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Role of fluorescence in situ hybridization in detecting mycobacterium avium complex presenting as fever in treatment failure HIV

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ABSTRACT

A 49-year-old male HIV positive patient on treatment failure presented with complaints of fever and dysphagia of three weeks duration and later on developed cervical lymphadenopathy along with severe vomiting and abdominal pain. Liver function tests were found to be worsening with severe drop in CD4 counts. An extensive workup for pyrexia was done. FNAC and biopsy of lymph node showed features suggestive of granulomatous lymphadenitis. CBNAAT of the lymph node aspirate was negative for MTB. Blood culture and lymph node cultures were negative for Mycobacterium Avium Complex (MAC). MAC was however, finally detected and reported positive on Fluorescence in Situ Hybridization (FISH) of the cervical lymph node aspirate. Prompt treatment for MAC was initiated with Ethambutol 800 mg OD and Azithromycin 500 mg OD following which fever spikes subsided and lymph node resolved. The Patient's condition gradually improved and was discharged shortly with a good recovery on subsequent follow ups.

Fever is one of the common symptoms in patients with MAC infection. Some other clinical manifestations include weight loss, hepatosplenomegaly and intra-abdominal lymphadenopathy. Diagnostic evaluation should be aggressive.

As there is a high risk for MAC infection in advanced HIV cases with poor HAART compliance, FISH can be a valuable and effective diagnostic tool in early detection and treatment of MAC.

1. Introduction

Fever in advanced HIV, although a common presenting complaint, can be a diagnostic conundrum [1]. The immune-compromised state predisposes one to an array of opportunistic infections. Mycobacterium Avium Complex infection (MAC) is one of the rare complications in the present era of HAART, worldwide. However, patients who have a poor compliance to HAART are at a high risk of developing MAC. The most important intervention in this setting is for early diagnosis and treatment of MAC. The identification of mycobacteria from cultures at the species level frequently relies on observing colony characteristics and growth [2]. FISH can distinguish closely related organisms by DNA sequencing [3]. FISH assays can be effective diagnostic tools for detecting Mycobacteria from solid and liquid cultures and for their identification

2. Case presentation

A 49-year-old male HIV positive patient for the last 15 years and driver by occupation presented with chief complaints of fever with chills and dysphagia since 3 weeks. Fever was insidious in onset, high grade associated with chills and rigors. Dysphagia was associated with throat pain. His CD4 T-cell as of 12/06/17 was 300. Patient was a known case of RVD for the last 15 years and was clinically stable on regular combination ART regimen of Zidovudine, Lamivudine and Efavirenz.

However, due to complaints of fatigability and drowsiness during his regular night duties, he stopped taking Efavirenz and then gradually stopped ART one month back, following which he presented with the above mentioned complaints. He had history of pulmonary tuberculosis 15 years back for which he had taken a complete 6 monthly course of ATT. He was also diabetic since 3 years on regular medications. No

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Table 1

Investigations	On admission (12/07/17)	(25/07/17)	(27/07/17)
Hemoglobin (gm/dl)	10.4	10.2	
White blood cell count (cells/mm^3)	5200	12,400	
Platelet (cells/mm^3)	1.5 Lakhs	1.22 Lakhs	
ESR (mm/hr)	52		
Creatinine (mg/dl)	0.7		
Urea (mg/dl)	19		
Total bilirubin (mg/dl)	0.33	4.36	5.09
Direct bilirubin (mg/dl)	0.15	3.83	4.67
Aspartate Transaminase (U/L)	28	206	415
Alanine Transaminase (U/L)	45	490	450
Alkaline Phosphatase (U/L)	350	402	576
Glycosylated Hemoglobin (%)	11		
CD4 count (cells/mm^3)	64		

history of any other comorbidities. On examination vitals were stable. Local examination of the oral cavity showed candidiasis and ulcer over posterior pharyngeal wall. Chest auscultation revealed vesicular breath sounds with no adventitious sounds. Other Systems examination did not reveal any abnormalities.

3. Investigations

Routine blood investigations at the time of admission and subsequent ones during hospital stay are as shown in Table 1.

Upper gastrointestinal endoscopy done in view of dysphagia showed Grade B GERD along with duodenal erosions and esophageal ulcer. Biopsy was taken from the ulcer site and was reported to be active esophagitis, which was inconclusive. PAS stain was negative for fungus.

Ultrasonography of abdomen and pelvis showed hepatomegaly, borderline splenomegaly and few sub-centimetric iliac lymph nodes.

The patient continued to have persistent fever, and later on developed cervical lymphadenopathy along with vomiting and abdominal pain during the hospital stay. LFT was found to be worsening. Peripheral smear showed microcytic hypochromic anaemia with thrombocytopenia and reactive lymphocytosis.

Sputum culture showed no AFB and no growth on culture. CBNAAT was negative for MTB. Repeated Blood cultures showed no growth. Lymph node cultures showed no growth. Stool routine was negative for opportunistic pathogens

Left Cervical Lymph Node FNAC done showed granulomatous lymphadenitis as shown in Fig. 1.

