PCR based methods for diagnosis of Mucormycosis

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Introduction

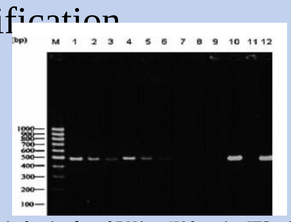
Invasive Mucormycosis (IM) is a life-threatening infection caused by *Mucorales*. However, rapid progression of the disease and absence of early and reliable diagnostic assay lead to high mortality and morbidity. The sensitivity of conventional methods including direct microscopy and culture is around 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

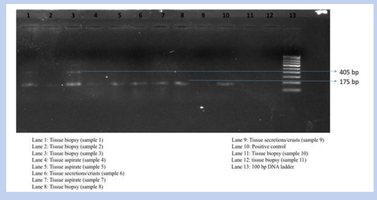
- This was a prospective study where clinically suspected cases of IM attending our tertiary care hospital from August 2015 – March 2018 were enrolled.
- All the cases were defined as proven/ probable/possible cases mucormycosis based on EORTC/MSG guidelines.
- Conventional identification was performed 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'- TCCGTAGGTGAACCTGCGG- 3'
  ITS4: 5'- TCCTCCGCTTATTGATATG- 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 TTC ACC TCT AG-3')

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



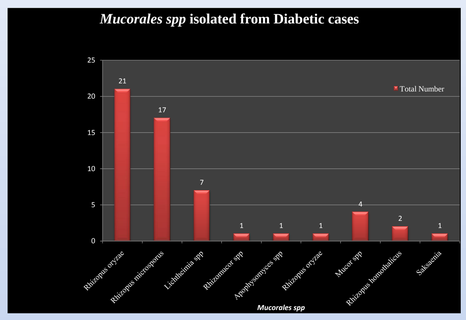
Gel electrophoresis showing fungal DNA at 450 bp using ITS primers; lane M is for molecular weight marker, while lanes 1-10 demonstrate PCR products of patients; lanes 1-6, and 10 are positive cases, and lanes 7 -9 are negative cases. Lane 11 is for the negative control, while lane 12 is for the positive control which was DNA extracted from *Aspergillus species*.



Gel electrophoresis of internal PCR product of 18S rDNA of mucorales fungi (from tissue samples)

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.



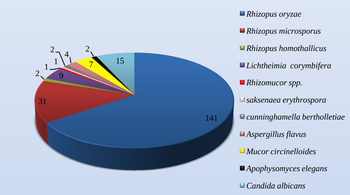
Distribution of *mucorales species* grown on culture (n=55)

Sensitivity and specificity of different diagnostic methods according to EORTC MSG criteria

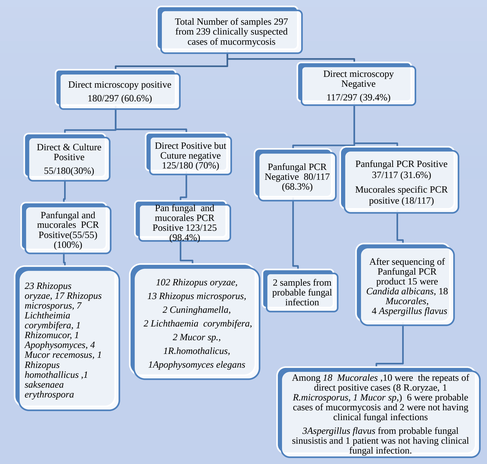
		Sensitivity 95% CI	Specificity 95% CI	NPV 95% CI	PPV 95% CI
Proven cases(n=11)	Conventional PCR (Panfungal)	100	100	100	100
	Mucorales specific PCR	100	100	100	100
	Direct Microscopy	100	100	100	100
	Culture	100	100	100	100
Probable (n=129)	Conventional PCR (Panfungal)	98.45	100	98.20	100
	Mucorales specific PCR	98.45	100	98.20	100
	Direct Microscopy	100	100	100	100
	Culture	42.64	100	59.78	100
Possible (n=99)	Conventional PCR (Panfungal)	70.59	82.93	93.15	46.15
	Mucorales specific PCR	47.06	100	90.11	100
	Direct Microscopy	0	100	82.83	0
	Culture	0	100	82.83	0

Sensitivity and specificity of panfungal PCR and *Mucorales* specific PCR according to direct Microscopy and conventional culture

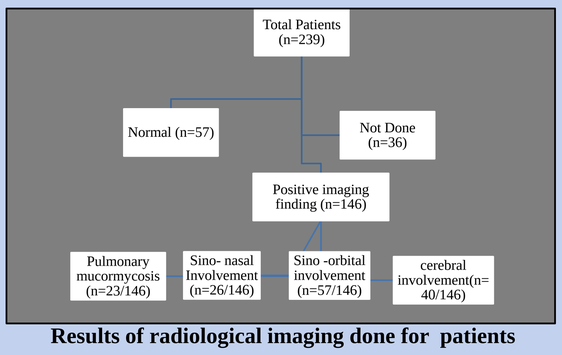
	Panfungal PCR	*Mucorales* Specific PCR	
Direct Microscopy as reference			
Sensitivity%	98.57	98.57	
Specificity%	73.74	91.92	
PPV%	84.15	94.52	
NPV%	97.33	97.85	
Concordance Rate%	88.28	95.82	
Conventional Culture as reference			
Sensitivity%	100	100	
Specificity%	40.54	50.27	
PPV%	32.93	36.99	
NPV%	100	100	
Concordance Rate%	53.97	61.51	



Identification of fungi from pan fungal PCR positive samples following sequencing:



Total number of samples collected and results obtained from conventional methods and conventional PCR after sequencing



Results of radiological imaging done for patients

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

Conclusion

- Pan fungal 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

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