



## Role of Fine Needle Aspiration Cytology In Diagnosis of Cutaneous & Sub-Cutaneous Parasitic And Fungal Infections

Dr. Manjari Kishore<sup>1</sup>, Dr. Avinash Kumar<sup>2</sup>, Gaurika Gupta<sup>3</sup>, Annu Nanda<sup>4</sup>

<sup>1,2</sup>Assistant Professor, <sup>3</sup>MBBS 3<sup>rd</sup> year Student, <sup>4</sup>Professor

<sup>1,4</sup>Department of Pathology, <sup>2</sup>Dept. of E.N.T.

<sup>1,2,3</sup>Noida International Institute of Medical Sciences (NIIMS),

Noida International University (NIU), Greater Noida, U.P

<sup>4</sup>ESIC Dental College & Hospital, Rohini, New Delhi

### \*Corresponding Author:

Dr. Manjari Kishore

Assistant Professor, Dept. of Pathology

Noida International Institute of Medical Sciences (NIIMS),

Noida International University (NIU), Greater Noida, U.P.

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

### Abstract

**Introduction:** Parasitic & fungal infections are commonly seen in our clinical set up. Even after extensive health care programmes, these infections are frequently found in association with poor hygiene & sanitation. In this series, we make an attempt to study the role of Fine needle aspiration cytology (FNAC) in detecting these infections. Timely diagnosis can aid in early management of these cases, thereby preventing further complications.

**Aims & Objectives:** To highlight the role of FNAC in diagnosis of fungal & parasitic infections and to study cytomorphology of various fungal & parasitic elements.

**Materials & Methods:** We included 22 confirmed cases of different fungal & parasitic infections in last 2 years in our study. A detailed clinic-pathological correlation was done. Pap & Giemsa-stained smears were examined for presence of various infectious agents highlighting their cytomorphology.

**Results:** We analysed 22 cases and found 10 cases of cysticercosis at different rare locations. We noted 4 cases of filariasis, 2 cases of candidiasis and 3 cases of aspergillosis. Two cases of cutaneous histoplasmosis and 1 case of cutaneous leishmaniasis were also found in our study.

**Conclusion:** FNAC is a simple, rapid and economical investigative modality in detection of these parasitic & fungal infections. This can also help in ruling out presence of any underlying malignancy based on cytological evaluation. Early diagnosis of these infections helps in guiding the clinician for an appropriate management. This early intervention can prevent any further complications in the patients.

**Keywords:** FNAC, aspergillus, cysticercosis, filariasis, leishmaniasis, histoplasma capsulatum, malignancy, Pap stain, Giemsa

### Introduction

Parasitic and fungal infections are still a major concern in our country in spite of having a lot of progress in the prevention & treatment of infectious disease.<sup>1</sup> Many health-care programmes have been implemented but still these cases are frequently noted

in the current clinical settings.<sup>2-5</sup> Many such infections present as subcutaneous & cutaneous swellings and are variably diagnosed by clinicians as cystic lesions, inflammatory lesions or neoplasms.<sup>3-5</sup> These cases are treated either in a conservative

manner or subjected to fine needle aspiration cytology (FNAC) or surgical excision for a definitive diagnosis.

Fine needle aspiration cytology offers a simple, easy, reliable and minimally invasive method in diagnosis of various infections caused by fungus and parasites.<sup>2-6</sup> It can be easily done as an out-patient procedure. The diagnostic role of FNAC was described by Kung et al in 1989.<sup>2-4</sup> This procedure can easily diagnose the inflammatory & infective lesions and more importantly exclude the possibility of any malignancy in suspected cases.<sup>3-6</sup> In the current study, we make an attempt to study the role of FNAC in diagnosis of fungal & parasitic infection presenting as superficial cutaneous & subcutaneous lesions.

**Aims & Objectives:**

1. To highlight the role of FNAC in diagnosis of fungal & parasitic infections.
2. To study the cytomorphology of various fungal & parasitic elements along with associated findings in cytological smears.

