Fine Needle Aspiration Diagnosis of Histoplasma Lymphadenitis using Multidisciplinary Expert Opinion through Telepathology

***B Satya Vara Prasad*[*], *Neeta Kumar*[*], *F A S Allibhoy*[**], *Reddy P Rao*[***], *Shahin Sayed*[*], *Gunturu Revathi*[****]**

Abstract

Background: Histoplasmosis is an opportunistic fungal infection commonly seen in immunocompromised patients, especially in AIDS patients, children and elderly patients. Early detection of these organisms can help in curbing the mortality and complications. Fine needle aspiration is an ideal cost effective diagnostic procedure. Diagnosis may be missed if the pathologist is not familiar with cytological features.

Case: We describe a case of histoplasma lymphadenitis in retroviral positive female where accurate diagnosis was made possible by using the availability of multidisciplinary expert opinion through referral and telepathology.

Conclusion: Diagnosing a fungal infection can prove challenging because of uncommon presentations. Availability of multidisciplinary expert opinion through referral or telecytology can be of great value to a solitary pathologist practicing in a remote community hospital.

Keywords: Histoplasma, Lymphadenitis, Fine needle aspiration, Ziehl neelsen, telepathology.

Introduction

Histoplasmosis is a fungal infection that occurs most frequently in small children, the elderly and immunosuppressed patients.[1] The clinical presentation of these opportunistic infections in immunosuppressed patients may be atypical because of decreased host response, which leads to diagnostic difficulties. There is a possibility of misdiagnosing it as tuberculosis due to overlapping clinicoradiological and histopathological features.[2] Early and accurate detection of the infective organisms is very important, so that the infection can be treated at an early stage and complications can be prevented.[3]

Fine needle aspiration (FNA) is an ideal first line cost effective approach to rapid and definitive diagnosis, as the organisms can easily be demonstrated in the cytology smears, resulting in prompt diagnosis and timely initiation of treatment. It is presumed that numerous cases occur in endemic areas of Africa but are not recognized.[4] The shortage of trained cytopathologists in East Africa puts the onus of diagnosis on histopathologists who may be unfamiliar with its cytomorphological features in FNA smears. This can lead to underdiagnosis of cases. The resultant delay in diagnosis significantly worsens the clinical outcome.

We report a case of histoplasmosis in a HIV positive patient where accurate diagnosis was achieved at a community hospital using a multidisciplinary approach through telepathology and the referral system. The aim is to increase awareness of cytomorphological features of Histoplasma, differentiating features from other fungi, with special emphasis on uncommon features observed in our case and to highlight the value of remote expert assistance through telepathology which is well established at our hospitals.

Case Report

A 44 years old female was admitted in our hospital with complaints of diarrhea, vomiting and fever

[*]Departments of Pathology, Aga Khan Hospital, Kisumu and Aga Khan University Hospital, Nairobi, Kenya
[**]Departments of Medicine, Aga Khan Hospital, Kisumu and Aga Khan University Hospital, Nairobi, Kenya
[***]Departments of Radiology, Aga Khan Hospital, Kisumu and Aga Khan University Hospital, Nairobi, Kenya
[****]Departments of Microbiology, Aga Khan Hospital, Kisumu and Aga Khan University Hospital, Nairobi, Kenya
Correspondence to: Dr B Satya Vara Prasad, Resident pathologist, Department of Pathology, Aga Khan Hospital, Kisumu, P. O. Box 530, Kenya. ***E- Mail Id:*** satyabsv@yahoo.com

© ADR Journals 2015. All Rights Reserved.

for 5 days. Routine investigations showed peripheral pancytopenia (R.B.C. 3.2 $x10^{12}$, Hematocrit 26, Hemoglobin 7.8 grams/dl, Mean corpuscular volume 79, Mean corpuscular hemoglobin 24, White blood cells count of 1.2 $x10^{9}$/liter, with a differential count of, Neutrophils 80%, Lymphocytes 14%, Monocytes 5%, Eosinophils 1%, and Platelets $91x10^{9}$/liter.). Urine examination (Microscopy & Culture) and serum electrolytes were normal. Blood tests for malaria and typhoid were negative.

Her HIV positive status was discovered four years ago. At the time of admission, her CD4 and CD8 count was low. Total T-Cell (CD 3) count was 191/cumm, CD 4 cell count was 10, and CD 8 cell count was 163. The CD4:CD 8 ratio was 0.06 and HIV -1 viral load was 3093 cps/ml with LOG UNITS 3.49 indicating marked immunosuppression.

