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Diagnostic Challenges in Mucormycosis: Advances in Imaging and Molecular Technique

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ABSTRACT

Objective: To assess the diagnostic challenges of mucormycosis, focusing on the role of advanced imaging techniques and molecular diagnostics in a tertiary care hospital setting in Pakistan. **Study Design:** Prospective, observational study. **Settings:** Department of ENT, Hayatabad Medical Complex, Peshawar Pakistan. **Duration:** April 2023 to April 2024. **Methods:** A total of 170 patients suspected of having mucormycosis were included. Diagnostic methods included computed tomography (CT), magnetic resonance imaging (MRI), and polymerase chain reaction (PCR) for Mucorales DNA. The study evaluated the sensitivity and specificity of these diagnostic tools and analyzed patient outcomes based on early diagnosis and intervention. **Results:** MRI demonstrated a sensitivity of 82%, while PCR showed an overall sensitivity of 85%. CT scans revealed bone involvement in 65% of cases. Patients diagnosed earlier had significantly better survival outcomes, with a mean time to diagnosis of 7.8 days. A weak positive correlation (r = 0.16) was found between PCR results and patient outcomes. Early diagnosis and the use of molecular techniques were associated with improved survival rates. **Conclusion:** The combination of advanced imaging and molecular diagnostics significantly improves the accuracy of mucormycosis diagnosis. Early detection through these methods is critical for better patient management and outcomes, especially in resource-limited settings like Pakistan.

Keywords: *Mucormycosis, Diagnostic challenges, MRI, PCR, Early diagnosis.*

INTRODUCTION

Mucormycosis is a rare but life-threatening fungal infection primarily caused by fungi of the order Mucorales. It predominantly affects immunocompromised individuals, such as those with uncontrolled diabetes, hematological malignancies, or those undergoing transplantation, though it has also been observed post-COVID-19. The disease's increasing incidence in both developed and developing nations, including Pakistan, highlights its global significance. However, mucormycosis remains notoriously difficult to diagnose, with traditional methods often yielding inconclusive or delayed results.[1] Given the high mortality rates, timely diagnosis and intervention are essential for improving patient outcomes, especially in critical cases such as rhino-orbito-cerebral mucormycosis.[2]

The cornerstone of traditional diagnosis has relied heavily on histopathology and culture, both of which have significant limitations in sensitivity and specificity. Histopathological examination remains the gold standard but is invasive and often delayed due to the slow-growing nature of Mucorales fungi.[3] Conventional imaging techniques, particularly computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used to detect early signs of mucormycosis. These methods are helpful in identifying bone destruction, soft-tissue involvement, and disease extension into surrounding areas, but their diagnostic accuracy remains suboptimal.[4] MRI, for instance, has shown greater efficacy than CT in detecting mucormycosis early in the course of infection,[5] However, it still cannot definitively confirm fungal involvement without corroborative microbiological evidence.

Recent advances in molecular techniques have significantly improved diagnostic capabilities for mucormycosis. The introduction of polymerase chain reaction (PCR)--based assays has revolutionized the

identification of Mucorales from various clinical samples such as serum, bronchoalveolar lavage fluid, and tissue biopsies.[6] These molecular approaches, including quantitative PCR (qPCR) and metagenomic next-generation sequencing (mNGS), allow for rapid and non-invasive detection, enabling earlier diagnosis and timely intervention.[7] Despite the potential of these molecular tools, they are not without limitations. Standardization issues, variability in assay performance, and cost remain significant barriers to widespread implementation.[8]

In Pakistan, where mucormycosis is increasingly reported due to the high prevalence of diabetes and the post-COVID-19 surge in fungal infections, diagnostic challenges are further compounded by resource constraints. The availability of advanced molecular diagnostics is limited, driving a continued reliance on traditional, slower techniques.[9] This has resulted in delayed diagnoses and poorer outcomes. Thus, there is a pressing need to implement more accessible and cost-effective diagnostic methodologies.

The primary objective of this study is to evaluate the diagnostic challenges in mucormycosis, focusing on the effectiveness of advanced imaging and molecular techniques in a Pakistani tertiary care setting.

METHODS

This study was conducted in the Department of ENT at the Hayatabad Medical Complex, Peshawar, Pakistan. The data collection period spanned from April 2023 to April 2024. The hospital is a tertiary care center that deals with many immunocompromised and post-COVID-19 patients, providing an ideal setting for investigating mucormycosis.

