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Pathology

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CLINICOPATHOLOGICAL SPECTRUM OF FUNGAL AND PARASITIC INFECTIONS ON FINE NEEDLE ASPIRATION CYTOLOGY: A TERTIARY CARE CENTER EXPERIENCE

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ABSTRACT

Background: The incidence many fungal and parasitic lesions are on the rise over the decades. In this study, we have analyzed the spectrum of fungal and parasitic lesions diagnosed on fine needle aspiration cytology (FNAC) in a tertiary health care center.
Aims: To study the clinicopathological spectrum of fungal and parasitic infections
Methods: This is a retrospectively over a 3.5 year period from June 2015 to December 2018.The clinical details were obtained from the biopsy request forms. The study included all aspirations of superficial and deep fungal and parasitic lesions in various parts of the body.Special stains including periodic acid Schiff (PAS), and Grocott's methenamine silver (GMS) were done for identification of parasite and fungus.
Results: In this study we had a total of 58 cases comprising of 46 parasitic cases and 12 fungal infections. Table I and II show detailed clinicopathological spectrum of these cases. The parasitic category included 26 cases of filariasis, 12 cases of leishmaniasis, 6 cases of Hydatid and 2 of cysticercosis. The fungal infections were dominated by 6 cases of candidiasis followed by 2 cases each of aspergillosis, mucormycosis and histoplasmosis.
Conclusions: FNAC is an easy, reliable, and minimally invasive method to diagnose and categorize the various fungal and parasitic lesions in the body for early and definitive treatment.

INTRODUCTION

Fine Needle aspiration cytology is a quick, effective and minimally invasive technique to diagnosis various inflammatory and infective conditions. Recent trends of therapy with widespread and prolonged use of broad spectrum antibiotics, chemotherapeutic agents, use of immunosuppressive drugs and radiotherapy have led to increase in incidence of fungal infections. Immunosuppression and breakdown of anatomical barriers such as the skin are the major risk factors for fungal infections. Fungi have been recognized as the causative agents of human disease earlier than bacteria. Fungal infections are difficult to diagnose by culture alone as it takes long time and all fungi cannot be cultured. More over microbiological examinations can be misguided by contamination of other fungi. Serological reactions lack complete specificity. In this scenario, cytology proves to be one of the major diagnostic tools in mycology, because it permits rapid, presumptive identification of fungal infections. Parasitic infections have wide range of clinical presentation. Specific parasites such as filariasis, hydatid disease, and cysticercosis can be easily detected by this technique as well as suggest parasitic infestation in those cases where the actual parasite could not be demonstrated. The present study was done to analyse the role of FNAC in the diagnosis of various fungal and parasitic infections in various parts of the body. **[1]**

MATERIALS AND METHODS

This is a retrospectively over a 3.5 year period from June 2015 to December 2018.The clinical details were obtained from the biopsy request forms. The study included all aspirations of superficial and deep fungal and parasitic lesions in various parts of the body. The superficial lesions were aspirated using 23G needle and 10ml syringe and deep lesions were aspirated by lumbar puncture needle under image guidance. In clinically suspected cases the residual material in the hub of the needle was sent for bacterial and fungal culture. Special stains including periodic acid Schiff (PAS), and Grocott's methenamine silver (GMS) were done wherever required. The varoius inflammatory response by the fungal infection or parasite was also noted in cases where the actual organism could not be detected.

RESULTS

In this study we had a total of 58 cases comprising of 46 parasitic cases and 12 fungal infections. Table I and II show detailed clinicopathological spectrum of these cases. The parasitic category included 26 cases of filariasis, 12 cases of leishmaniasis, 6 cases of Hydatid and 2 of cysticercosis. The fungal infections were dominated by 6 cases of candidiasis followed by 2 cases each of aspergillosis, mucormycosis and histoplasmosis.