Chest radiograph showed blurring of left costophrenic angle with minimal left pleural effusion. The right lung appeared normal. Ultrasound showed few small abdominal lymph nodes, mild splenomegaly with no focal lesions, and minimal free fluid in peritoneal cavity.

With the above findings, the patient was started on empirical anti tuberculous treatment. During the course she developed jaundice (Total bilirubin of 211 µmol/L, bilirubin direct 57 µmol/L, Alkaline phosphatase 778 µl/L, Gamma GT 91 U/L, SGOT 62 U/L, SGPT 41 U/L) and hypoproteinemia (Total protein of 43 G/L, Albumin 16 G/L). The CT scan of the lungs was normal.

A submandibular swelling was noted on the second day of admission, which was gradually increasing (fig. 1). FNA from this mass revealed uniform round to oval budding yeasts, with surrounding clear zone, which were 2-4 µm in diameter (fig. 3). The organisms were present both intra as well as extracellularely and were found within the histiocytes and neutrophils (figs. 2, 4). These features were suggestive of a fungal infection. The reporting pathologist (SVP) considered Cryptococcus on the basis of morphology of budding forms. Ziehl Neelsen stain was negative. The culture from FNA material did not show any growth.

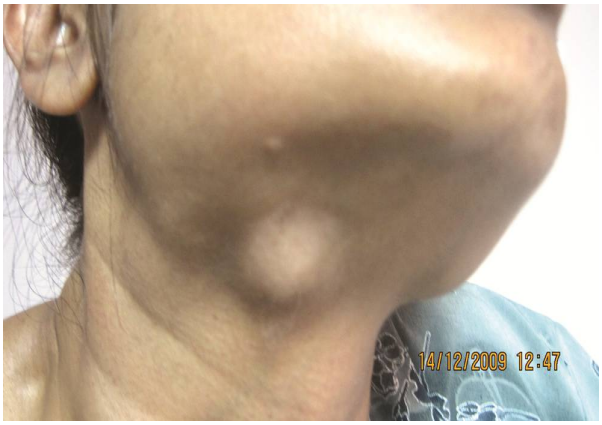

Figure 1.Right submandibular mass measuring 4x3 cm

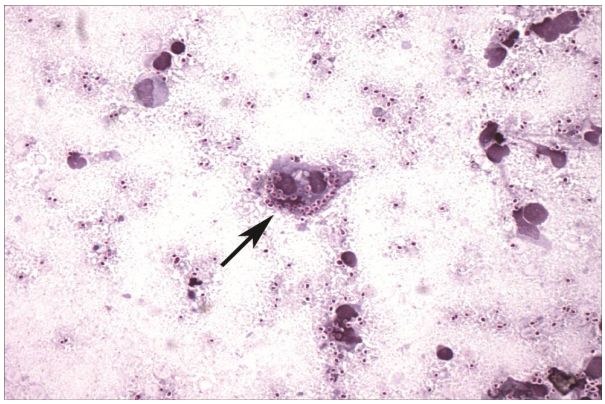

Figure 2.The arrow indicates a neutrophil containing several organisms (Papanicolaou, 400x)

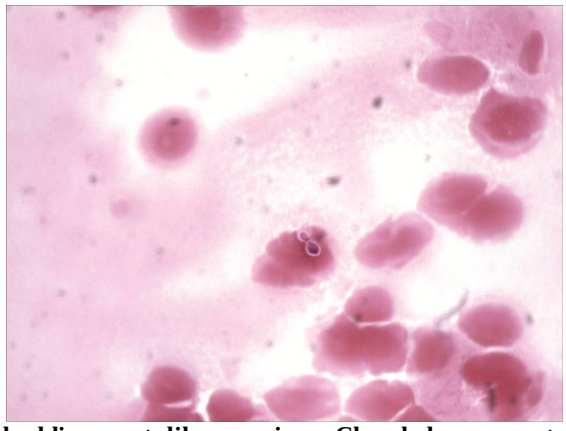

Figure 3.Extracellular budding yeast- like organisms. Clear halo represents capsule (H&E, 1000X)

