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Cutaneous fungal infections in elderly population from Bhopal

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Objectives:

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 Microbiology 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 antifungals topical or systemic were excluded. Data extraction was based on predesigned proforma for a detailed history and clinical examination entries. Necessary ethical approval and patient consent were obtained. Samples of skin, hair, nail, and exudates were processed for direct microscopy, culture isolation in suitable media, and identification phenotypically.

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 360/480 (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. *Tinea corporis* 140/480 cases (29.17%) was most common clinical type followed by onychomycosis 64/480 cases (13.33%), ulcerations 62/480 (12.92%), *T. cruris* 56/480 (11.67%), *T. pedis* 35/480 (7.29%), *T. mannum* 25/480 (5.21%), and *T. facei* 12/480 (2.50%) in suspected cases of cutaneous fungal infection. Majority of cases were found in non-dependent 253/480 (52.70%) population. Out of 480 clinically suspected cases of cutaneous fungal infection was demonstrated in 193 cases (40.20%) either by direct microscopy and/or culture.

A total of 176/480 cases (36.67%) were KOH positive and 113(23.54%) cases were culture positive.

Taking culture as a gold standard sensitivity and specificity of KOH in diagnosing fungal infection was 84.96% and 78.2% respectively. Among 113 culture isolates dermatophytes 53.10% (60/113) were most common mold isolates followed by non-dermatophyte molds 28.3% (32/113), and yeasts 18.59% (21/113). *Trichophyton* mentagrophytes most common 21.24% followed by *T. tonsurans* 9.73%, and *T. violaceum* 7.96% are the common dermatophyte isolates. *Aspergillus* species is the most common non-dermatophyte mold isolated.

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

Conclusion: This study showed the prevalence of cutaneous fungal infection among elderly visiting AIIMS, Bhopal as 23.54% (113/480). With increase in elderly population, changing environmental conditions, and association with non-communicable diseases it becomes important that all elderly patients visiting hospital OPD and those hospitalized for long should be evaluated for fungal infections especially cutaneous.

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

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Objectives: Long pentraxin-3 (PTX3) is a soluble pattern-recognition receptor secreted by phagocytes and non-immune cells at sites of inflammation. It has been reported to have a nonredundant 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 risk 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* opsonization 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