Lymph node biopsy was done which revealed Bacterial Infective lymphadenitis as shown in Fig. 2.

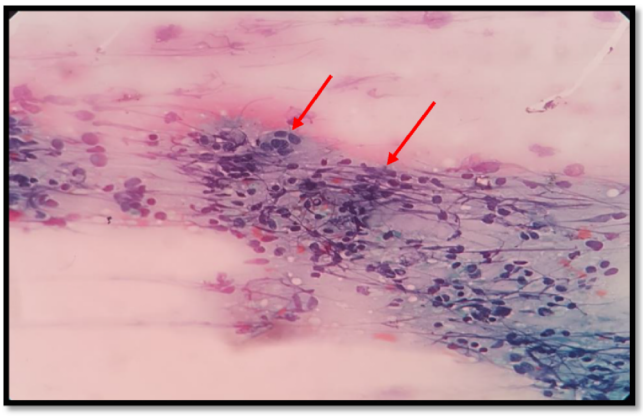

Fig. 1. Lymph node FNAC showing granulomas (red arrows) suggestive of granulomatous lymphadenitis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

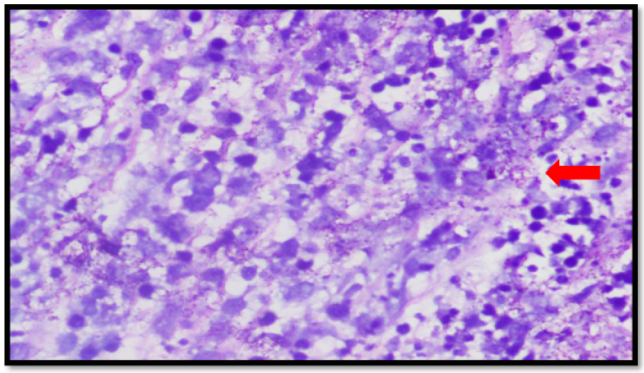

Fig. 2. Giemsa stain showing bacterial aggregates (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

FISH study was done and was reported positive for Atypical mycobacterium – Mycobacterium Avium Complex (MAC).

4. Outcome and follow up

Patient started on MAC treatment. Ethambutol 800 mg OD and Azithromycin 500 mg OD started. Patient restarted on ART treatment with Tenofovir, Lamivudine with Atazanavir/Ritonavir on 03/08/2017. Fever spikes subsided; Lymph node resolved. On 10/08/17, LFT showed AST – 22 U/L, ALT- 94 U/L and ALP- 642 U/L. Patient symptomatically better. Showed good clinical improvement and was discharged shortly. Patient showed good recovery and is now on regular follow ups.

5. Discussion

Mycobacterium avium Complex (MAC) is a serologic complex of Mycobacterium avium and Mycobacterium intracellulare. Infection with MAC is extremely common in the late stages of HIV disease [1,4]. The risk of infection increases linearly with decreasing CD4 counts below 50 cells/mm^3 and with interruption of antiretroviral therapy [5,6].

After the entry of MAC organisms from the environment through inhalation or ingestion, the infection spreads via local lymphatics, disseminating haematogenously. The bacteria are taken up by mononuclear phagocytic cells throughout the body, and reticuloendothelial organs such as the liver, spleen, and bone marrow are the most frequently affected sites [7].

Fever can be seen in around 85% of patients with MAC infection. Some other clinical manifestations include weight loss, hepatosplenomegaly and intra-abdominal lymphadenopathy. Night sweats, diarrhoea, nausea and vomiting, and abdominal pain have also been reported [1,4]. Laboratory tests may show anaemia and elevated levels of alkaline phosphatase and lactate dehydrogenase [1,5].

A rise in SGOT, SGPT was reported in this case, which could be part of granulomatous hepatitis due to disseminated MAC infection.

A marked elevation in alkaline phosphatase is a special characteristic of disseminated MAC infection. The underlying pathology for increased alkaline phosphatase in HIV with disseminated MAC infection is due to the granulomatous infiltration and obstruction of the terminal branches of the biliary tree [6].

Although, a single positive blood culture is considered evidence for disseminated MAC disease; intermittent bacteraemia in the presence of disease may delay positive identification of MAC by this method [8]. Fever in HIV patients should not be attributed to HIV infection itself. Diagnostic evaluation should be aggressive, and is most effectively guided by clinical symptoms, associated conditions, and stage of HIV disease [1]. Investigations with high diagnostic yield include blood, sputum cultures for mycobacteria, lymph node aspiration and biopsy,

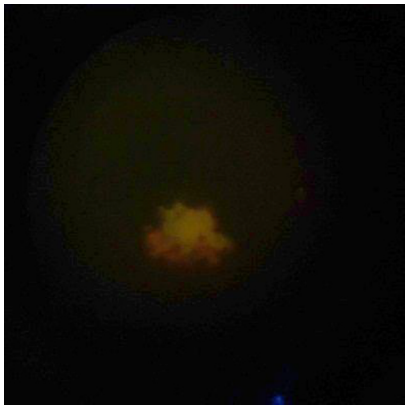

Fig. 3. Absence of Mycobacterium tuberculosis complex hence no fluorescence is seen.