**Materials & Methods:**

This is a retrospective study done over a period of 2 years. We evaluated 50 cases which presented with cutaneous or subcutaneous swellings lesions in Departments of ENT, Medicine & Surgery. These cases were given provisional diagnosis of suspected cystic lesion and fungal or parasitic lesions on the

basis of clinical findings and radiological assessment. All these cases were sent for FNAC to Dept of Pathology. FNA was done using 22-gauge needle under aseptic conditions. We found 22 cases of fungal & parasitic lesions on cytology. Pap & giemsa stained smears were used to stain these cases. Centrifugation was done in cases of fluid aspirate and then slides were prepared according from the sediments. A proper record of patient’s age, sex and site of the infections were kept. The FNAC smears were examined for various cytomorphological features and background details which favoured the diagnosis of such fungal & parasitic lesions.

Periodic acid Schiff (PAS) stain and gomori methenamine silver (GMS) stain along with serology and culture were applied accordingly after evaluation of Giemsa & Pap stained slides.

**Results:**

We received a total of 50 suspected cases of subcutaneous infections (fungal/ parasitic) during our study period. Of these 50 cases which underwent FNAC, we finally made a diagnosis of fungal or parasitic infections in 22 cases. Rest 28 cases were diagnosed as lipoma, epidermal cyst, abscess on cytology. In our study, we included only these 22 cases with definitive diagnosis of either fungal or parasitic infections. The detailed clinical features including age, sex and site of all 22 cases are depicted in Table 1 & Table 2, respectively.

**Table 1: Clinical & cytological details of all cases included in the study (n=22)**

S. No.	Cytological diagnosis on FNAC	Total no. of cases	Age range & median age [in years]	Sex ratio (M:F) [Male-M; Female- F]
1.	Cysticercosis	10	4-40 & 25.4	2.3:1
2.	Filariasis	04	24-45 & 32	1:3
	a. Filaria with tuberculosis in a lymph node			
	b. Filaria with neurofibroma			
	c. Filaria with			

	metastasis d. Filaria in a reactive lymph node			
3.	Candidiasis	02	10-42 & 31	1:1
4.	Aspergillosis	03	35-58 & 46.5	1:2
5.	Cutaneous histoplasmosis	02	35-40 & 37.5	2 cases (both in males)
6.	Cutaneous leishmaniasis with multibacillary leprosy	01	32	Single case (in male)

The age of patients ranged from 4 years to 58 years and median age being 31 years in our study. The most common sites of these infections were extremities and uncommon sites like neck, cheek, temporal region, scalp, abdomen, etc. Grossly, the size of these lesions varied from 1 cm to 7-8 cm in diameter. Majority of our patients were male (15 of 25 cases) with male: female ratio being 1.5:1.

**Table 2: Various sites of all the parasitic & fungal infections are highlighted according to each case in our study [n=22]:**

S. No.	Cytological diagnosis	Various sites
1.	Cysticercosis [10]	Left arm, right arm, left cheek (2), submental region, left upper eyelid, abdominal wall, left neck, upper back, right lower cervical.
2.	Filariasis [4]	Left cervical LN, right cervical LN, scalp, left foot.
3.	Candidiasis [2]	Left temporal region, right supraclavicular region.
4.	Aspergillosis [3]	Right thumb, left arm, right arm.
5.	Cutaneous histoplasmosis [2]	Multiple skin nodules on extremities, left leg.
6.	Cutaneous leishmaniasis [1]	Left arm

