ORIGINAL RESEARCH PAPER

Pathology

KEY WORDS: deep mycosis, necrotizing inflammation, neutrophillic infiltrate

CLINICOPATHOLOGICAL SPECTRUM OF DEEP FUNGAL INFECTIONS: A TERTIARY CARE CENTER EXPERIENCE

Dr.Vijay Kumar Gupta Assistant Professor,MD,DCP,DMRD Department of Pathology Patna Medical College and Hospital Patna Bihar India

Dr.Pallavi Agrawal* Assistant Professor,MD,DNB,PDCC Department of Pathology Patna Medical College and Hospital Patna Bihar India *Corresponding Author

Dr.N.K.Bariar Head, Department of Pathology Head of the Department Department of Pathology Patna Medical College and Hospital Patna Bihar India

ABSTRACT

Background: The deep cutaneous mycosis involves the dermis and subcutaneous tissues and often spread to systemic organ systems. A histopathological examination is mandatory for definite diagnosis which helps in recognition of the fungus and early treatment of the infection avoiding dissemination and fatality in untreated infections

Aims: To study the clinicopathological spectrum of deep fungal infections

Methods: A total of 22 cases were studied in detail with a confirmed histopathologcal diagnosis of deep fungal infection. All the biopsies were fixed in 10% formalin and sent for histopathological examination. The biopsies were stained with hematoxylin and eosin (H and E). Special stains including periodic acid Schiff (PAS), and Grocott's methenamine silver (GMS) were done for identification of fungus. The histopathological and cytological features were noted.

Results: Among these cases, 18/22 (%) cases had clinical suspicion of fungal infection. The clinical symptoms varied from nodules, ulcerative plaque, pustule, to sinus tract formation. The dermis showed varied tissue reactions which included necrotizing inflammation, neutrophillic infiltrate, giant cell reaction, granulomas and lymphoplasmacytic infiltrate.

Conclusions: We conclude that PAS and Grocott's methenamine stains are essential in addition to a well fixed skin biopsy for H&E for a definite diagnosis of deep fungal infections.

Introduction

The primary cutaneous fungal infections are divided arbitrarily into the superficial and deep mycosis. The third group includes colonization of skin by superficial dermatophytes. The superficial fungi are localized in the keratin layer of the epidermis and follicles in the form of hyphae, pseudohyphae and sometimes yeast forms. There may be minimal tissue reaction or mild spongiosis to psoriasiform spongiosis along with mixed dermal inflammatory infiltrate in superficial cutaneous fungal infections. **[1]**

The deep mycosis on the contrary, involves the dermis and subcutaneous tissues, and often spread to systemic organ systems. They are usually acquired either via inhalation of fungal spores or by direct inoculation of the fungus into the skin. They may present primarily as cutaneous lesions in immunocompetent persons and show mixed dermal inflammatory cell infiltrate with mild fibrosis and pseudoepitheliomatous hyperplasia. **[2]** The immune status of the host plays an important role in modifying the cutaneous histologic reaction pattern in both superficial and deep mycosis.

The diagnosis of deep cutaneous mycosis mainly relies on histopathological examination and special stains which remains the gold standard for definite diagnosis. Early diagnosis and prompt recognition of the fungus helps in early treatment of the infection which avoids dissemination and fatality in untreated infections.

Materials and Methods

All skin biopsies were analysed retrospectively over a 2.5 year period from June 2015 to December 2017. The tissue biopsies were taken from representative lesions and fixed in 10% formalin, and sent for histopathology. The clinical details were obtained from the biopsy request forms. The biopsies were routinely processed and sections were stained with hematoxylin and eosin (H and E). Special stains including periodic acid Schiff (PAS), and Grocott's methenamine silver (GMS) were done for identification of fungus in all the cases. The histopathological features were noted in respect to epidermal changes, type of dermal inflammatory response, presence of necrotizing/non-necrotizing granulomas, giant cell reaction (GCR), angioinvasion and absence or presence of fungal profiles. Cytological details were also collected as and when available.

Results

A total of 22 cases were studied in detail with a confirmed histopathologcal diagnosis of deep fungal infection. Among these, 18/22 cases had clinical suspicion of fungal infection. The clinical symptoms varied from nodules, ulcerative plaque, pustule, to sinus tract formation. The clinical details, histopathological features and percentage of the lesions are given in **[Table 1].**

Table 1 Clinicopathological Spectrum of Deep Fungal Infections (n=22)

