Invasive Mucormycosis (IM) is a life-threatening infection caused by Mucorales. However, rapid progression of the disease and absence of early and reliable diagnostic assay lead to high mortality and morbidity. The sensitivity of conventional methods including direct microscopy and culture is around 50% and data utilizing molecular assays for diagnosis is very limited. Therefore the present study was conducted to assess the diagnostic utility of (Panfungal PCR in combination with mucorales specific PCR) among suspected cases of IM.

**Material & Method**

This was a prospective study where clinically suspected cases of IM attending our tertiary care hospital from August 2015 – March 2018 were enrolled.

All the cases were defined as proven/ probable/possible cases mucormycosis based on EORTC/MSG guidelines.

Conventional identification was performed using direct microscopy and culture.

Panfungal and mucorales specific PCR assay were performed simultaneously on all the collected specimens using primers for ribosomal DNA region of fungi.

A nested PCR assay was done using primers targeting the V4 and V5 variable regions of the 18S rDNA of Mucorales fungi. (Bialek J et al., 2005).

**Panfungal Primers**

ITS1: 5'- TCCGTAGGTAGACCTGCGG-3'

ITS4: 5'- TCCCTCCGCTTATTGATATG-3'

External PCR: ZM1 (5'-ATT ACC ATG AGC AAA TCA GA-3')

ZM2 (5'-TCC GTC AAT TCC TTT AAG TTT C-3')

Internal PCR: ZM1 (5'-ATT ACC ATG AGC AAA TCA GA-3')

ZM3 (5'-CAA TCC AAG AAT TAC ACC TCT AG-3')

The amplified products were further subjected for sequencing to confirm species identification.

**Result**

A total of 297 clinical samples were collected from 239 clinically suspected cases of IM. Diabetes mellitus was the most common underlying predisposing condition documented in 90.79% (217/239) cases. As per EORTC/MSG guidelines, 11 (4.6%) cases were classified as proven, 134(53.97%) as probable and 99 (41.42%) cases were classified as possible mucormycosis.

**Discussion**

In this study, we demonstrated the clinical significance of panfungal and mucorales specific PCR from clinical samples for the diagnosis of mucormycosis.

Sensitivity and specificity of the panfungal PCR and mucorales specific PCR in this research was 100% in proven cases of mucormycosis.

Considering direct microscopy as reference, the specificity of panfungal and mucorales specific PCR was 73.74% and 91.92% respectively.

The reason for the low specificity of panfungal PCR was that 15 Candida albicans were found after sequencing.

Considering Calbicans (n=15) as commensal flora isolated from nasal tissue, the specificity of panfungal PCR increased to 86.27%.

**Conclusion**

Panfungal PCR in combination with mucorales specific PCR followed by sequencing may play a significant role in diagnosis of mucormycosis in suspected cases of mucormycosis especially among those with negative direct microscopy and culture.

In highly suspected cases of mucormycosis, mucorales specific PCR is better than the panfungal PCR.

**Reference**