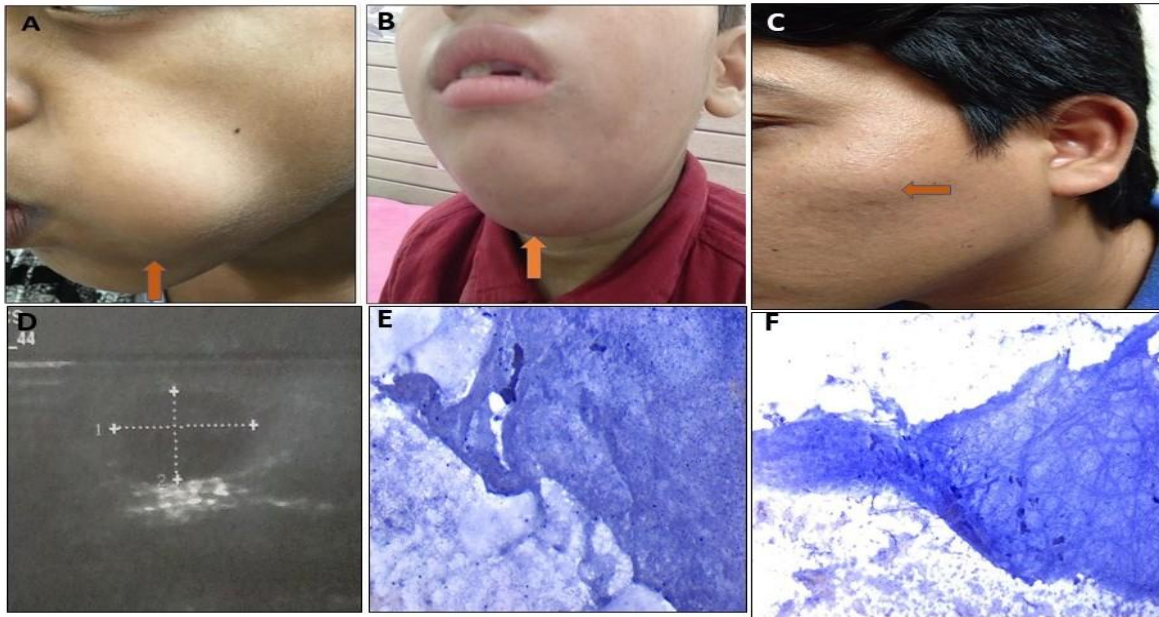
Twenty of our total 22 cases revealed proper parasitic and fungal structures on smears and this could help us in making a definitive diagnosis. Two of our remaining cases were suggestive of parasitic infection but no definitive structures were found. In these 2 cases, the background details like dense mixed inflammation comprising of mainly polymorphs, lymphoid cells, eosinophils, macrophages and presence of giant cells provided us the clue for underlying parasitic infections. Further

with help of PAS stain we could very well appreciate the presence of parasitic elements and make our definitive diagnosis.

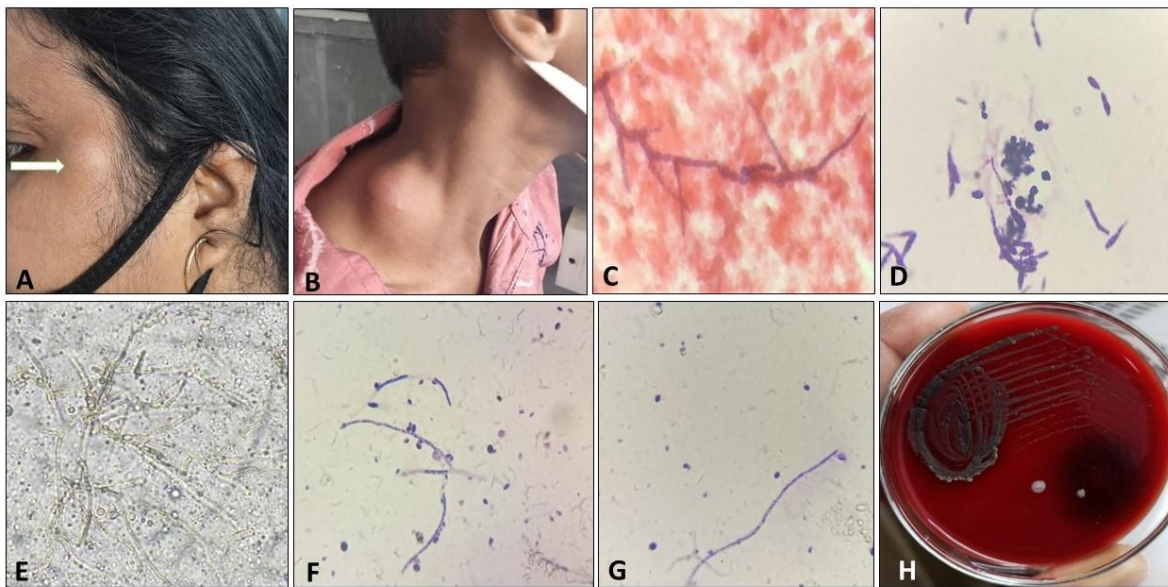
In our study we noted various parasitic and fungal infections like, candidiasis, aspergillosis, cysticercosis, filariasis, cutaneous leishmaniasis and cutaneous histoplasmosis. The detailed frequency of these cases is shown in a pie-diagram [Figure 1A-F]. The majority of our infectious cases were cysticercosis (10 of 22). The region involved in