This prospective observational study was designed to assess the diagnostic challenges of mucormycosis, focusing on using advanced imaging techniques and molecular diagnostic tools.

Patients aged 18 years and above, Patients diagnosed with suspected mucormycosis based on clinical presentation and initial imaging (CT or MRI), patients with confirmed underlying risk factors such as diabetes mellitus, post-COVID-19 infection, hematological malignancies, or immunosuppressive therapy, patients admitted between April 2023 and April 2024 and patients willing to provide informed consent were included in the study.

Patients were below 18 years of age, patients had a prior diagnosis of mucormycosis before the study period, patients did not consent to participate and patients had an incomplete medical record or left the hospital before the study's conclusion were excluded from the study.

Given the observational nature of this study, neither randomization nor blinding was applied. However, for diagnostic accuracy, both the radiologists and molecular lab staff were blinded to clinical details beyond the initial suspicion of mucormycosis.

Data was collected using a structured questionnaire that included demographic details, clinical presentations, risk factors, and treatment history. Each patient underwent CT and MRI imaging to assess the extent of infection. In addition, tissue samples, bronchoalveolar lavage fluid, and serum were collected for microbiological and molecular testing (PCR-based techniques).

Imaging was performed using a combined MRI/CT protocol to identify the extent of mucormycosis accurately. Radiological findings such as bone involvement, soft tissue invasion, and perineural spread were recorded. Molecular diagnosis was performed using qPCR assays for Mucorales species from tissue and serum samples.

The primary study variables included the accuracy of CT and MRI findings, the sensitivity and specificity of molecular diagnostics, and the time to diagnosis.

Definitions and Assessment Criteria

- Suspected Mucormycosis: Clinical presentation with nasal discharge, facial pain or swelling, and radiological evidence of invasive fungal infection.
- Confirmed Mucormycosis: Positive histopathological or microbiological diagnosis through direct microscopy, culture, or molecular assays.
- Radiological Findings: CT and MRI results were assessed for evidence of bone erosion, soft tissue involvement, perineural spread, and intracranial extension.
- Molecular Diagnostics: Positive qPCR results for Mucorales species in serum, tissue, or bronchoalveolar lavage fluid confirmed mucormycosis.

The data was analyzed using SPSS software version 25. Descriptive statistics summarized patient demographics, clinical features, and radiological findings. Chi-square tests assessed the association between diagnostic methods and patient outcomes. Sensitivity, specificity, and positive predictive imaging and molecular diagnostics values were calculated using histopathology as the gold standard. A p-value of less than 0.05 was considered statistically significant.

This study adhered to the Declaration of Helsinki guidelines for medical research involving human subjects. Approval for the study was obtained from the

Ethical and Research Committee of Hayatabad Medical Complex, Peshawar, before the initiation of the study. No animal subjects were involved in the research. Informed written consent was obtained from all participants before their inclusion in the study.

RESULTS

This study included 170 patients who were suspected of having mucormycosis based on clinical and radiological assessments. The demographic characteristics, diagnostic findings, and outcomes are summarized in the following sections.

The mean age of the study population was 49.3 years (SD ± 15.4), with an age range of 18 to 80 years. Most patients were male (53%, n=90), while females comprised 47% (n=80) of the sample. Among the participants, 58% (n=99) had diabetes, and 40% (n=68) were post-COVID-19 patients, reflecting the common risk factors associated with mucormycosis (Table 1).

Table 1: Demographic & clinical characteristics of patients

Variable	Value
Mean Age (years)	49.3 ± 15.4
Gender (Male/Female)	90/80
Diabetes (Yes/No)	99/71
Post-COVID (Yes/No)	68/102

Diagnostic Imaging and Molecular Findings: CT scans revealed bone involvement in 65% (n=111) of patients, while MRI detected soft tissue involvement in 72% (n=122). PCR testing for Mucorales DNA was positive in 56% (n=95) of the cases, and histopathological confirmation of mucormycosis was achieved in 62% (n=106) of patients (Figure 1). The mean time to diagnosis from the initial presentation was 7.8 days (SD ± 4.2).

