing and Staining Techniques: All specimens had been fixed in 10% neutral buffered formalin immediately after collection and subsequently processed using standard histopathological techniques. Tissue sections of 4–5 μm thickness were prepared from paraffin-embedded blocks and routinely stained with Hematoxylin and Eosin (H&E) for initial microscopic evaluation [7].

Special fungal stains were employed whenever fungal infection was suspected or when confirmation of fungal elements was required. Periodic Acid-Schiff (PAS) stain was utilized to demonstrate fungal cell walls by staining polysaccharide-rich structures magenta, thereby enhancing fungal visualization within tissues. Gomori Methenamine Silver (GMS) stain was used to highlight fungal hyphae and yeast forms as black structures against a pale green background, facilitating identification of fungal morphology and tissue invasion. These special stains significantly improved the sensitivity of histopathological diagnosis, particularly in cases with sparse fungal organisms.[8-10]

Microscopic Evaluation: All stained slides were reviewed by experienced pathologists. Microscopic examination focused on the identification and characterization of fungal organisms and associated host tissue responses [11].

The morphological features assessed included the size, shape, branching pattern, septation, and arrangement of fungal hyphae or yeast forms. Tissue reaction patterns such as acute inflammation, chronic inflammation, granulomatous inflammation, suppuration, necrosis, fibrosis, and giant-cell response were carefully documented.

Particular attention was given to evidence of tissue invasion, vascular invasion

(angioinvasion), thrombosis, and destruction of surrounding structures, as these features are often associated with invasive fungal disease and adverse clinical outcomes. Based on morphological characteristics and staining properties, fungal organisms were categorized into various groups including Aspergillus species, Candida species, Mucorales, Cryptococcus species, Rhinosporidium seeberi, dermatophytes, and other less common fungal pathogens whenever possible.

Statistical Analysis

Data were entered into Microsoft Excel and subsequently analyzed using Statistical Package for Social Sciences (SPSS) version 22.0. Descriptive statistical methods were used to summarize demographic, clinical, and pathological variables. Continuous variables were expressed as mean ± standard deviation (SD) and median values where appropriate, while categorical variables were presented as frequencies and percentages.

Associations between categorical variables such as age group, sex distribution, anatomical site, and fungal type were analyzed using the Chi-square test. Statistical significance was determined at a p-value of less than 0.05 with a 95% confidence interval. The findings were presented using tables, graphs, and descriptive summaries to facilitate comprehensive interpretation of the clinicopathological characteristics of fungal infections diagnosed during the study period.

Results

Demographic Characteristics: A total of 60 cases of fungal infections were identified.

Table 1: Age and Sex Distribution of Cases

Age Group (Years)	Male	Female	Total
11–20	1	0	1
21–30	4	3	7
31–40	6	7	13
41–50	12	4	16
51–60	8	2	10
61–70	1	4	5
71–80	7	1	8
Total	**37**	**23**	**60**

The majority of patients belonged to the 41–50 years age group (26.7%), followed by the 31–40 years age group (21.7%). Males predominated with a male-to-female ratio of 1.6:1 (Table 1).

Table 2: Anatomical Distribution of Fungal Infections

Site	Number (%)
Skin & Subcutaneous Tissue	20 (33.3)
Respiratory Tract	13 (21.7)
Sinonasal Region	11 (18.3)
Gastrointestinal Tract	5 (8.3)
Oral Cavity	4 (6.7)
Central Nervous System	3 (5.0)
Genitourinary Tract	2 (3.3)
Miscellaneous	2 (3.3)
Total	60 (100)

Skin and subcutaneous tissue were the most frequently involved sites, accounting for one-

third of all cases (Table 2).

Table 3: Spectrum of Fungal Organisms Identified

Organism	Cases (%)
Aspergillus spp.	18 (30.0)
Candida spp.	15 (25.0)
Mucorales	10 (16.7)
Rhinosporidium seeberi	8 (13.3)
Cryptococcus spp.	4 (6.7)
Dermatophytes	3 (5.0)
Others	2 (3.3)
Total	60 (100)

Aspergillosis emerged as the most common fungal infection followed by candidiasis (Table 3).

Table 4: Predisposing Risk Factors

Risk Factor	Cases (%)
Diabetes Mellitus	18 (30.0)
Immunosuppression	12 (20.0)
Malignancy	8 (13.3)
Chronic Lung Disease	7 (11.7)
HIV Infection	5 (8.3)
Organ Transplantation	2 (3.3)
No Identifiable Risk Factor	8 (13.3)

Diabetes mellitus represented the most common predisposing condition.

Discussion

The present study evaluated 60 histopathologically confirmed fungal infections over a four-year period. Similar to studies by Low and Rotstein, a male predominance was observed, possibly attributable to increased environmental exposure and occupational risk factors [12-13]. The peak incidence in the fifth decade corresponds with findings reported by Vallabhaneni et al. and Richardson et al., who demonstrated increased susceptibility among middle-aged and older adults due to comorbid conditions and immune dysfunction[15-17[. Skin and subcutaneous tissues were the most commonly affected sites in our study. Comparable observations have been reported by Lamps et al. and Firacative, emphasizing the diversity of fungal infections and their ability to affect multiple organ systems [18-19].

Aspergillus species constituted the largest proportion of fungal isolates. Similar findings have been reported by Bongomin et al., who highlighted Aspergillus as one of the leading causes of invasive fungal disease worldwide [20].

Diabetes mellitus emerged as the most important predisposing factor. This observation is supported by numerous studies demonstrating impaired neutrophil function and altered immune responses in diabetic patients [21].

Histopathology proved invaluable for rapid diagnosis. PAS and GMS stains significantly enhanced fungal detection, corroborating observations by Antinori et al. and Buchheidt et al. regarding the diagnostic utility of special stains in resource-limited settings [22]. A noteworthy finding was that several cases were diagnosed incidentally during routine histopathological examination, underscoring the importance of meticulous tissue evaluation even when fungal

infection is not clinically suspected.

Limitations

1. Retrospective study design.
2. Single-center study.
3. Limited availability of culture confirmation.
4. Lack of molecular diagnostic correlation.

Strengths

1. Four-year study period.
2. Histopathological confirmation of all cases.
3. Comprehensive clinicopathological evaluation.
4. Use of special fungal stains.

Conclusion

Fungal infections constitute an important cause of pathology encountered in tertiary care hospitals. Middle-aged males were most frequently affected, with skin, subcutaneous tissue, and respiratory tract being common sites of involvement. Aspergillus and Candida species predominated. Histopathological examination combined with PAS and GMS staining remains an indispensable diagnostic tool for early detection and management of fungal infections. Increased awareness among clinicians and pathologists is essential to improve diagnostic yield and patient outcomes.

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