cysticercosis were extremities, abdominal wall, upper back, neck region, cheek, eyelid and submental region. In 7 cases, definitive structures were noted on Pap and Giemsa-stained smears which led to diagnosis of cysticercosis on cytology. In 3 cases we need help of special stain, i.e., Per-iodic acid Schiff

(PAS) stain to highlight the presence of these parasitic elements [Figure 1]. Here, the role of background dense inflammation and presence of eosinophils provide a clue to pathologists to have diligent search for presence of these infectious elements.



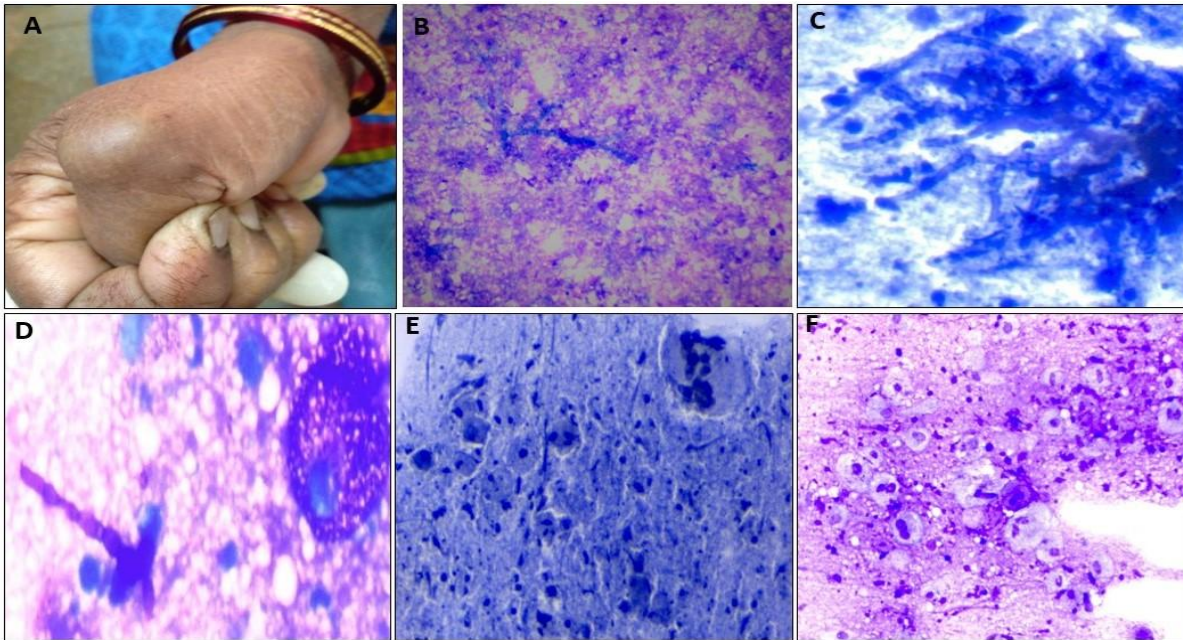
**Figure 1A-F:** A-C showing left lower cheek, submental & left upper cheek swellings of cysticercosis; D-USG image of the cystic lesion; E-F: Cytological smears revealing bladder wall of cysticercosis (Pap-20X).