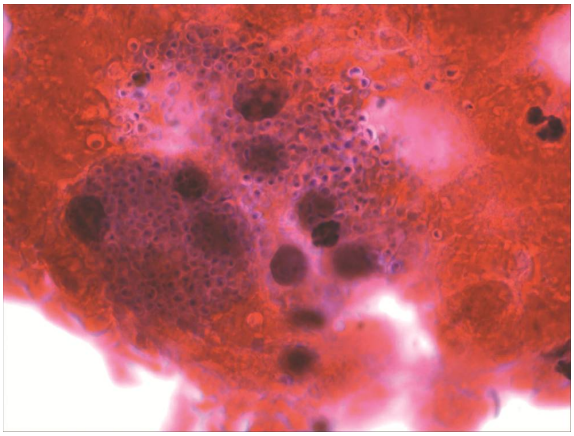

Figure 4.Histiocytes are filled with numerous intracellular yeast- like organisms (H&E, 1000X)

Two images of extracellular organisms were referred to cytopathologist at referral hospital, for telepathology consultation, who considered Cryptococcus but asked for glass slides and special stains for review. After reviewing glass slides and special stains and consultation with histopatholgists and microbiologists at the referral hospital, the case was confirmed as Histoplasmosis. Treatment with itraconazole 400 mg/day was given for 12 weeks.

The lymph node regressed initially, but later increased in size after 4 weeks of complete antifungal treatment. At this point FNA was repeated and cytopathologist at referral hospital confirmed that there were no residual organisms. Culture from this second FNA material did not show any growth. A course of intravenous antibiotics was given for one week to treat superadded bacterial infection. After two weeks, the lymph node subsided completely. The patient is well after two months and is under regular follow up.

Discussion

Histoplasmosis is widely distributed throughout the world, occurring in more than 60 temperate and tropical countries in USA, Africa and Australia.[5] Histoplasma infection is frequently associated with exposure to birds and bat droppings, which are thought to alter soil characteristics, favoring organism proliferation.[6] The association of African Histoplasmosis (Histoplasma capsulatum var. duboisii) with AIDS is known in a limited number of cases. The first few cases were described among heterosexual Belgians who were long time residents in Africa.[4]

Histoplasmosis is a disease involving primarily the reticuloendothelial system. In most cases it is subclinical or benign involving the respiratory system as fibro- cavitatory pneumonia or less commonly, as disseminated infection involving spleen, bone marrow, adrenal glands, brain, kidney, pancreas, ileum, descending colon and lymph nodes.[1,5] Histoplasmosis is three times

more common than tuberculosis as a cause of serious infection in HIV patients who are receiving tumor necrosis factor (TNF) blockers. It is also the most frequent invasive fungal infection in such patients, responsible for a mortality rate of 20%. [7] This implies that delay in treatment may increase the risk of a life threatening or fatal outcome.

Cryptococcus neoformans, Histoplasma capsulatum, Coccidiodes immitis and Blastomycosis dermatitidis are the four common yeast forms that form the differential diagnosis on cytomorphology. Features that distinguish these fungi from each other include the presence and nature of daughter buds or endospores, size of the yeast, and staining characteristics (table 1).[1,8]

S. No.	Microorganisms	Size	Morphology	Papanicolaou stain	Diff quik stain	Special staining
1	H. Capsulatum	3-6µm	Thin based buds, histiocytes filled with small organisms, when extracellular, comets or shooting star shaped.	Dark out line	Dark blue	GMS, PAS
2	C. neoformans	5-10µm	Tear drop shaped buds, distinct mucopolysaccharide capsule.	Pale, Cyanophilic (halo around organism)	Blue, purple(halo around organism)	GMS+, Mucicarmine, PAS (Bright pink rim), AB (capsule)
3	B. dermatitidis	7-20µm	Broad based buds, out of plane of focus, thick double contoured cell wall.	Blue- green	Pale blue	GMS, PAS
4	C. immitis	20-100µm	No budding, Production of endospores with in spherule that eventually ruptures.	Orangeophilic (occ)	Pale blue. color less	PAS, GMS

GMS: Gamori Methenamine Silver, PAS: Periodic Acid Schiff, AB: Alcian Blue

Table 1.Morphological features of the fungi in the differential diagnosis of histoplasma[1,8]

On cytological smears, Histoplasma is characterized by a variable load of uniform, round to oval, about 2-4 µm in diameter, budding yeast forms. Histiocytes are filled with these small organisms. When extracellular, these small organisms have been described as small comets or shooting stars. Methenamine silver or PAS stains are the best stains to visualize histoplasma (fig. 5). A rare and unique feature of our case was the presence of histoplasma organisms in neutrophils, which suggested the severity and heavy load of infection. This morphological feature is more commonly seen in immunocompromised host as compared to immunocompetent host.