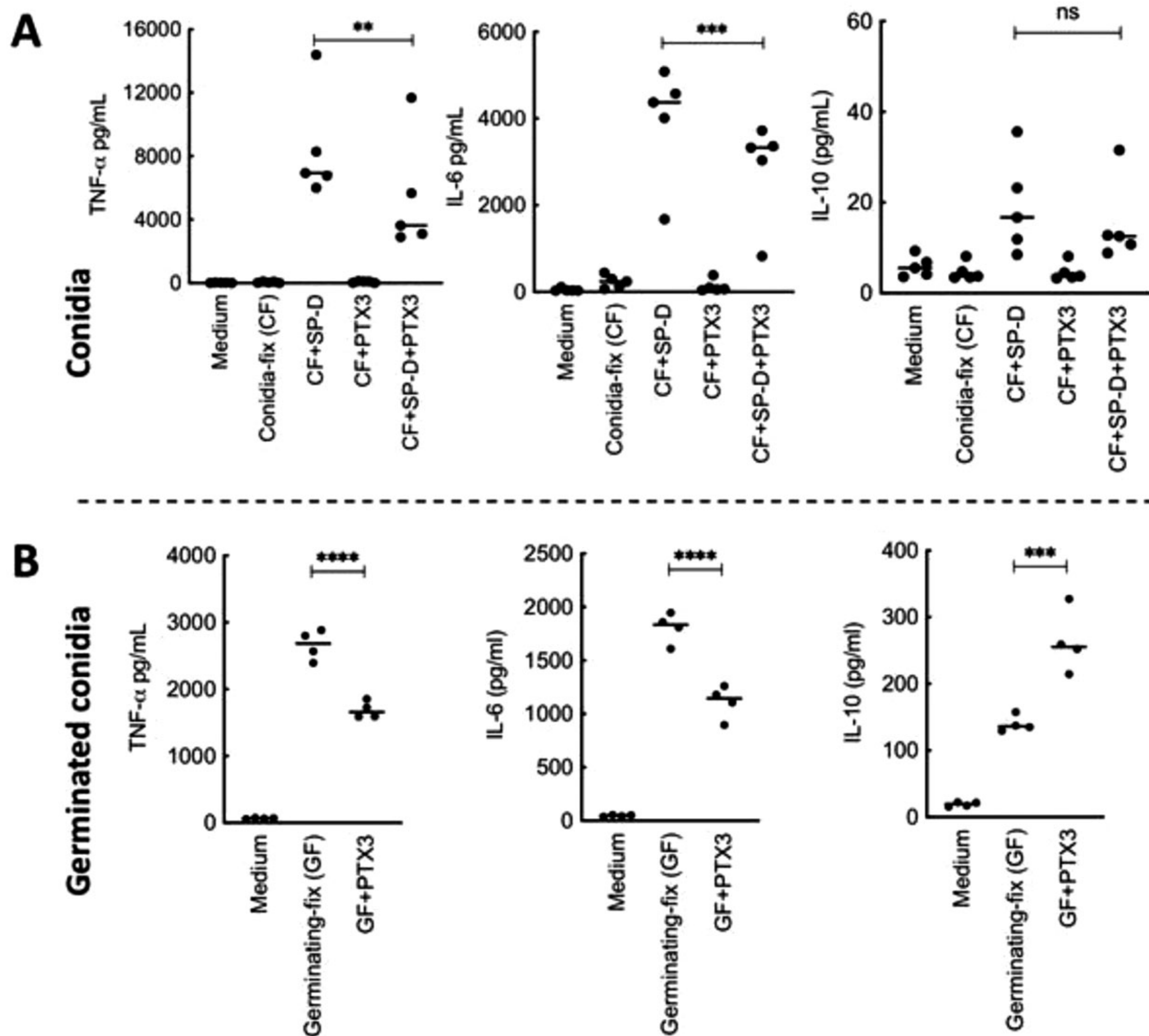

Figure 1. (A) Human monocyte derived macrophages (hMDM) cytokine secretion when stimulated with SP-D, PTX3 or SP-D+PTX3 opsonized PFA-fixed conidia (B) hMDM cytokine secretion when stimulated with PFA-fixed and PTX3 opsonized germinating conidia (***$p < 0.001$; ****$p < 0.0001$; ns-non-significant).

PTX3 was performed by ELISA. Fixed conidia and germinated conidia were opsonized with different serum factors and co-incubated with human monocyte-derived macrophages (hMDM) for 24 h at 37°C. Culture supernatants were collected, and pro-/anti-inflammatory cytokines were measured by sandwich ELISA.

Results: PTX3 did not bind *A. fumigatus* conidia directly but in the presence of human serum, purified collectins [surfactant protein D (SP-D) or C1q], and complement products (C3b). Pre-opsonization of conidia with these complement proteins or SP-D stimulated proinflammatory cytokine secretion by hMDM upon interaction (Fig. 1a). In contrast, secondary opsonization of complement proteins or SP-D opsonized conidia with PTX3 significantly reduced pro-inflammatory cytokines and increased anti-inflammatory cytokine secretion from hMDM. PTX3 opsonized PFA-fixed germinating conidia significantly reduced pro-inflammatory cytokine and increased anti-inflammatory cytokines secretion from hMDM (Fig. 1b).

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 immunoregulatory 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 deleterious and facilitate *A. fumigatus* infection.

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Dysregulated IL-23R and CD25 T cells in post -COVID-19 Rhino-Orbital Mucormycosis patients

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Objectives: To study the IL-23R (Th17) and CD25+ (Treg) in CD4 + T cell populations in rhino-orbital mucormycosis post-COVID-19 patients and in healthy controls.

Methods: The study included 20 cases of mucormycosis and 20 healthy controls. Nasal crust, collected post-surgery was subjected to KOH/culture for mycological identification. Venous blood sample (3 ml) was collected in EDTA vials from cases and controls and stained with different monoclonal antibodies such as CD3, CD4, CD25, and IL-23R for analyzing the expression of Th17 and Treg cells by flow cytometry. The assays were performed at the time of enrolment of patients and repeat blood samples were taken from each patient for staining 3 months later after treatment prescribed by Otorhinolaryngologists. Statistical analysis was done using SPSS software and the *P*-value ≤ .05 considered as significant. All the data are expressed as the mean ± SD.

Results: All the cases were found positive by KOH and confirmed for *Rhizopus arrhizus* by culture.

The flow cytometry analysis showed that the percentage of CD4 + IL-23R+ (Th17) cells was significantly high in patient before treatment compared to healthy controls and found to be lower post 3 months of antifungal treatment. The percentage positivity of CD4 + CD25+ (Tregs) cells was decreased in patients (before treatment) as compared to controls and after treatment groups. The percentage positivity of CD4 + CD25 + cells was significantly increased in patients after treatment.