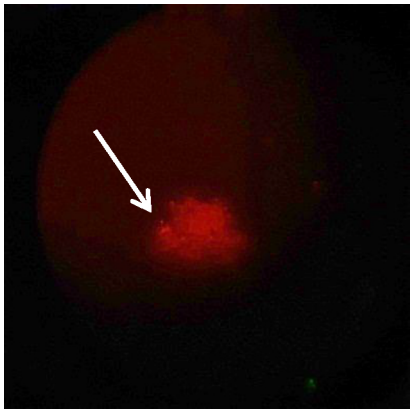

Fig. 4. Presence of Mycobacterium Avium Intracellulare complex as orange fluorescence by the bacilli is seen (white arrow).

and bone marrow biopsy. FISH will help reduce delays in diagnosis and initiating treatment of patients in many tuberculosis-endemic countries. [9]

5.1. Role of FISH in diagnosis

Fluorescent in situ hybridisation (FISH) is a molecular cytogenetic technique that utilizes short complementary fluorescent probes to localize and detect specific target DNA or RNA sequences. The presence of multiple copies of rRNA in the cytoplasm enables the reaction with specific probes to be visualized without PCR amplification of the target sequence [9].

MTBC-MAC FISH assay was used in this case. Initially, the cervical lymph node aspirate was smeared on to a glass slide. The slide was then treated with green and orange fluorescent probes specific for Mycobacterium tuberculosis complex (MTBC) and Mycobacterium avium intracellulare complex respectively [9,10].

The slide was observed under fluorescence microscope with dual filters (blue and green), since the bacilli fluoresced under green filter it was considered as Mycobacterium avium intracellulare complex as shown in Figs. 3 and 4.

This technique helps to detect and identify MTB and atypical mycobacteria, thus making it an important diagnostic tool [9].

6. Conclusion

In late stages of HIV infection, in those with low CD4 counts, the risk of MAC infection is higher. In this case, even though the patient was on HAART for the last 15 years, due to non-compliance to ART, he developed MAC infection. Routine diagnostic methods such as blood culture and lymph node cultures were negative for MAC. MAC was however, finally detected and reported positive on FISH. A high index of clinical suspicion for MAC must be maintained in advanced HIV for its early identification and prompt initiation of treatment. FISH can therefore be a valuable and effective diagnostic tool in helping identify MAC.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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CRediT authorship contribution statement

Vikas Prabhu: Investigation, Writing - original draft, Visualization. **Steffi Coelho:** Investigation, Writing - review & editing. **Basavaprabhu Achappa:** Conceptualization, Methodology, Supervision. **Shrikala Baliga:** Investigation, Resources. **Leesha Sharon:** Investigation, Resources. **Jyotsna Shah:** Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Gregor L. Case report: mycobacterium avium complex infection as a cause of fever of unknown origin in HIV disease. Dalhousie Med J 1997;25(2):10–3.
[2] Asmar S, Drancourt M. Rapid culture-based diagnosis of pulmonary tuberculosis in developed and developing countries. Front Microbiol 2015;6:1184.
[3] DeLong EF, Wickham GS, Pace NR. Phylogenetic stains: ribosomal RNA— based probes for the identification of single cells. Science 1989;1243:1360–3.
[4] Horsburgh CR. Mycobacterium avium complex infection in the acquired immunodeficiency syndrome. N Engl J Med 1991;324(19):1332–8.
[5] Karakousis PC, Moore RD, Chaisson RE. Mycobacterium avium complex in patients with HIV infection in the era of highly active antiretroviral therapy. Lancet Infect Dis 2004;4(9):557–65.
[6] El Chaer F, Harris N, El Sahly H, Hemmige V, Martinez Blanco E, Woc-Colburn L. Mycobacterium avium complex-associated cholecystitis in AIDS patient: a case description and review of literature. Int J STD AIDS 2016;27(13):1218–22.
[7] Horsburgh Jr. CR. The pathophysiology of disseminated Mycobacterium avium complex disease in AIDS. J Infect Dis 1999;179(suppl 3):S461–5.
[8] Benson CA, Ellner JJ. Mycobacterium avium complex infection and AIDS: advances in theory and practice. Clin Infect Dis 1993;17(1):7–20.
[9] Shah J, Weltman H, Narciso P, Murphy C, Poruri A, Baliga S, et al. Dual color fluorescence in situ hybridization (FISH) assays for detecting mycobacterium tuberculosis and mycobacterium avium complexes and related pathogens in cultures. PLoS ONE 2017;12(4):1–14.
[10] Baliga S, Murphy C, Sharon L, et al. Rapid method for detecting and differentiating Mycobacterium tuberculosis complex and non-tuberculous mycobacteria in sputum by fluorescence in situ hybridization with DNA probes. Int J Infect Dis 2018;75:1–7. https://doi.org/10.1016/j.ijid.2018.07.011.