Evaluation of diagnostic potential of recombinant secretory asparaginase proteinase 2 (rSap2) protein from Candida parapsilosis for in-use systemic candidiasis

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Objective: Systemic candidiasis is the fourth most common bloodstream infection in EU patients worldwide. Along with Candida albicans, infections caused by non-albicans Candida (NAC) species are increasingly becoming more prevalent globally along with the emergence of drug resistance. The diagnosis of systemic candidiasis is difficult due to the absence of significant clinical symptoms in patients. Since conventional diagnostics methods for candidiasis show less sensitivity and specificity, novel immunodiagnostic techniques are needed for early diagnosis. We investigated the diagnostic potential of recombinant secretory asparaginase proteinase 2 (rSap2) from C. parapsilosis for the detection of Candida infection.

Methods: Genomic DNA was isolated from C. parapsilosis, followed by PCR amplification of sap2 gene using degenerate gene-specific primers. Sap2 protein expression and purification was performed using Ni-NTA affinity chromatography under denaturing conditions. The purified protein was subsequently refolded in a multi-step dialysis procedure. CD and FTIR studies were performed to confirm refolding. Assays were immunized with rSap2 protein and serum ELISA assays were performed for testing immunogenicity. Immunoblotting assays and human serum ELISAs were performed using whole-cell Candida and rSap2-protein in proven systemic candidiasis patient serum and controls, recruited in PGIMER, Chandigarh.

Results: Sap2 protein from C. parapsilosis was successfully cloned and expressed using an E. coli-based prokaryotic expression system. Protein refolding was performed in vitro using step-wise dialysis. Structural analysis by CD and FTIR spectroscopy revealed the refolded protein to be in its native tertiary conformation. Immunogenicity analysis demonstrated the rSap2 protein to be highly immunogenic as evident from significantly high titers of IgG-specific antibodies in antibodies-immunized BALB/c mice, compared to sham-immunized controls. The diagnostic potential of rSap2 protein was evaluated using immunoblotting and ELISA assays using serum from proven systemic candidiasis patients and controls. Our immunoblotting results demonstrate that the recombinant Sap2 protein was recognized as a single band of ~41 kDa by systemic candidiasis patient serum samples and no cross-reactivity was observed in healthy controls. On evaluating the diagnostic potential of the rSap2 antigen using an ELISA-based approach, our results show that anti-Sap2 IgG and IgM antibodies could be detected in the sera of proven candidiasis patients. Of note, the differences in Sap2 antibody titers observed amongst patients and controls were similar to the serological response observed when heat-killed whole-cell Candida was used as a coating antigen.

Conclusions: In summary, the rSap2 protein from C. parapsilosis has the potential to be used in the diagnosis of systemic candidiasis, providing a rapid, convenient, accurate, and cost-effective strategy. Our results indicate that the rSap2 protein from C. parapsilosis can be used to detect and diagnose systemic candidiasis infection in human patients and can be used as an alternative/replication of whole-cell Candida-based ELISA procedures, which are currently in use.

Clinical utility of semi-nested conventional PCR for diagnosis of mucormycosis in fresh clinical samples

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Objective: Our aim was to assess the clinical utility of the semi-nested conventional PCR in smear positive, culture-negative clinical samples for diagnosis of mucormycosis.

Methods: This prospective study was conducted for a period of 3 months (April-June 2021). A total of 218 clinical samples were included from patients in whom smear was positive for aspergillus hyphae, but the culture failed to grow within 2 days of incubation or smear was negative but had high suspicion of mucormycosis. Molecular diagnosis was attempted using semi-nested PCR with Mucorales-specific primers targeting 18S rRNA, described previously by Bhale et al.1. Fisher's exact test was performed to find significant differences in the PCR positivity between the samples. The overall sensitivity and specificity of the PCR protocol were calculated.

Results: Among 218 patients with suspected mucormycosis included in this study, the major organisms were rhino-orbito-cerebral mucormycosis (ROCM), (77.7%, n = 169), followed by pulmonary mucormycosis (19.2%, n = 42), cutaneous (6.2%, n = 4), and gastro-intestinal (GI) mucormycosis (0.9%, n = 3). In 24 samples, the presence of both aspergillus and aspergillus hyphae was seen under microscopic examination raising the possibility of mixed infection. On microscopic examination, 90.5% samples (197/218) had aspergillus hyphae while the remaining samples were smear-negative but had strong clinical suspicion of mucormycosis. The molecular technique was able to identify causative agent in all culture-negative samples (95.8%, 158/165) and 52.4% (11/21) in smear-negative cases. Among 218 patients, only 20 samples show delayed growth of Mucorales, and on comparison with molecular results 100% concordance was observed. In 218 patients with strong clinical suspicion for mucormycosis, 23/24 patients with both conventional and molecular methods. The low culture positivity necessitates the molecular diagnosis based on semi-nested PCR using above-mentioned primers followed by longer sequencing. In the case of 24 samples with-smear-negative but-potential mucormycosis samples, PCR correctly identified 23/24 (95.8%) Mucorales as a causative agent. The overall turn-around-time from the sample receiving to diagnosis was ~48 h. Overall, R. arrhizus (83/143), 5.9% were most commonly associated with ROCM, while R. microsporus (15/198, 7.6%) and R. homothallicus (5/58, 8.6%) were seen mainly with pulmonary mucormycosis. Atypical/phycomycosis and Saksenaea genus were associated with GI and cutaneous mucormycoses.

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