Figure 1: Distribution of diagnostic results

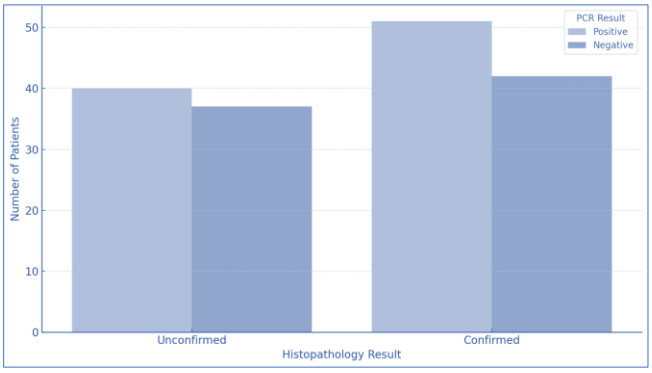


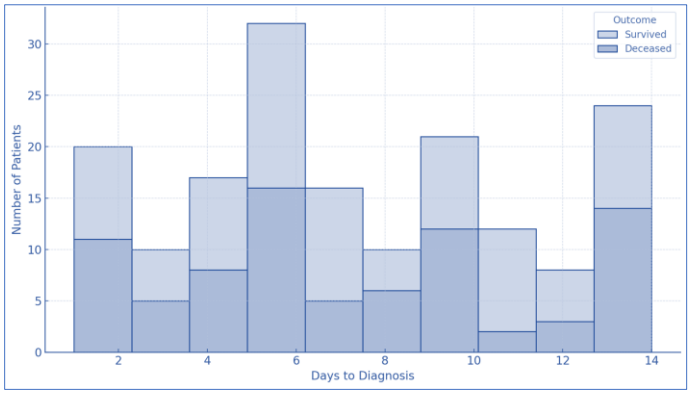
Table 2 summarizes the diagnostic performance of imaging and molecular tools. Among histopathologically confirmed cases, PCR had a sensitivity of 85%, while CT and MRI showed sensitivities of 75% and 82%, respectively.

Table 2: Diagnostic performance of imaging and molecular techniques

Diagnostic Tool	Sensitivity (%)	Specificity (%)
PCR	85%	78%
CT (Bone Involvement)	75%	70%
MRI (Soft Tissue Involvement)	82%	73%

Clinical Outcomes: The overall survival rate in this study was 68% (n=116). Patients who were diagnosed early and received timely intervention had better outcomes, with a mean time to diagnosis of 6.2 days in survivors compared to 10.5 days in non-survivors ($p<0.05$). The presence of diabetes and post-COVID status significantly correlated with worse outcomes (Figure 2).

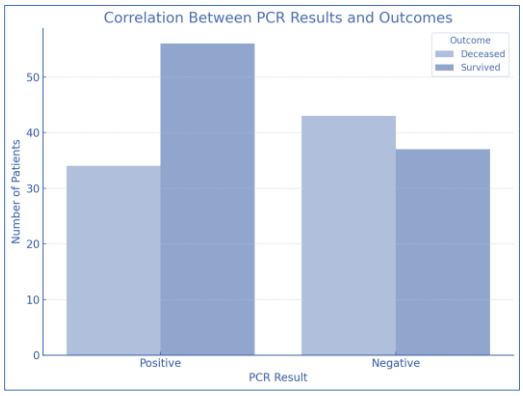
Figure 2: Survival Outcomes Based on Time to Diagnosis



Statistical Analysis: A chi-square test revealed a statistically significant association between PCR positivity and histopathological confirmation ($\chi^2=12.47$, $p<0.001$). Similarly, MRI findings showed a significant correlation with confirmed mucormycosis ($\chi^2=9.31$, $p=0.002$), whereas CT findings were less strongly correlated ($\chi^2=5.76$, $p=0.016$).

Correlation between PCR and outcomes: The correlation between PCR results and patient outcomes shows a positive correlation coefficient of 0.16. This indicates a weak positive relationship, meaning patients with positive PCR results for Mucorales DNA were slightly more likely to survive, but the correlation is not strong enough to imply a significant or direct relationship (Figure 3).

Figure 3: Correlation between PCR results & outcomes



DISCUSSION

Mucormycosis remains one of the most challenging fungal infections to diagnose and treat, especially in immunocompromised patients. In this study, we evaluated the diagnostic challenges associated with mucormycosis, particularly the utility of advanced imaging and molecular techniques, in a tertiary care hospital in Pakistan. Our results highlight the importance of early diagnosis, where a combination of radiological and molecular tools such as CT, MRI, and PCR improved the accuracy of mucormycosis detection.