Table 1 Clinicopathological spectrum of parasitic lesions (n=46)

Diagnosis	No.of cases	Age (yrs)	M:F	Clinical presentation	Sites of aspiration	Type of aspirate	Cytological findings
Filariasis	26	12-57	4:1	Fever, malaise,swelling	LN,Thyroid, lung, breast	Fluidy/blood mixed	Microfilaria, neutrophils, eosinophils
Leishmaniasis	12	26-72	3:1	Spleen Fever	Spleen	Blood mixed	Intra and extracellular LD bodies
Hydatid	6	16-59	2:1	Pain abdomen	Liver	Fluidy necrotic	Acellular lamellated membranes with hooklets
Cysticercosis	2	8-27	1:0	Subcutaneous swelling	Arm nodule	Clear fluidy	Tegment layer, neutrophils

Table II Clinicopathological spectrum of Fungal lesions (n=12)

Diagnosis	No.of cases	Age (yrs)	M:F	Clinical presentation	Site of Aspirate	Type of Aspirate	Cytological findings
Candidiasis	6	12-68	1:2	Oral thrush	Mouth	Whitish	Spores and pseudohyphae
Aspergillus	2	22-32	1:0	Chronic cough	Lung	Necrotic	Septate hyphae with acute angle branching
Mucormycosis	2	28-36	1:0	Nasal mass	Paranasal sinus	Necrotic	Broad stout aseptate, folded hyphae
Histoplasmosis	2	28-31	1:0	Fever ,pain abdomen	Adrenal	Blood mixed	Capsulated fungal bodies within the histiocytes

Parasitic infections dominated by filarial infectation was seen in various sites predominantly superficial palpable nodules and lymph nodes followed by rare cases of breast, thyroid, liver,lung and abdominal wall. Majority lesions yielded blood mixed fluidy aspirate except pus in few cases with secondary infection. Peripheral smear revealed eosinophilia in 7 cases. Smears prepared from aspirates revealed sheathed microfilariae with a central column of nuclei. Cephalic and tail ends were free from nuclei. Host response could also be identified in the form of inflammation comprising of lymphocytes, eosinophils, histiocytes, granulomas and giant cell reaction. **[Figure1A&B, Figure2A]**

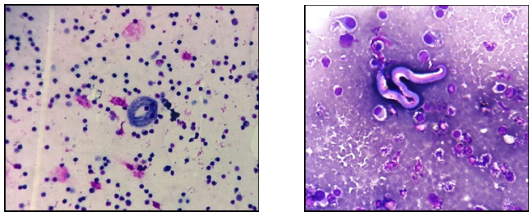

Figure 1A Microfilaria in a lymph node in a fluidy reactive background (Giemsa 40x)

Figure 1B Microfilaria in thyroid in a colloid background with benign follicular epithelial cells (Giemsa 40x)

Leishmaniasis was diagnosed mainly on splenic aspirates with numerous intracellular and extracellular organisms. A nuclear body with distinct kinetoplast was appreciated in each case. Host response could be identified in the form of granulomatous rection and increased plasma cells and histiocytes. [Figure 2B]

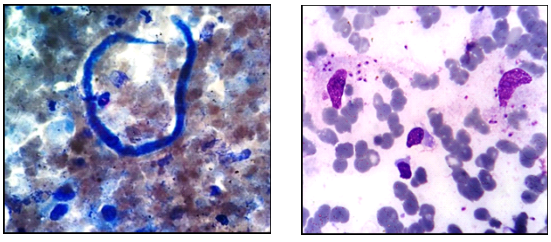

Figure 2A Incidental detection of microfilaria with lung adenocarcinoma (Giemsa 40x)

Figure 2B Spleenic smear showing intracellular and extracellular amastigote with nucleus and kinetoplast (Giemsa 40x)

Hydatid predominatly involved liver and lungs with a rare presentation of spinal cord involvement in the cervical region. It was diagnosed by fragments of acellular lamellated membranes and scattered dagger shaped hooklets. Host response was seen in the form of inflammatory and necrotic background. The hydatid serology was negative in all the cases. **[Figure 3A]** Cysticercosis presented with painless slow growing nodule, soft to firm in consistency with the provisional diagnoses of neurofibroma and lipoma. It was identified by fragments of tegmentum layer and host response in the form of eosinophils, neutrophils, palisading histiocytes and giant cells. Hooklets were not identified in any of the cases. **[Figure 3B]**

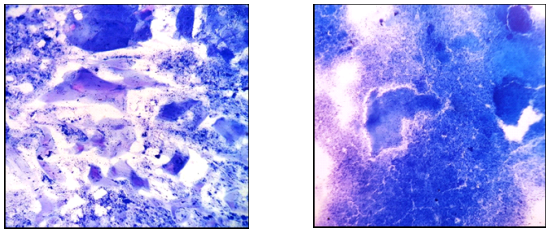

Figure 3A FNAC smears demonstrating fragments of lamellated membranes of hydatid cyst in liver (Giemsa 40x)

Figure 3B Smear showing outer acellular pinkish layer, following which were seen subcuticular or tegumental cells with small pyknotic-looking nuclei set in a loose fibrillary parenchyma (Giemsa 40x)