Fungal lesion (No.of cases)	Age	Sex M:F	Clinical presentation	Tissue Response
Mucormycosis (8/22)	8-66 years	3:1	Sinus tract Ulcer	Necrotizing inflammation, granuloma, angioinvasion, thrombi, vasculitis
Aspergillosis (9/22)	3-68 years	1:1	Ulcer Nodule Sinus	Neutrophillic infiltrate, vasculitis, thrombi
Rhinosporidiosis(3/22)	22-30 years	Male	Nodular swelling	Varying size globular cyst Sporangia, occasional fibrin thrombi
Histoplasmosis (2/22)	26-50 years	2.5:1	Nodule Patch	Hyperkeratosis, acanthosis, ulceration, histiocytic infiltration

None of the patients had any current or past history of debilitating diseases such as diabetes mellitus, tuberculosis or HIV infection to cause immunosuppression.The commonest fungus identified was mucormycosis 9/22 , aspergillosis 8/22 , histoplasmosis 2/22 and rhinosporidiosis 3/22 . Histomorphologically common tissue reactions observed were necrotizing inflammation, neutrophillic infiltrate, giant cell reaction, granulomas and lymphoplasmacytic infiltrate.

Majority patients with mucormycosis infection presented with ulcerated swelling and or sinus tract formation. Clinically mucormycosis was suspected in 6/9 cases with a differential diagnosis of necrotizing fasciitis, atypical mycobacterial infection,

pyoderma gangrenosum and herpes infection. Angioinvasion was observed in 2/9 cases, along with fungal vasculitis in 1/9 and fungal thrombi in 1/9 cases. **[Figure 1A&B]**

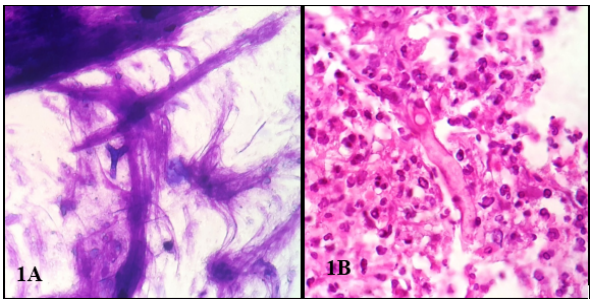

Figure 1A Crush smear showing fungal hyphae with broad, stout, aseptate, ribbon like, right angled hyphae (Giemsa 100x)

Figure 1B Section showing broad stout aseptate hyphae of mucormycosis with marked neurophillic infiltrate (H&E 100x)

Aspergillosis was identified in 8/22 cases with associated findings in the form of dermal neutrophillic infiltrate, granulomas, vasculitis and vascular thrombi. Unlike most deep cutaneous fungal infections, pseudoepitheliomatous epidermal hyperplasia was not a characteristic feature of cutaneous aspergillosis. The hyphae measured 2 to 4µm in diameter, often arranged in a radiate fashion, septate, and showed branching at an acute angle. The most common clinical presentation was nodule formation followed by ulcerated lesions with sinus formation. **[Figure 2A&B]**

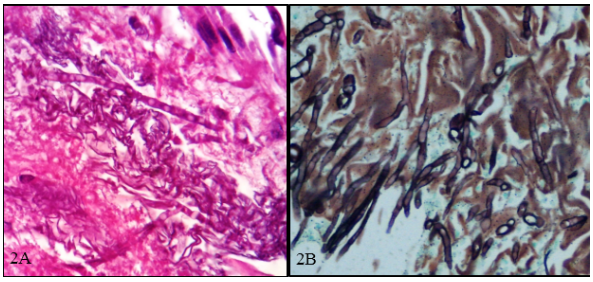

Figure2A Section showing septate acute angled hyphae of Aspergillus (H&E 100x)

Figure 2B Grocott's methenamine silver highlighting the morphology of Aspergillus (GMS100x)

A diagnosis of histoplasmosis was confirmed in (2/22) cases of which one presented with nodular lesion and other with a patch. Histologically there was predominant histiocytic infiltrate in the deeper dermis admixed with lymphoplasmacytic cells. The organism could be identified intracellularly which are round to oval with clear halo and positive for PAS stain. None of the cases showed granulomatous reaction. **[Figure 3A&B]**

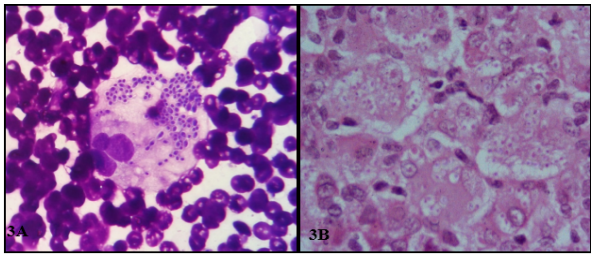

Figure 3A Smear examined show intracellular within the histiocyte rounded organism with clear corresponding to Histoplasma (Giemsa 100x)

Figure 3B Section examined show both intracellular and extracellular histoplasma in the histiocytes (H&E100x)

