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Multiplex Real Time PCR for detection and identification of *Aspergillus* and *Mucormycetes* spp. in native and formalin-fixed paraffin-embedded tissue samples of patients with mycosis

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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 simultaneous detection and identification of *Aspergillus* and *Mucormycetes* spp. in tissue samples.

Methods

The study included 44 native and formalin-fixed paraffin-embedded tissue samples from 34 patients with aspergillosis and 12 clinical samples from 10 patients with mucormycosis in Saint-Petersburg between 2013 and 2019 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-isoamyl 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, Mucor racemosus, Rhizomucor pusillus, Lichtheimia corymbifera* (Fig.1).

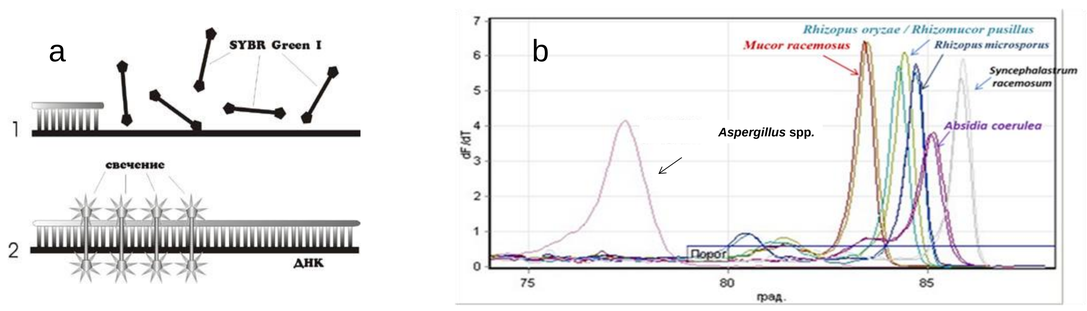


Fig.1. a: Real time PCR with use SYBR GREEN;

b: Melting temperature of PCR - products: Tm *Aspergillus sp.* - 76 - 79° ,

Tm *Mucormycetes* - small thermal spices – 81 – 82,5° , big - 84 – 87°.

The study included 56 biological specimen: tissue from sinuses - 13, lungs - 25 , central nervous system - 4, bowel - 4, heart - 1, spleen - 1, liver - 6, kidney - 1 and omentum -1. In patients with aspergillosis direct microscopy of 15 native tissue samples was positive in 80% cases. *Aspergillus fumigatus* was isolated in 80% and *Aspergillus flavus* - in 20% cases.

Results

Only in 1 of 3 native tissue samples of patients with mucormycosis and positive direct microscopy *Lichtheimia corymbifera* was isolated. PCR assay was positive in native and formalin-fixed paraffin-embedded tissue samples of 97, 0% patients with IA and 100% patients with mucormycosis. mHRM-RT-PCR allowed to identify the representatives of mucormycetes: *Lichtheimia corymbifera* in 6 and *Rhizomucor pusillus* in 3, *Rhizopus microsporus* in 1 from 12 samples. In biological specimens of 2 patients the PCR assay detected a mixed infection by *Aspergillus* and *Mucormycetes* spp.*: Aspergillus* spp.+ *Rhizopus microsporus* and *Aspergillus* spp. + *Rhizopus arrhizus*. The positive results of PCR assay in patients with aspergillosis in 95% and mucormycosis 100% of cases correlated with traditional methods. In 21 control tissue samples PCR test was negative.

<u>Example 1.</u>The investigation of the autopsy material of liver tissue from patient T. with non-Hodgkin's lymphoma and mixed mycosis.

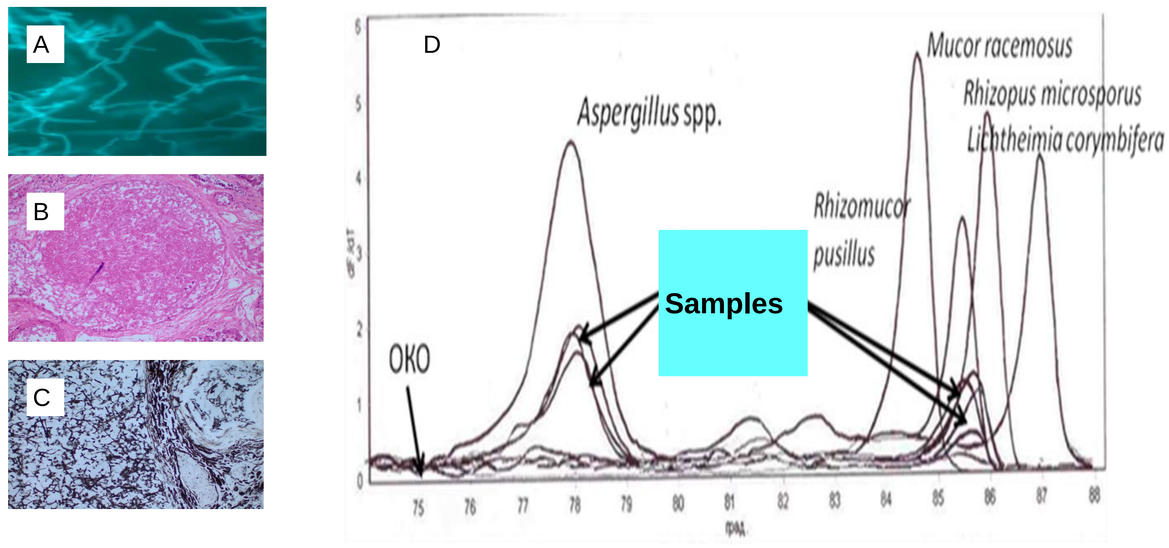


Fig.2 . A-direct microscopy: non-septate mycelium; Histologic study (B - PAS, C –Gomori – Grocott) - non-septate mycelium; D –mHRM-RT-PCR –revealed DNA *Aspergillus spp.*+ *Rhizopus microsporus.*

<u>Example 2.</u>The investigation of the biopsy material of intestine and brain tissue from patient G. with leukemia and mucormycosis.

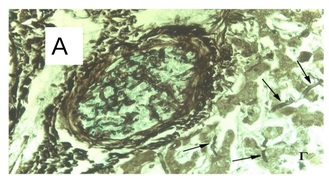
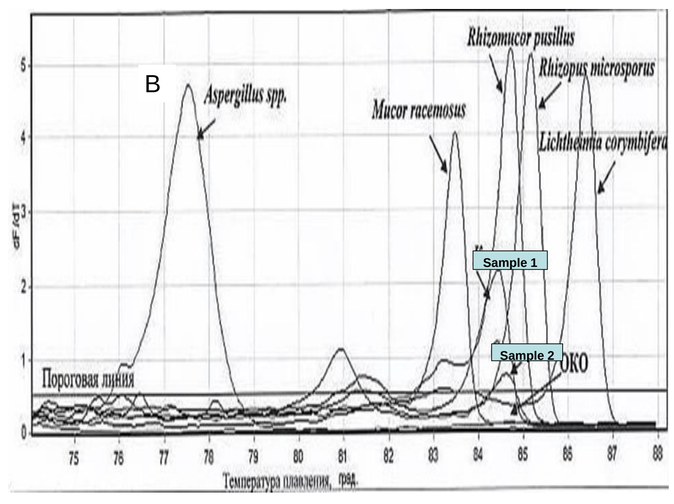


Fig.3 . A – histologic study – non-septate mycelium;

B –mHRM-RT-PCR –revealed DNA *Rhizomucor pusillus:*

Sample 1-intestine

Sample 2 - omentum



Conclusions

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

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