Sometimes parasitic infections are indicated by the presence of Charcot leyden crystals. Charcot leyden crystals are present within the primary granules of eosinophils and basophils and are composed of lysophospholipase enzyme. They are hexagonal, bipyramidal structures, about 50 microns in size. It was seen in one of the case of liver abscess. **[Figure 4A]**

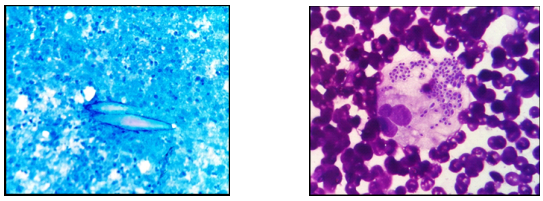

Figure 4A FNAC smears demonstrating clusters of Charcot layden crystals indicating evidence of parasitic etiology in a necrotic background. (Giemsa 40x)

Figure 4B Smear examined show intracellular within the histiocyte rounded organism with clear corresponding to Histoplasma (Giemsa 100x)

Both the cases of histoplasma were aspirations from adrenals. These are round to oval organisms with clear halo measuring 3-5µm present intracellular histiocytes or scattered extracelullary. **[Figure 4B]**Fungal infections predominated by candidiasis was easy to identify because of the spores and pseudohyphae. Aspergillus and mucormycosis predominantly involved the head and neck region followed by respiratory tract. The aspergillus hyphae had parallel wall, septations and acute angle branching while mucormycosis is broad, stout, folded, aseptate, irregular wall and wide angled branching. Both the patients with mucormycosis were diabetics. The host response was seen in the form of necrosis, degenerated polymorphs and occasional eosinophils. **[Figure 5A&B]**

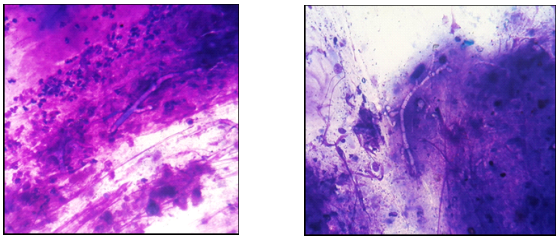

Figure 5A Crush smear showing fungal hyphae with broad, stout, aseptate, ribbon like, right angled hyphae of mucormycosis (Giemsa 100x)

Figure 5B Smear showing septate acute angled hyphae of Aspergillus (Giemsa 40x)

DISCUSSION

Filariasis is a major public health problem in tropical countries. It is caused by slender, thread-like nematodes *(Onchocerca volvulus and Loa loa)* or the lymphatic system *(Wuchereria bancrofti and Brugia malayi).* India contributes ~40% of the global burden and accounts for ~50% of the people at risk of infection. The different types of microfilaria found in humans are sheathed and unsheathed. Microfilariae bancrofti, Microfilariae malayi and Microfilariae loa are the sheathed Microfilaria. Microfilariae perstans and Microfilariae ozzardi are the unsheathed variety. Incidental detection of filarial organism has been reported in cytological smears from almost any part of the body. As the parasite circulate in the lymphatic and vascular systems, appearance of filarial organism in tissue fluids and exfoliated surface material probably occurs due to conditions causing lymphovascular obstruction resulting into extravasations of blood and release of microfilariae. Cytology has an important role in the diagnosis of subclinical filariasis and can demonstrate these extravasated larvae in tissue spaces or fluids. **[2,3]**