There were 3/22 cases of cutaneous rhinosporidiosis. Rhinosporidiosis showed numerous globular cyst of varying shape, representing sporangia in different stages of development with dense mixed inflammatory cell infiltrate. **[Figure 4A&B]**

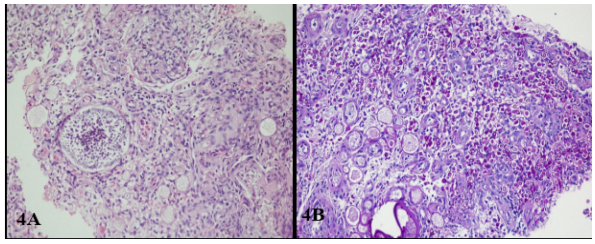

Figure 4A Section showing sporangium of Rhinosporidium with numerous individual spores (H&E40x)

Figure 4B PAS stain highlighting the spores of rhinosporidium (PAS40x)

Discussion

Deep cutaneous mycosis is uncommon and fairly limited to a few fungal species with varied manifestations. It is from the cutaneous lesions that the diagnosis of a systemic fungal infection is often made. [1] Deep mycosis can be primarily a cutaneous fungal infection or be a part of systemic infections such as those involving the respiratory system or reticuloendothelial system, especially in immunocompromised hosts. Mostly the fungi enter the body via the lungs, through gut, paranasal sinuses or skin. The fungi can then spread via the bloodstream to multiple organs including the skin, often causing multiple organs to fail and eventually cause death of the patient. **[3, 4, 5, 6]**

A variety of skin changes may be seen in association with systemic mycosis. The skin lesions depend partly on the type and virulence of the fungus. Generally the characteristic histologic pattern is pseudoepitheliomatous hyperplasia with extensive suppurative and granulomatous inflammation in the dermis. Other findings are small neutrophilic abscesses surrounded by varying numbers of lymphocytes, plasma cells, epithelioid histiocytes, and multinucleated giant cells, involvement of the subcutaneous fat resulting in a lobular pattern of panniculitis with suppuration and granulomatous reaction. Causative fungal organisms can be found in the cytoplasm of the histiocytes or within the abscesses. **[1]**

Mucormycosis is caused by fungi of the orders Mucorales most commonly by Rhizopus or Mucor. Primary cutaneous infection may occur following burns, trauma or diabetes. The lesions may begin as erythematous macules that blister and ulcerate or may form indurated nodule. Entomophthorales infection causes slowly progressive subcutaneous firm or hard nodules, often affecting the nose or sinuses. **[7]** Mucormycosis frequently involves the sinuses, brain, or lungs. **[8]** The hallmark is vascular invasion by long, nonseptate hyphae with thrombosis and infarction which was seen in all of our cases. Primary cutaneous mucormycosis is uncommon and occurs mainly in immunosupressed or diabetic patients or after local trauma. Benito et al **[8]** described 7 cases of primary cutaneous mucormycosis with the clinical presentation of necrotic ulcer and the histological pattern of ulcerated epidermis with a variable inflammatory infiltrate and dermal necrosis. Since the prognosis of mucormycosis is poor with overall mortality of 55% depending on its form and severity, an early diagnosis of mucormyocosis is essential to start immediate therapy to reduce morbidity and mortality.

Primary cutaneous aspergillosis is rare and involves sites of skin injury, intravenous access catheter sites, and sites associated with occlusive dressings, burns, or surgery. Secondary cutaneous lesions result either from contiguous extension to the skin from infected underlying structures or from widespread blood-borne seeding of the skin. It is mostly caused by A. flavus, A. fumigatus, and rarely, by A. Niger. Clinically, the lesion is characterized by macules, papules, plaques or haemorrhagic bullae, which may progress into necrotic ulcers that are covered with a heavy black

eschar.**[9,10]** The fungus was seen and confirmed on Grocott's stain in all the cases with sepatation and acute angle branching.

Histoplasma is caused by Histoplasma capsulatum and occurs in tropical, subtropical and temperate areas of the world: however, the ecologic niche for this infection remains unclear. **[1]** Cutaneous histoplasmosis occurs in upto 17% of American patients with dissemination. **[11]** Tissue diagnosis of histoplasmosis is based on the identification of fairly uniform round to oval 2- to 4-µm spores with clear halo and hyphae within histiocytes and extracellularly in the stroma. Similar, intracellular parasites also occur in granuloma inguinale, rhinoscleroma, and leishmaniasis. Thus a histochemical stain is mandatory for a confirmatory diagnosis of histoplasmosis. H. capsulatum is the only pathogen to parasitize macrophages that stains with the PAS and Grocott's methenamine silver stain. **[12,13,14]** Except in cases of primary inoculation of histoplasmosis, cutaneous lesions signify disseminated disease. Hence a skin biopsy is useful in establishing an early diagnosis for aggressive treatment and to reduce dissemination and mortality.