**Figure 2:** A&B showing left temporal region and right supraclavicular swellings of candidiasis; C-Giemsa stained smear showing candidial pseudohyphae (Giemsa, 20X); D-G PAS (D, 20X) stained, KOH mount (E, 20X) & Gomori's methenamine stained (F&G, 20X) smears showing spores & pseudohyphae of *Candida* spp respectively; H- Culture confirming the presence of *Candida* spp.



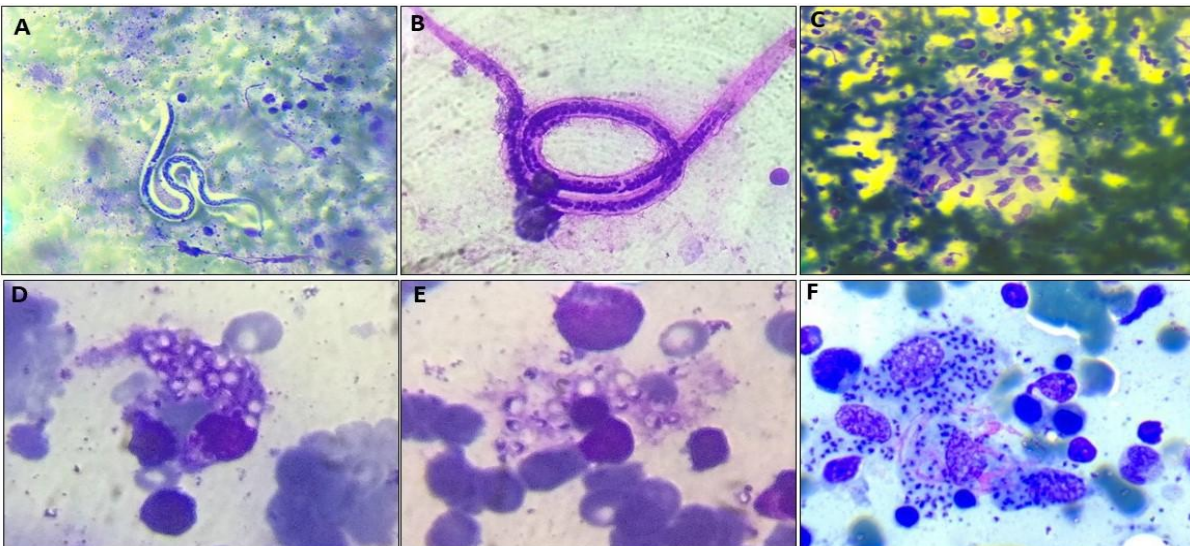
Two cases of Candida were seen on rare location, i.e., left temporal region and right supraclavicular region [Figure 2].



**Figure 3: A- Clinical image of right thumb swelling; B-D: Giemsa (B, 20X), Pap (C, 20X) & PAS (D, 40X) stained smears showing acute angle branched fungal elements of Aspergillus; E&-F: Pap (E, 20X) & Giemsa (F, 20X) stained smears showing sheets of histiocytes and multinucleated giant cells in the background.**

Three cases of cutaneous aspergillosis (Figure 3) were noted in left arm, right thumb and left foot region.

Of 4 cases of filariasis, 3 were seen in association with tuberculosis, neurofibroma and metastasis, respectively. Two cases of cutaneous histoplasmosis were noted where an adult male presented with multiple skin nodules. One case of cutaneous leishmaniasis was noted in an adult female who also had leprosy in left leg.



