Detection and Identification of Fungi causing Invasive Fungal Infections (IFI) in Fixed Formalin Paraffin Embedded Tissue (FFPE) Samples Using Polymerase Chain Reaction (PCR)

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INTRODUCTION: One of the greatest challenge in the management of invasive fungal infection (IFI) is making accurate diagnosis in a timely manner. Conventional methods are not sensitive enough and time consuming. Various non-culture methods have been used to augment accurate identifications of invasive fungal pathogens including employing PCR techniques to detect the internal transcribed spacer (ITS) rRNA genes of pathogenic fungi.

Aim: This study was conducted to evaluate PCR and DNA sequencing as a method for detection and identification of fungal pathogens from formalin fixed paraffin embedded (FFPE) tissue samples.

METHOD: Fresh and archived FFPE tissues (2014-2017) reported as fungal infections or granulomatous lesions were evaluated. A pan-fungal PCR assay was optimised and used to detect fungal DNA from the FFPE tissues. Following treatment with deparafinisation solution, DNA was extracted using the QIAamp DNA FFPE Tissue Kit. Polymerase chain reaction was performed using 2 universal primer sets ITS1/ITS2 and ITS3/ITS4 that amplified the Internal Transcribed Spacer regions of the rRNA genes ITS1 and ITS2 respectively. Amplified PCR products were purified and sequenced. The DNA sequences obtained were analysed and compared with nucleotide sequences in the NCBI Databases.

RESULT and DISCUSSION:

A total of 78 samples from were collected from the Histopathology Laboratory, Hospital Kuala Lumpur (HKL) for this study. Positive PCR were obtained from 63 (80.7%) samples. Summary of the positive results are as shown in Table 1.

The common fungi identified were *Sporothrix schenkii* (15.9%) *Penicillium marneffei* (11.1%), *Candida albicans* (7.9%), *Rhizopus* spp. (7.9%), *Trametes polyzona* (6.3%), and *Aspergillus* spp. (6.3%). Besides these, several interesting findings were revealed which included opportunistic infections due to certain environmental fungi which are better recognised as plant pathogens and edible fungi. Some examples were infections caused by *Schizophyllum commune* (2 cases), *Coprinopsis cenerea* (1 case) and *Peniophora* species (1 case).

The hallmark of invasive fungal infections is the presence of fungal elements in deep-tissue sections associated with tissue damages. The exact identification of fungi present in the tissue is often not possible because fresh tissue specimens are not always available for culture, and when available, cultures often fail to grow fungal pathogens. Histopathological examination based on their tissue microscopic morphology is often inconclusive due to low discrimination power and high error rates. Exact identification is almost impossible in most cases as demonstrated in this study.

Patients with invasive infections need timely targeted antifungal therapy. The choice of antifungal drugs is largely dependent on the classification of fungi because different fungi are treated with different antifungal drugs. Investigators have attempted to use molecular approaches to identify fungi present in FFPE tissues to the correct genus/species level. Molecular methods included pan-fungal PCR and DNA sequencing, real-time PCR assay and *in situ* hybridization using peptide nucleic acid probes have been used.

In this study, by employing a simple PCR sequencing assay using panfungal primers, we found that 89.7% of the samples identified as fungal infections by HPE were also identified as fungal infections by PCR. In addition, PCR was able to identify causative agents to genus/species level. For samples identified only as granulomatous tissue on HPE, PCR was able to identify 35.3% of them which could be an added benefit in management of patients. The quality of fungal DNA extracted from FFPE tissues may not always be optimal, as degradation has been observed. The samples from 2014 showed the least positive results (37%) compared to a 100% identification for 2017 and 2018 samples.

CONCLUSION: This study revealed that the Polymerase Chain Reaction (PCR) amplifying the Internal Transcribed Spacer (ITS) gene sequences can be used for accurate identification of fungi causing invasive and opportunistic infections from FFPE tissue sample and may provide useful information for better management of IFIs.

No. cases	Ages (yrs)	Site samples	Histopatholy Reports	PCR-sequencing identification	Underlying diseases
10 cases	17-73	Skin over leg, arms or leg	Granulomatous tissue, eosinophilic infiltrations	<i>Sporothrix schenkii</i>	No significant underlying disease
7 cases	28-43	Skin at Cheek, face, body	Features suggestive of histoplasmosis/pe nicilliosis / granulomatous tissue	<i>Penicillium marneffei</i>	Pulmonary tuberculosis (PTB) Retroviral Disease (RVD)
5	45-73	Esophagus /bronchial biopsy, skin	Fungal infections, candidiasis	<i>Candida albicans</i>	RVD, Diabetes(D M), cancer
4	2-59	Skin, nasal, maxillary	Granulomatous tissue, suggestive of mucor infection	<i>Trametes polyzona</i>	NP Carcinoma, DM
5 cases	58-72	Adrenal (1), sinus,	Chronic sinusitis, disseminated fungal infection	<i>Rhizopus oryzae</i> (3), <i>R. delamar</i> (1), <i>R. microspores</i> (1)	DM in 4 cases, Aplastic anemia (1)
4 cases	26-47	Bronchial, lung, brain	Fungal infection, Fungal granuloma	<i>Aspergillus flavus</i> (2), <i>A fumigatus</i> , <i>A oryzae</i> (2)	DM, PTB, leukaemia
3 cases	33-53	Lymph node, bronchial, adrenal	Fungal infection, granuloma, suggestive of histoplasma	<i>Histoplasma capsulatum</i>	RVD
2 cases	8-32	Lung, abdomen	Lung abscess, chronic fibrosis	<i>Schizophyllum commune</i>	Jobs syndr, foreign body
3 cases	23-53	Skin	Fungal infections	<i>Malassezia restricta</i> , <i>M globosa</i>	N/A
20 cases	33-72	Various tissues: lung, nasal, pleura, skin	Chronic inflammation, Chronic granuloma,	<i>C. glabrata</i> (2), <i>Paracorniothyrium</i> (2), <i>Cladosporium</i> (1), <i>Alternaria</i> (1), <i>Curvularia</i> (1), <i>Fusarium</i> sp.(1), <i>Coprinopsis cinerea</i> (1), <i>Pericornia byssoides</i> (1), <i>Readeriella sp.</i> (1), <i>Rhodotorula sp</i> (3), <i>Peniophora sp</i> (1), <i>Polymyxa graminis</i> (1), <i>others</i> (4)	DM, Leukemia, TB, RVD, and others

References:

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