Rhinosporidiosis is a chronic granulomatous disease caused by Rhinosporidium seeberi. It is endemic in India and Sri Lanka but has also been reported from United States, South America and Iran. In India, it is found more commonly in southern and central regions. **[2]** It may present as satellite lesions, in which skin adjacent to the nasal rhinosporidiosis is involved secondarily, generalized cutaneous type with or without nasal involvement, occurring through hematogenous dissemination of the organism; and primary cutaneous type associated with direct inoculation of organisms on to the skin. **[23]** Apart from warty and nodular lesions it can rarely present as large soft tissue mass mimicking soft tissue sarcomas. The ulcerated lesions have to be distinguished from squamous cell carcinoma, basal cell carcinoma, verruca vulgaris, tuberculosis verrucosa cutis, veneral warts and donovanosis. This disease must also be differentiated from coccidiomycosis which has a different clinical presentation and smaller fungal spherules (30-60µm). Hence a high degree of clinical suspicion and skin biopsy is essential for an early diagnosis and prompt management of such cases. **[15,16]**

Thus, we conclude that skin biopsy is mandatory in deep cutaneous mycosis and a definite diagnosis is often possible by histochemical stains to demonstrate the fungi e.g. PAS which is rapid and easy and Grocott's methenamine silver stain for exact morphological identification. However, fungal elements may sometimes be mistaken for artefacts like Russel bodies, calcific bodies, karyorrhectic debris and elastic as well as reticulin fibers and thus it is important to recognize them. This will help in early diagnosis and prompt management especially with disseminated fungal infection to reduce mortality.

References

1. Hinshaw M, Longley JB. Fungal Diseases. In: Elder DE, editor. Lever's Histopathology of the Skin, 9th ed. Lippincott Williams & Wilkins; 2005.p.603-34.
2. Mohan H, Bal A, Aulakh R. Evaluation of skin biopsies for fungal infections: role of routine fungal staining. J Cutan Pathol 2008;35:1097–99. Gonzalez Santiago TM
3. Pritt B, Gibson LE, Comfere NI. Diagnosis of deep cutaneous fungal infections: correlation between skin tissue culture and histopathology. J Am Acad Dermatol. 2014 Aug;71(2):293-301. doi: 10.1016/j.jaad.2014.03.042. Epub 2014 May 15. Gochhait D
4. Dey P, Rajwanshi A, Nijhawan R, Radhika S, Gupta N. Spectrum of fungal and parasitic infections on fine needle aspiration cytology. Diagn Cytopathol. 2015 Jun;43(6):450-5. doi: 10.1002/dc.23247. Epub 2015 Jan 21.
5. Chapman SW, Daniel CR. Cutaneous manifestations of fungal infection. Infect Dis Clin N Am 1994;8:879-910.
6. Kaliyamoorthy S, Srinivasan S. Histopathological study of cutaneous and soft tissue fungal infections. International J of Res Med Sci. 2016;4(6):1933-37
7. Chander J, Kaur J, Attri A, Mohan H. Primary cutaneous zygomycosis from a tertiary care centre in north-west India. Indian J Med Res 2010;131:765-70.
8. Benito A, Cornejo P, Lopez-Rios F, Gonzalez-Lios C, Rodriguez-Peralto JL. Primary cutaneous mucormycosis. American Journal of Dermatopathology.1998;20:594. Van Burik
9. JH, Colven R, Spach DH. Cutaneous Aspergillosis. J Clin Microbiol 1998;36:3115-21.
10. Stevans DA, Kan VL, Judson MA, Morrison VA, Dummer S, Denning DW, et al. Practice guidelines for diseases caused by Aspergillus. Clin Infect Dis 2000;30:696-9.
11. Nanda S, Grover C, Goel A, Reddy B, Khurana N. Primary cutaneous histoplasmosis in an immunocompetent host from a non-endemic area. Indian J Dermatol 2006;51:63-5.
12. Dijkstra JWE. Histoplasmosis. Dermatol Clin 1989;7:251-8.
13. S Dhar, S. Dhar , Dilip KM, Manas KB. Histopathological features of deep fungal infections: An analysis of sixteen skin biopsies Indian J Dermatol 2003;48(1):33–35.
14. Wheat LJ. Laboratory diagnosis of histoplasmosis: Update 2000. Seminars in respiratory infections 2001;16:131-40.
15. Houreih MA, Eyden B, Lucas SB, Roy S, Banerjee SS. Rhinosporidium seeberi: a case clinically mimicking soft tissue sarcoma. Histopathology 2006;49:208-10
16. Avadhani A, Pai K, Mohanty SP. Cutaneous rhinosporidiosis clinically masquerading as a soft tissue sarcoma- a rare occurrence. International J of Dermatol 2008;47:1153-4