Conclusion: We observed a noticeable immune imbalance, with elevated CD4 + IL-23R Th17 and diminished CD4 + CD25 + T regulatory cells. The findings imminently indicate the mechanism of immune dysregulation involving Th17 and Treg pathways in mucormycosis and provide evidence that restoration of Th17/Treg may be considered as a therapeutic option for long-term benefit. Recovery of CD4 + CD25 + T cells after treatment indicated a favorable phenotype outcome.

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Vaccine induced protection by secreted aspartyl proteinase 2 from *Candida parapsilosis* in *Candida tropicalis* mediated murine systemic candidiasis: a role of B-cells and antibodies

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Objectives: Non-*albicans Candida* species are increasingly becoming more prevalent globally and emergence of drug resistance is another cause of concern. These challenges merit the need for developing novel immune therapies against *Candida* infections. We investigated the protective potential of secreted aspartyl proteinase (Sap2), a leading Candidate vaccine antigen identified from research based on *C. albicans*, in murine systemic candidiasis caused by non-*albicans Candida* species. Moreover, while the role of cellular immune responses in anti-*Candida* immunity is well established, less is known about the role of humoral immunity against systemic candidiasis. As such, we also investigated the role of humoral immunity in vaccine-mediated protection during murine systemic candidiasis.

Methods: The Sap2 genes were successfully cloned and expressed as recombinant proteins from *C. albicans*, *C. tropicalis* and *C. parapsilosis* strains prevalent in India. Groups of wild-type BALb/c mice were vaccinated with individual rSap2 protein along with alum as adjuvant, followed by systemic infection with a lethal dose of *C. tropicalis*. The protective potential of each rSap2 protein was evaluated using survival analysis and estimation of organ fungal burden. Histopathological assessment was performed using H&E and PAS staining. Serum cytokine levels and antigen-specific antibody titers were measured by ELISA. Cellular responses were analyzed in detail using flow cytometry. Functional evaluation of antibody role was performed using *in vitro* (biofilm inhibition and neutrophil-mediated killing) and *in vivo* (passive transfer) studies. B-cell epitope analysis was carried out using immunoinformatics approaches.

Results: Mice vaccinated with rSap2 cloned from *C. parapsilosis* (Sap2-*parapsilosis*) showed highest increase in survival time ($P = .02$) and maximum reduction in organ fungal burden (spleen, kidney, lungs, brain) ($P < .05$); compared with sham immunized controls. Vaccination with rSap2 cloned from *C. albicans* did not improve survival in non-*albicans C. tropicalis* infection, despite the protein having ~60% homology across species. Mice vaccinated with rSap2-*parapsilosis* also exhibited significantly higher levels of IFN gamma, IL-17, and IL-4 cytokine levels just before infection, which correlated with protection. In addition, rSap2-*parapsilosis* vaccination induced high titers of Sap2-specific antibodies, and a fraction of antibodies could bind whole fungus (which were predominantly of IgM isotype). Notably, sera from rSap2-*parapsilosis* vaccinated mice exhibited increased *C. tropicalis* biofilm inhibition ability and enhanced neutrophil-mediated fungal killing in vitro. Sap2-specificity was further confirmed by depleting antigen-specific antibodies. Passive transfer of Sap2-*parapsilosis* immune serum significantly reduced fungal burdens in naive mice, as compared to mice receiving sham-immune serum, upon infection. Higher numbers of total CD19 + B-cells, plasma cells, and *Candida*-binding B-cells in rSap2-*parapsilosis* vaccinated mice indicate a role of B-cells during early stages of Sap2-mediated immune response. Epitope analysis performed using identified B-cell epitopes provides insights about including important IgM and IgG epitopes, when designing multivalent or multi-epitope anti-*Candida* vaccine/s.

Conclusion: Mice immunized with recombinant Sap2 cloned from *C. parapsilosis* exhibited increased survival during *C. tropicalis* mediated systemic candidiasis. Both Th1/Th17 mediated cellular immunity and humoral immunity contribute toward protection. Our results establish a role of B-cells and antibodies in generation of protective immune responses against Sap2 vaccine antigen during non-*albicans Candida* mediated murine systemic candidiasis.

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Effect of corticosteroids on the host innate immune response and *in vitro* growth characters during dermatophyte infection.