**Figure 4: A-B showing microfilaria, suggestive of *W. bancrofti* (A, Pap-20X; B, Giemsa-40X); C- Well formed granuloma was noted along with presence of microfilaria in the background (Giemsa, 20X); D-E- Cytological image of *Histoplasma capsulatum* (Giemsa, D&E, 40X); F- *Leishmania donovani* bodies seen in the smears with inflammatory cells in the background (Giemsa, 40X)**

## Discussion:

FNAC is a simple, low-cost, reliable and minimally invasive diagnostic procedure which needs no local anaesthesia or admission of patients in hospital.<sup>2-4</sup> The diagnostic role of FNAC was described by Kung et al in 1989.<sup>2</sup> This procedure can easily diagnose inflammatory & infective lesions, thereby excluding the possibility of malignancy in any suspected cases. In fact, it can even eliminate the need of open biopsy or histological evaluation in certain cases.

Increased fungal infections have been noted in the recent trend which may be attributed to increase in the immunocompromised patients (e.g., HIV, transplant recipients, cancer patients, patients undergoing invasive procedure & with prolonged hospital stay).<sup>3-9</sup> Increased use of broad-spectrum antibiotics, immunosuppressants and chemotherapeutic drugs have also been implicated in association with these fungal & parasitic infections.<sup>4-21</sup>

In all such cases, diagnosis by fungal-culture takes a lot of time or results may get delayed due to contamination with other fungi or bacteria. Also, serology lacks the complete specificity.<sup>2-5</sup> Here, comes the role of cytology, which poses to be a major diagnostic technique in giving a rapid diagnosis by presumptive identification of such fungal elements or different parasites by detailed cytopathological evaluation.

Parasitic infections occur due to poor socio-economic condition, contaminated water supply, inadequate sanitation and personal hygiene. Few common parasitic infections like cysticercosis, filariasis, hydatid disease can be very easily identified on careful examination of cytological smears. In present study, we evaluated presence of many parasitic and fungal infections by studying the proper cytomorphological features of such agents. We examined 22 confirmed cases of parasitic & fungal infections. In our study, majority of patients were male and more common age group involved was 20-30 years of age. Singhal et al noted the presence of majority of cysticercosis in children and young adults.<sup>3</sup>

Of 22 cases, we had 10 cases of cysticercosis infection which is seen due to the infection by larval form of cestode, *Taenia solium*. Singhal et al also

stated cysticercosis as most common infection in their study.<sup>3</sup> It presents as solitary nodules and can be found in many regions, usually noted in skeletal muscle, subcutaneous tissue, nervous system, eyes, etc.<sup>4-7</sup> We found cysticercosis over extremities, cheek, submental region, abdominal wall, back, etc. However, with only clinical and radiological evaluation, a definitive diagnosis of cysticercosis is difficult to be made. A provisional diagnosis of epidermal cyst, lipoma or infectious lesions can be given.

FNAC gives a reliable technique in early diagnosis of this infection and thereby preventing its neurological complications.<sup>8-9</sup> A viable cyst will yield clear fluid on aspiration and fragments of bladder wall can be noted in the acellular proteinaceous background. The parasitic portions can be appreciated as fibrillary bodies which are the part of bladder wall along with parasitic nuclei, appearing as small dot-like spaces. On cytology, we can see fragments of larval cuticle, parenchyma and scolex. The presence of background elements like dense acute inflammation and eosinophils point to such diagnosis.

Serology is helpful in positive cases but in negative cases serology cannot rule out the presence of cysticercosis infection. False positive cases can be seen in serology in cases with past parasitic infection or cross reactivity with other helminths. Here, FNAC makes the way much simpler in giving an early and appropriate diagnosis so that early medical or surgical intervention can be done.

