Detection and identification of mucormycosis agents in native and formalin-fixed paraffin-embedded tissue samples of patients with mycosis using multiplex real time PCR

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Purpose

The aim of the study was to test a multiplex real time PCR with High Resolution Melt analysis (mHRM-RT-PCR) on clinical samples for detection and identification of Mucormycetes spp. in tissue samples.

Methods

The study included 8 native and 22 formalin-fixed paraffin-embedded tissue samples from 25 patients with mucormycosis in Saint-Petersburg between 2013 and 2021 yy. As controls, 21 tissue samples were collected from patients without mycoses. We investigated the native tissue samples by direct microscopy method with calcofluor white. Fungal cultures were obtained by sample inoculation on Sabouraud glucose agar. Histological sections of tissue samples were stained by PAS and Grocott - Gomori's technique. Fungal DNA was extracted from clinical samples by a chloroform-isooamyl extraction method. DNA amplification was performed using Aspergillus - and Mucormycetes - specific primers pairs separately and EvaGreen based mHRM-RT-PCR on Rotor-Gene 6000 cycler.

Results

The mHRM-RT-PCR allows to identify in clinical samples from patients with aspergillosis and mucormycosis the representatives of Aspergillus to the genus and Mucormycetes to the species level: Rhizopus arrhizus, Rhizopus microsporus, Mucor racemosus, Rhizomucor pusillus, Lichtheimia corymbifera (Fig.1).

In patients with mucormycosis direct microscopy of 8 native tissue samples was positive in 75% cases. Lichtheimia corymbifera was isolated in 12,5% and Rhizopus arrhizus - in 37,5% cases.

Conclusions

The multiplex RT-PCR has high sensitivity and specificity in patients with mucormycosis. This study indicated that the mHRM-RT-PCR may be a very useful tool for detection of etiologic agents of mycoses, particularly in the case of a mixed infection caused by Aspergillus spp. and of the order Mucorales.