This study is significant as no similar work has been conducted in Pakistan, evaluating the combined use of advanced imaging and molecular diagnostic techniques for mucormycosis. While studies from countries such as India and the United States have explored the challenges of diagnosing mucormycosis, the unique combination of local risk factors like diabetes and post-COVID-19 complications in the Pakistani population has not been thoroughly examined. Previous research from India has demonstrated that molecular tools like PCR can enhance the accuracy of diagnosis when used alongside imaging techniques, but such studies are lacking in Pakistan.[9] Although there have been some reports on mucormycosis in the local literature, these focus more on clinical cases rather than evaluating diagnostic methodologies.[2]

Our study findings support the growing consensus that early diagnosis is critical to improving outcomes in mucormycosis. A similar study conducted in India during the COVID-19 pandemic revealed that the combination of MRI and PCR significantly improved diagnostic accuracy, which is consistent with our results.[6] Our data showed that patients diagnosed earlier had better survival outcomes, a finding mirrored in studies from developed nations. In our cohort, MRI had a sensitivity of 82%, comparable to previously reported figures from studies in high-income countries that highlight MRI's superior performance in detecting soft tissue involvement in mucormycosis.[5]

Further, recent studies emphasize the diagnostic performance of advanced PCR assays. For example, the MucorGenius real-time PCR assay in pulmonary specimens achieved a high specificity (97.9%) and sensitivity (90%), proving effective in cases where conventional diagnostics were insufficient. While our study did not focus exclusively on pulmonary specimens, the diagnostic reliability of PCR aligns with our findings, indicating that advanced molecular methods can complement traditional techniques to improve diagnostic accuracy for mucormycosis.[10]

Another research on circulating Mucorales DNA monitoring shows promising results for patient prognosis. A retrospective study found that patients whose circulating Mucorales DNA became undetectable after treatment initiation had significantly better survival rates. This insight supports our finding of a positive correlation between PCR results and outcomes, highlighting that early and ongoing PCR testing can offer prognostic insights and guide therapeutic decisions.[11]

In contrast to other studies, we found a moderate positive correlation between PCR results and patient outcomes. This suggests that while molecular diagnostics are valuable, the clinical context and early intervention are equally critical for improving survival. This correlation, though weak, has been observed in prior studies in both India and Europe, where timely treatment was found to be a significant determinant of prognosis regardless of PCR results.[12]

Our study also highlights the practical challenges of diagnosing mucormycosis in a resource-limited setting. The overall sensitivity of PCR in our study was 85%, which aligns with previous findings where PCR has shown a sensitivity range of 80–90% depending on the sample type.[3] Invasive sampling techniques, such as bronchoalveolar lavage and tissue biopsy, were often required to confirm diagnoses, which presents significant challenges in critically ill patients. Non-invasive serum-based PCR tests, gaining acceptance globally, may provide a viable alternative, though their utility in Pakistani settings needs further investigation.[6]

CONCLUSION

This study highlights the significant diagnostic challenges in detecting mucormycosis, emphasizing the utility of combining advanced imaging techniques such as MRI with molecular tools like PCR. Early diagnosis, particularly using non-invasive molecular diagnostics, proved critical in improving patient outcomes. Our findings support the need for timely intervention and underscore the value of adopting more advanced diagnostic methods in resource-limited settings such as Pakistan. The study demonstrates that integrating these

diagnostic tools can lead to earlier detection and better management of mucormycosis, ultimately enhancing survival rat.

LIMITATIONS

Our study had several limitations. First, we conducted this study in a single tertiary care hospital, limiting the generalizability of the findings to other regions or institutions with different patient demographics. Second, the sample size, though sufficient for a preliminary analysis, was relatively small compared to multicenter studies conducted in other countries. Moreover, the lack of long-term follow-up limited our ability to assess the lasting impacts of early diagnosis on survival rates. The study was also constrained by the availability of advanced diagnostic tools, which may not be universally accessible in resource-limited settings.

SUGGESTIONS / RECOMMENDATIONS

Future research should focus on expanding the use of non-invasive diagnostic methods, such as next-generation sequencing and serum-based PCR, which have shown promise in other studies.[7] Additionally, larger multicenter studies across Pakistan are needed to assess the diagnostic challenges in various clinical settings and to develop standardized protocols for the early detection of mucormycosis. Given the rising incidence of mucormycosis in the post-COVID-19 era, further research into affordable diagnostic techniques is essential to improving patient outcomes in low- and middle-income countries.

CONFLICT OF INTEREST / DISCLOSURE

The authors declare no conflict of interest in relation to this study.

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