Visceral leishmaniasis (VL) is a severe disease caused by *Leishmania infantum* in the Mediterranean basin, and is associated with considerable morbidity and mortality. The parasites spread systemically to propagate in the macrophages of internal organs such as the liver, spleen, bone marrow, and lymph nodes. It is usually diagnosed by serological tests and parasite detection, the latter including parasitological and molecular methods. Morphological detection remains the gold standard. **[4]** Hydatid cysts (echinococcosis) are caused by an infestation with larval tapeworms of the genus *Echinococcus.* A hydatid cyst is white, spherical, and filled with fluid. It varies from a few millimeters to many centimeters in diameter. Because of its slow growth, the diagnosis may be delayed for months to years after the initial infection. Cytologically, protoscolices, hooklets, and fragments of the laminated membrane are commonly found in hydatid cysts. The differential diagnosis of hepatic hydatid cyst includes abscess, hemangioma, and non-parasitic cysts such as solitary bile duct cyst and hepatobiliary cystadenoma. **[5]** Cysticercosis, a parasitic tissue infection caused by the larva of *Taenia solium*. Cestodes have a lifecycle characterized by two stages- larva and adult, besides an egg phase. The human is a definite host and pig is an intermediate host. The cysticercus secretes certain substances locally (e.g., paramyosin, taeniastatin), which alter the host immune response. Both cellular as well as humoral immunity are affected. Demonstration of fragment of larval bladder wall, hooklets and calcareous corpuscles confirms the diagnosis of cysticercosis. **[6]** The local immune response of the host to the parasites is extremely variable and ranges from an insignificant response to marked inflammatory cell infiltration with histiocytes and formation of epithelioid cell granulomas. Charcot Leyden crystals are colorless, hexagonal, bipyramidal crystals formed from aggregation of material from disintegrating eosinophils. Eosinophilic infiltrate along with the presence of Charcot Leyden crystals is an indirect evidence of parasitic infestation. **[7]**

Histoplasmosis is an infective condition caused by a dimorphic, saprophytic fungus, *Histoplasma capsulatum* and is acquired by inhalation of its spores. Soil rich in bird and bat dropping is its natural habitat, and it exists as a mycelium in the atmosphere. There are three major clinical presentations: pulmonary, progressive disseminated and primary cutaneous histoplasmosis. FNAC can differentiate between tuberculosis and histoplasmosis. *H. capsulatum* is an intracellular dimorphic fungus which is commonly seen within the cytoplasm of the macrophages and exhibit narrow based budding. Rapid onsite cytopathological evaluation can suggest immediate diagnosis of the infection and hence the treatment can be started at the earliest. **[8]**

Rhinocerebral mucormycosis is a rapidly progressing, often fatal fungal infection that occurs commonly in diabetics and immunocompromised individuals. Cytomorphology is broad, ribbonlike, aseptate hyphae with right-angled branching consistent with the Zygomycetes class of fungi, which includes Rhizopus and Mucor species. Early diagnosis of mucormycosis is important, and prompt therapeutic intervention may prevent progressive tissue invasion and its sequelae . These sequelae include (1) angioinvasion and direct tissue injury of the respiratory tract, (2) direct extension from lungs into the great vessels, (3) invasion from the paranasal sinuses into the orbit and brain, and (4) hematogenous dissemination to central nervous system tissues. **[9]** Aspergillosis is seen in warm and dry climatic conditions. It is particularly common in young men from rural background in north India.Invasive aspergillosis of the paranasal sinuses in immunocompetent hosts was reported for the first time by Miloshev et al. Inhalation is the usual mode of infection but the disease is not contagious. The pathogenesis is attributed to local invasiveness and Types I and III hypersensitivity reactions. *Aspergillus rhinosinusitis* occurs exclusively with Aspergillus flavus and can be divided into non-invasive (allergic fungal rhinosinusitis and fungal balls) and chronic invasive fungal rhinosinusitis (with and without granulomatous reaction) and acute fulminant rhinosinusitis.*Aspergillus* produces hyaline (nonpigmented) hyphae with regular septations. They display acute angle branching as well as dichotomous branching and may show angioinvasion. **[10]**

CONCLUSION

Despite high incidence of filariasis, microfilaria in fine needle aspiration cytology is not a very common finding. Careful screening of FNAC smears is helpful in detecting microfilaria even in asymptomatic patients. spleen nodules represent a possible hallmark of VL, especially in infants, thus contributing to diagnosis of systemic *L. infantum* infection in children. It is important to include hydatid cyst in the differential diagnosis of cystic lesions in the liver, particularly in those who have lived or traveled in endemic areas. fine-needle aspiration (FNA) which provides direct and specific diagnosis of cysticercosis remains one of the ideal diagnostic procedure wherever the lesion can be approached easily by FNAC. Adrenal FNAC can suggest the diagnosis of histoplasmosis which is further confirmed by culture, polymerase chain reaction, and urine antigen. FNAC and scrape smears can give a conclusive diagnosis of mucormycosis, and the patient can be treated with appropriate antifungal therapy and surgical debridement. In most cases, differentiation between the two most common offending fungi i.e. aspergillus and mucor can be made on cytology with the help of special stains like PAS and Grocotts. Thus, preoperative FNA diagnosis obviates the need for a diagnostic biopsy, allows rapid diagnosis as cultures take time and helps to plan proper treatment to suit individual patients.

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