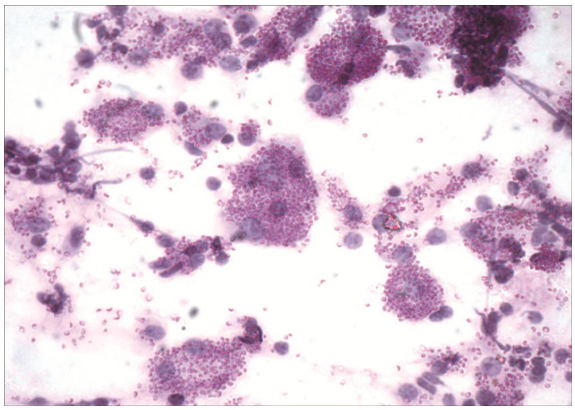

Figure 5.Periodic Acid Schiff (PAS) stain delineates the morphological features, with positive staining of numerous intracellular yeast-like forms (400x)

The paucity of intracellular organisms in the initial digital images sent for telecytology consultation and their limited number misled the cytopathologist regarding the size of the organisms. Finally the accurate diagnosis was achieved through multidisciplinary consultation with cytopathologist, histopathologist and microbiologist on glass slides and special stains and culture provided by our referral hospital. This case is an example of a likely pitfall in telecytology where image selection by referring pathologists is limited and key feature is not adequately photographed. This should be kept in mind when giving opinion on static images.

Although fungal culture is a useful tool in diagnosing Histoplasmosis, the organism itself, is fastidious.[5] The culture yielded negative result in our case. A commercial assay for histoplasma antigen in the blood or urine is useful as a diagnostic tool and also for monitoring response to therapy in a patient with disseminated infection. Surveys carried out in Africa using the histoplasmin skin test to investigate the prevalence of the disease have shown the rate of reactors to be from 0.0% to 28%, the rate varying not only from one country to another but also from one village to another within the same region.[4] Antigen detection of the histoplasma requires confirmation by culture or histopathology because of possibility of false positives in some cases. A highly specific and sensitive immunohistochemistry test using rabbit antibody against Histoplasma for in situ identification of fungal antigen can be used on FNA material thereby avoiding the need for biopsy.[9] We do not have the facility for this test.

Conclusion

Diagnosing a fungal infection can prove challenging because signs of infection may be mild and slow in onset. For an accurate diagnosis, FNA identification of organisms in smear is essential. Availability of multidisciplinary expert opinion through referral or telecytology can be of great value to a solitary pathologist practicing in a remote community hospital.

Acknowledgment

Our sincere thanks to IHPA and Aga khan foundation for their generous help in establishing the telepathology services in our hospitals.

References

1. Gupta N, Arora SK, Rajwanshi A et al. Histoplasmosis: cytodiagnosis and review of literature with special emphasis on differential diagnosis on cytomorphology. *Cytopathology* 2010; 21(4): 240-44.
2. Kumar N, Jain S, Singh ZN. Disseminated histoplasmosis with reactive emophagocytosis: Aspiration cytology findings in two cases. *Diagnostic Cytopathology* 2000; 23(6): 422-24.
3. Smith GD, Shatford RAD. Histoplasmosis infection presenting as an isolated subcutaneous periarticular upper limb swelling in the immunosuppressed patient. *J Hand Surgery* 2005; 30B:2: 229-32.
4. Gugnani HC. Histoplasmosis in Africa: A Review. *(Indian) J Chest Dis Allied Sci* 2000; 42: 271-77.
5. Dhar S, Dutta Roy RK, Todi SK et al. Seven cases of Histoplasmosis: cutaneous and extra cutaneous involvements. *Indian J Dermatology* 2006: 51(2); 137-39.
6. Youness H, Miche RG, Pitha JV et al. Tracheal and Endobronchial involvement in disseminated Histoplasmosis: case report. *Chest* 2009; 136: 1650-53.
7. Hage CA, Bowyer S, Tarvin SE et al. Recognition, Diagnosis, and Treatment of Histoplasmosis complicating tumor Necrosis factor Blocker therapy. *Clinical Infectious Diseases* 2010; 50: 85-92.
8. Atkins KA, Powers CN. The Cytopathology of Infectious Diseases. *Adv Ana Pathol* 2002; 9(1): 52-64.
9. Tuon F, Gomes V, Pagliari C et al. Isolated lymphadenitis due to Histoplasma capsulatum diagnosed by fine needle aspiration biopsy and immunohistochemistry. *Rev Iberoam Micol* 2008; 25: 50-51.