DIAGNOSTIC VALUE OF MOLECULAR METHODS IN DIAGNOSIS OF MUCORMYCOSIS

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AIM OF THE STUDY

- To evaluate molecular methods in tissue samples for diagnosis of mucormycosis.

BACKGROUND

- Incidence of Invasive Mucormycosis is very less (<0.1%) but is on rise now since a decade.
- Increasing trends correlate with recent reports of breakthrough infections in those receiving prophylaxis (Kriengkauyiat et al., 2011).
- Rise in incidence is two-fold in India and is alarming. It has increased from 12.9 cases/year during 1991 through 2000 to 35.6 cases/ year during 2001 through 2005 (Chakrabarti et al., 2008).
- Renal involvement has been reported in about 20% of the cases in India.
- Majority of the patients are immunocompromised and only upto 12% have been found immunocompetent.
- Major risk factors: diabetes mellitus, diabetic ketoacidosis, alcoholism, systemic steroid usage, burn wounds, organ transplants, haematological malignancies etc.
- Direct Microscopy & Histopathology is the “gold standard” for diagnosis. However, detection of fungal DNA in tissue samples by PCR may improve the diagnosis of Mucormycosis.
- Delay in treatment can lead upto >50% mortality (Llorente A et al., 2011).

METHODOLOGY

- Study period: January, 2015 – December, 2017
- Tissue samples were collected from suspected cases of mucormycosis in diabetes mellitus patients.
- Conventional identification was done using direct microscopy and culture.
- Molecular diagnosis were performed by nested PCR assay using primers targeting V4 and V5 variable regions of 18S rDNA of *mucorales* (figure 1)
- PCR for endogenous β -globin gene was done to validate DNA extraction method (figure 2)

RESULTS

297 tissue samples were collected from 239 clinically suspected cases of mucormycosis

Direct microscopy **POSITIVE** for broad aseptate hyphae
180/297 samples, 60.6%

Direct microscopy **NEGATIVE** for broad aseptate hyphae
117/297 samples, 39.4%

Culture positive
42/180, 23.3%

Mucorales specific
PCR positive
178/180, 98.8%

Culture positive
0/117

Mucorales specific
PCR positive
37/117, 31.6%

Total samples: 297

Lane 1: Tissue Biopsy (sample 1)
Lane 2: Tissue Biopsy (sample 2)
Lane 3: Tissue Biopsy (sample 3)
Lane 4: Tissue Biopsy (sample 4)
Lane 5: Tissue Biopsy (sample 5)
Lane 6: Tissue Biopsy (sample 6)
Lane 7: Tissue Biopsy (sample 7)
Lane 8: Tissue Biopsy (sample 8)
Lane 9: Tissue Biopsy (sample 9)
Lane 10: Positive control
Lane 11: Tissue Biopsy (sample 11)
Lane 12: Tissue Biopsy (sample 12)
Lane 13: 100 bp DNA Ladder

Fig 1: Gel electrophoresis of internal PCR product of 18S rDNA of *mucorales* from tissue samples

Lane 1-5 : Tissue samples positive for *Mucorales* fungi
Lane 6 : Blank
Lane 7-11: Tissue samples Negative for *Mucorales* fungi
Lane 12 : Negative Control
Lane 13 : 100 bp DNA Ladder

Fig 2: Gel electrophoresis of PCR for human beta globin gene

CONCLUSIONS

- Mucorales* specific PCR (178+37= 215/297, 72.4%) proved to be more useful over the conventional methods as an additional 37 (31.6%) cases of mucormycosis were diagnosed with the former.

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