Filariasis is a highly prevalent parasitic infection seen in tropical and subtropical nations.<sup>10-12</sup> Most of the cases in India are caused by two species: *W. bancrofti* (95%) and *Brugia malayi* (5%).<sup>10-12</sup> On cytology, microfilaria can be demonstrated on smears as well as peripheral blood smear along with eosinophilia and dense inflammation.<sup>10-12</sup>

Considering cases of Candidiasis, we reported 2 cases in rare subcutaneous regions. Association of immunocompromised states have been noted in their occurrence but in our study, both these cases were seen in immunocompetent individuals as soft tissue lesions.<sup>13-15</sup> As invasive candidiasis is associated with a high mortality rate, diagnosis of this entity if done fast will make an early intervention, thereby preventing any further complications.<sup>17</sup> However,



culture technique should be used to confirm the diagnosis.

Primary cutaneous aspergillosis is also very rarely noted and that too in immunocompetent individuals.<sup>16-17</sup> These infections pose a diagnostic challenge on clinical examination alone. Here, a simple technique, like FNAC can make the diagnosis much faster and accurate by identifying the fungal elements, i.e., acute angled branching filamentous fungi along with dense necrotic background with presence of inflammation comprising of polymorphs & eosinophils. If not detected on time, these can become invasive and destroy the underlying bony structures.

We reported 3 such cases of cutaneous aspergillosis in immunocompetent patients. Smears from these infections showed acute & chronic inflammatory cells, along with cystic macrophages and multinucleated giant cells in a necrotic background. Presence of many septate fungal hyphae with acute angle branching were noted which were positive for Periodic acid-schiff stain. The pus was also sent for culture study and confirmed the presence of *Aspergillus flavus* on Sabouraud dextrose agar (SDA) medium at 37°C & 25°C.

On cytology, histoplasmosis infection can be detected by the characteristic cytomorphological features of *Histoplasma capsulatum*. The yeast forms of this organism are 2-5 micromm in size with a basophilic crescent shaped nucleus seen within the macrophages, with pericellular halo; can be seen extracellularly.<sup>18-19</sup> It can be easily differentiated from leishmaniasis which are 2-4 micromm, which is a small intracellular protozoa and has a kinetoplast.

In leishmaniasis, diagnosis is usually based on the presence of epithelioid cells with granuloma, inflammatory cells like polymorphs, lymphocytes, eosinophils, macrophages with Leishman-donovan (LD) bodies and also extracellular LD bodies.<sup>20-21</sup> In our study all cases of histoplasmosis and leishmaniasis were confirmed on histology as well. These are very rarely reported on cytology.

### Conclusion:

To conclude, parasitic & fungal infections are difficult to be diagnosed just on the basis of clinical or radiological examination. Hence a simple, economical & rapid diagnostic technique, i.e., FNAC

becomes important in such cases. Based on a detailed cytomorphological evaluation, presence of these fungal & parasitic elements can very well be appreciated in cytological smears. In fact the background details like presence of epithelioid cells with or without granuloma, inflammatory cells like neutrophils, lymphocytes, eosinophils, macrophages and necrosis can provide an essential clues in guiding for an eminent search for the presence of these organisms in smears. In suspicious cases, special stains like PAS can help in making a definitive diagnosis. Serology and also histopathology may be required in certain cases.

