Cutaneous fungal infections in elderly population from Bhopal

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Objective:
1. To know the distribution of fungal infections based on various demographic characteristics.
2. To study the clinical presentation of the lesions and their distribution.
3. To isolate and identify the fungal pathogens.

Methods: Hospital-based cross-sectional study for 5-year duration January 2017-December 2021. Study location is the department of Dermatology, AIIMS, Bhopal. Elderly patients (60 years and above) visiting as Outpatient and Inpatient of dermatology and venereology, General Medicine, and other departments with cutaneous infections suspected to be of fungal etiology and fulfilling the inclusion criteria comprised the study population. Patients already on antifungal topical or systemic were excluded. Data extraction was based on predesigned proformas for a detailed history and clinical examination criteria. Necessary ethical approval and patient consent were obtained. Samples of skin, hair, nail, and exudates were processed for direct microscopy, culture isolation in satellite media, and identification photo cytically.

Results: A total of 480 elderly patients clinically suspected of cutaneous fungal infections were included in the study. Majority of the suspected cases were in 60-70 years age group 364/480 (75%). Males were 349/480 (72.71%) and females were 131/480 (27.29%) of the total suspected cases. Male to female ratio in study population was 2.66:1. Three conidia 140/480 cases (29.37%) was most common clinical type followed by rhynchospora 64/480 cases (13.55%), ellaria 62/480 (12.92%), C. nardiflavus 56/480 (11.47%), T. purpurea 31/480 (7.28%), T. marneffei 25/480 (5.21%), and T. farciminosus 12/480 (2.50%) in suspected cases of cutaneous fungal infections. Majority of cases were found in non-dependent 253/480 (52.70%) population. Out of 480 clinically suspected cases of cutaneous fungal infection was documented in 193 cases (40.20%) either by direct microscopy and/or culture.

A total of 1764800 cases (36.47%) were KOH positive and 1121534 cases were culture positive.

Elding culture as a gold standard sensitivity and specificity of KOH in diagnosing fungal infection was 84.96% and 74.22% respectively. Among 153 culture isolates dermatophytes 53.10% (80/153) were most common mold isolates followed by non-dermatophytes molds 28.31% (52/153), and yeasts 15.95% (23/153). Trihydroxypropyl nimotopoglyres most common 21.24% followed by T. mannus 9.77%, and T. violaceum 7.69% are the common dermatophyte isolates. Aspergillus species is the most common non-dermatophyte mold isolated.

Diabetes was the most common comorbidity condition in culture-confirmed cases followed by hypertension and thyroid disorders.

Conclusion: The study showed the prevalence of cutaneous fungal infection among elderly visiting AIIMS, Bhopal as 25.54% (113/480). With increase in elderly population, changing environmental conditions, and association with non-commensal disease it becomes important that all elderly patients visiting hospital OPD and those hospitalized for long should be evaluated for fungal infections especially concomitant.

Poster Presentations

PT18

Pentraxin-3 interacts with Aspergillus fumigatus conidia to regulate pro-inflammatory cytokine production

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Objective: Long pentraxin-3 (PTX3)- isoform pattern-recognition receptor secreted by phagocytes and non-immune cells at sites of inflammation. It has been reported to have a non-cellular role in the immune response against Aspergillus fumigatus. Indeed, PTX3 knock-out mice show an increased susceptibility to invasive pulmonary aspergillosis (IPA) with a higher mortality rate. In humans, PTX3 genetic deficiency or single nucleotide polymorphism has also been associated with an increased role of IPA. However, the way in which PTX3 interacts with A. fumigatus and its mechanism of action has yet to be elucidated. The aim of the study was to investigate potential A. fumigatus ligands for PTX3 and the impact of A. fumigatus recognition by PTX3 on modulating the immune response.

Methods: Aspergillus fumigatus conidia, the infective morphotype, were incubated with PTX3 with or without human serum, stained with anti-PTX3 antibody, and studied by immunofluorescence. Identification of potential fungal ligands for
PTX3 was performed by ELISA. Final control and germinated control were spotted with different serum factors and co- incubated with antigen preparations for 24 h at 24°C. Immunoblot and immunoprecipitation were performed using anti-MAP2 antibody (FMP2) and anti-PTX3 antibody, respectively. Western blot and immunoprecipitation were performed with a 24°C incubation.

Results: PTX3 did not help A. fumigatus control directly but in the presence of human serum, purified colistin (0.1 mg/ml), and complement proteins (C5). Pre-incubation of control with these components produced a significant increase in PTX3 stimulated control and significantly reduced anti-microbial activity of PTX3. Pre-inoculated PTX3-fa germinated control significantly reduced pro-inflammatory cytokine and increased anti-inflammatory cytokine from A. fumigatus (Fig. 1).