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

Methodology: Patients ($n = 15$) were recruited in three groups; proven cases of dermatophytosis with a history of corticosteroid usage for >30 days (Group A), dermatophytosis with no history of corticosteroid usage for >30 days (Group B), and patients without dermatophytosis and expected to have normal skin (Group C). Skin biopsies were collected and subjected to scanning electron microscopy (SEM) and cytokine expression study. All *in vitro* experiments were performed with HaCaT Keratinocytes cell line co-cultured with *Trichophyton* mentagrophytes complex conidia isolated from dermatophytosis ($n = 4$) and standard strain ($n = 1$, ATCC 18748). Biopsies were fixed in 2.5% glutaraldehyde and dehydrated through (50%-100%) ethanol gradient. RT-PCR expression of pro-inflammatory cytokines (IL-6, TNF-a, IL-1β, IL-1α, IFN-ϒ, TLR-2, and TLR-4) from skin biopsies and HaCaT cells were conducted using beta-actin as reference gene. The viability and Cell-cycle analysis of HaCaT cells in the presence and absence of clobetasol propionate (0.05% w/w) was performed by MTT assay and Propidium Iodide (PI) staining via flow cytometry, respectively. Growth kinetics of dermatophytes was performed for 96 h in presence and absence of corticosteroid. Expression of sulfite efflux pump gene (ssu1) and pH response gene (pacC), involved in virulence of *Trichophyton* mentagrophytes complex clinical isolates from classical and atypical lesions ($n = 3$ each) as well as standard ATCC 18748 was studied by RT-PCR. All results were statistically analyzed using GraphPad Prism 6 software.

Results: SEM results showed skin atrophy in skin biopsies from patients with steroid usage. Relative gene expression (2-Δct) of pro-inflammatory cytokines from skin biopsies was significantly reduced in IL-6, IL-1β, IFN-ϒ, TLR-2 (*P*-values = .001; .005; .004; .001) in steroid-modified tinea group. Similarly, a difference was observed in keratinocytes *in vitro*. According to *in vitro* analysis clobetasol propionate treatment significantly arrests HaCaT cells in the S/G2M phase (*P*-value = .04). Corticosteroid slows down the growth of dermatophytes in the presence of corticosteroid. A significant upregulation was observed in ssu1 during co-culture of dermatophytes with HaCaT cells as well when co-culture was treated with corticosteroid as compared to the culture alone whereas significant changes were not observed with pacC in similar conditions.

Conclusion: Increased atrophy caused by corticosteroids allows dermatophytes to thrive on the intact keratin when steroid pressure is removed. Reduced cytokine response, viability, and S-phase arrest in host correlate with delayed clearance of dermatophyte infection from skin. Delayed growth of dermatophytes in the presence of corticosteroids and upregulation of ssu1 in dermatophytes when co-cultured with keratinocytes and corticosteroid correlates with recurrent infection. In addition, increased production of sulfite ions that degrade keratin may lead to the formation of widespread lesions.

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

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Background: Mucormycosis is a deadly fungal infection that emerges in patients affected with COVID-19. All fungal illnesses are caused by dysregulated adaptive immunity, but Myeloid-derived suppressor cells (MDSC) have added a new dimension to the chronic inflammatory response.

Objective: We attempted to enumerate the MDSC immune response in rhino-orbital mucormycosis patients before and after treatment and compared the data with healthy control.

Methods: A total of 3 ml of blood samples were taken in an EDTA vial from 20 patients with mucormycosis and 20 age-matched healthy control. A second blood sample was collected to examine the immune system post three months of treatment. Mycological identification was performed on nasal crust retrieved after surgery using KOH/culture. The expression of the MDSC marker was analyzed by immunostaining with the antibodies against CD14, HLA-DR, CD11b, CD33, CD66 (Biolegend). Fluorescence profiles were recorded by Flow Cytometer (BD FACSAria^{TM} III) and analyzed by Flow Jo software (BD Biosciences). The percentage of positive cells is used to express the results. The GraphPad Prism (version 8, GraphPad software, LaJolla, CA, USA) was used to analyze the data. All of the results were considered significant when $P < .05$.

Results: All of the patients tested positive for *Rhizopus arrhizus*, which was confirmed by the culture. The percentages of Monocytic-MDSC (mMDSC: CD14 + HLA-DR-/low) cells were significantly high in patients compared to healthy control. In post-3-month treatment, the percentages of mMDSC were found significantly low and comparable with healthy control. Granulocytic MDSC (gMDSC: HLA-DR-/low CD33 + CD11b + CD66+) cell population was higher in patients compared with healthy control and patients with post-3-month treatment.

Conclusion: MDSC regulates T cells and other immune cells with a different mode of action. The findings in this study imminently indicate the mechanism of immune dysregulation involving MDSC pathways in mucormycosis and provide evidence that restoration of immune balance causes reduction of MDSC cells may be considered a therapeutic option for long-term benefit.