### References:

1. Singh BB, Sharma R, Sharma JK, et al. Parasitic zoonoses in India: an overview. *Rev Sci Tech* 2010;29:629-37.
2. Kung It, Lee D, Yu HC. Soft tissue cysticercosis- Diagnosis by fine-needle aspiration. *Am J Clin Pathol*. 1989;92:834-5.
3. Singhal S, Kamra R. Parasitic and fungal infections presenting as subcutaneous lesions diagnosed by fine needle aspiration cytology- a study of one year. *International Journal of Research and Review* 2019;6(10):163-7.
4. Adhikari RC, Aryal G, Jha A et al. Diagnosis of subcutaneous cysticercosis in fine needle aspirates : a study of 10 cases. *Nepal Medical College J* 2007;9(4):234-8.
5. Agarwal R. Soft tissue cysticercosis: study of 21 cases. *J Clin Diagn Res* 2012;6(10):1669-71.
6. Handa U, Garg S, Mohan H. Fine needle aspiration in the diagnosis of subcutaneous cysticercosis. *Diagn Cytopathol* 2008;36(3):183-7.
7. Goyal P, Sehgal S, Ghosh S et al. A cytological study of palpable superficial nodules of parasitic origin: A study of 41 cases. *Pathology Res Int* 2014;14:373-472.
8. Parakh R, Gupta S, Singh S, Garewal J et al. Role of FNAC in the diagnosis of cysticercosis. *Int J Med Res Rev* 2016;4(3):407-13.
9. Singh N, Arora VK, Bhatia A. Are all subcutaneous parasitic cysts cysticercosis? *Acta Cytol* 2006;50(1):114-5.

10. Pal S, Mondal S, Pradhan R, Bose K, Chakrabarti S, Sikder M. Cytological findings of microfilariae in different sites: A retrospective review of 22 cases from endemic region. *Trop Parasitol.* 2018 ;8(1):24-28.
11. Nanda A, Gupta N, Lamba S, Sethi D. Subcutaneous filariasis: An unusual presentation with an adult gravid worm on aspiration. *Trop Parasitol.* 2018 Jul-Dec;8(2):121-123.
12. Khare P, Kala P, Jha A, Chauhan N, Chand P. Incidental Diagnosis of Filariasis in Superficial Location by FNAC: A Retrospective Study of 10 Years. *J Clin Diagn Res.* 2014 Dec;8(12):FC05-8.
13. Palese E, Nudo M, Zino G, et al. Cutaneous candidiasis caused by *Candida albicans* in a young non-immunosuppressed patient: an unusual presentation. *International Journal of Immunopathology and Pharmacology.* 2018;32. doi:10.1177/2058738418781368.
14. Kishore M, Kumar A, Gupta R. Candidiasis mimicking soft tissue lesions in immunocompetent patients: A report of 2 rare cases. *Int J of Medical Sciences and Current Research* 2022;5(1):277-80.
15. Taudorf EH, Jemec GBE, Hay RJ, Saunte DML. Cutaneous candidiasis - an evidence-based review of topical and systemic treatments to inform clinical practice. *J Eur Acad Dermatol Venereol.* 2019 Oct;33(10):1863-1873.
16. Kishore M, Gupta P, Bhardwaj M. Cytomorphological diagnosis of isolated cutaneous aspergillosis in an immunocompetent host. *Indian Dermatol Online J* 2018;9:206-8.
17. Chaturvedi R, Kolhe A, Pardeshi K, Naik L, Wanjare S. Primary cutaneous aspergillosis, mimicking malignancy, a rare presentation in an immunocompetent patient. *Diagn Cytopathol.* 2018 May;46(5):434-437.
18. Chandra S, Raina MK, Gaur DS, Agarwal VK. Cytological diagnosis of primary cutaneous histoplasmosis with hemophagocytosis in immunocompetent patient - A rare case from non-endemic region. *Indian J Pathol Microbiol.* 2020 Apr-Jun;63(2):309-311.
19. Santosh, T., Kothari, K., Singhal, S.S. et al. Disseminated histoplasmosis in an immunocompetent patient - utility of skin scrape cytology in diagnosis: a case report. *J Med Case Reports* 2018;12(7):1543-5.
20. Kishore M, Madaan GB. Visceral Leishmaniasis in an Immunocompromised Patient: Diagnostic Bone Marrow Cytomorphology. *Journal of Clinical and Diagnostic Research* 2017;11(6): EL01-EL02.
21. Kashi M, Tareen I, Qazi A et al. Fine needle aspiration cytology in the diagnosis of cutaneous leishmaniasis. *Ann Saudi Med* 2004;24(2):93-98.