Conclusion: PTX3 is an acute phase protein expressed in response to pro-inflammatory stimuli during infection and that is increased in bronchoalveolar lavage of patients with aspergillosis. Our recent data with A. fumigatus suggest that PTX3 is an immunosuppressive protein that reduces pro-inflammatory response. Although an inflammatory response is necessary to fight against fungal pathogens, the tissue damage associated with enhanced inflammation can be detrimental and facilitates A. fumigatus infection.

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Effect of corticosteroids on the host innate immune responses and in vitro growth characteristics during dermatophyte infection.

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Objective: During the current epidemic of dermatomycoses, dermatologists in India are noticing atypical-clinical presentations of dermatophytosis. Though fixed drug combinations of topical application containing corticosteroids-antifungal antibacterial drugs are attributed to this phenomenon, it is still not clear about its exact role. Corticosteroids alluring but not clear off dermatophytosis infections from the skin surfaces, which may lead to a collapse of dermatophytosis. Therefore, we analyzed the effect of corticosteroids on host immune response and pathology in vitro during dermatophyte infection.

Methods: Pathogens (n = 5) were incubated in three groups: three groups: patients’ samples of dermatomycosis with a history of corticosteroid usage for > 30 days (Group A), dermatomycosis with no history of corticosteroid usage for > 30 days (Group B) and without patients and dermatophytosis and expected to have normal skin (Group C). Skin biopsies were collected and subjected to molecular-electron microscopy (SEM) and cytokine expression analysis. All in vitro experiments were performed with T. massee Keratinocytes cell line co-cultured with T. massee dermatophytes complex isolated from clinical specimens of dermatomycosis (n = 4) and standard strain (n = 1, ATCC 1846). Biopsies were fixed in 2.5% gluteraldehyde and dehydrated through (50%) ethanol gradient. R.T.P.C. expression of pro-inflammatory cytokines (IL-6, TNF-α, IL-12, IL-6, IFN-γ, IL-1B, and TGF-β1) from skin biopsies and HAT-gf cells were conducted using beta-actin as reference gene. The viability and cell-cycle analysis of HAT-gf cells in the presence and absence of diborated phosphate (0.05%) was performed by MTT assay and Annexin V (AV) staining in flow cytometry, respectively. Growth kinetics of dermatophytosis was performed for 96 h in presence and absence of corticosteroid. Expression of sulfite efflux pump gene mru (sulf) and pH response gene (paCl), involved in resistance of T. massee dermatophytes complex clinical isolates from classical and atypical lesions (n = 3) as well as standard strain (n = 1, ATCC 1846) was studied by RT-PCR. All results were statistically analyzed using Graph Pad Prism 6 software.

Results: Our observations revealed that T. massee dermatophytes complex isolated from clinical specimens of dermatomycosis (n = 4) and standard strain (n = 1, ATCC 1846) were found to be susceptible to the antifungal drugs when tested against the clinical isolates. These findings are in agreement with previous studies that reported the susceptibility of dermatophytes isolates to the antifungal drugs.

Conclusion: Increased atopy caused by corticosteroids allows dermatophytosis to thrive on the intact keratin and when proinflammatory cytokines are neutralized, exposed keratinocytes can trigger cytokine- and/or chemotactic chemoattractants for neutrophils and eosinophils that are involved in dermal clearance of dermatophyte infection from skin. Reduced growth of dermatophytosis in the presence of corticosteroids and upregulation of sulfite efflux pump expression in dermatomycosis when co-cultured with keratinocytes and corticosteroids correlates with recurrent infection. In addition, increased production of sulfite ions that degrade keratin may lead to the formation of widespread lesions.

P120
Myeloid-derived suppressor cells as a potential biomarker and therapeutic target in rhino-orbital mucormycosis patients.

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Objective: Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of myeloid cells that include immature myeloid cells and are important in inhibiting the anti-tumor immune response. MDSCs are increased in various inflammatory and infectious conditions, and are associated with a poor prognosis and a decreased response to immunotherapy. In the present study, we aimed to investigate the role of MDSCs in the pathogenesis of rhino-orbital mucormycosis.

Materials and Methods: Peripheral blood samples were collected from 50 patients with rhino-orbital mucormycosis and 50 healthy controls. The percentage of MDSCs was determined by flow cytometry using the following antibodies: CD14, CD16, CD33, CD66b, and CD15. The data were analyzed using the GraphPad Prism 6 software.

Results: The percentage of MDSCs in the patient group was significantly higher (P < 0.05) compared to the control group. The percentage of MDSCs was positively correlated with the severity of the disease, as assessed by the Clinical and Erythema Scores.

Conclusion: Myeloid-derived suppressor cells may play a role in the pathogenesis of rhino-orbital mucormycosis and could be a potential biomarker and therapeutic target in this disease.