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Clinicopathological Conference Report

Coexistence of *Mycobacterium tuberculosis* and *Mycobacterium avium* with Disseminated Cryptococcosis in a HIV Positive Patient

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This case was discussed on 18th January 2012 as a staff clinicopathological exercise at Postgraduate Institute of Medical Education and Research, Chandigarh, India

Clinical Protocol (Dr Aman Sharma)

This 42-year-old male came with complaints of fever of 15 to 20 days duration which was low grade, intermittent with no diurnal variation. There was headache for the same duration which was frontal, insidious in onset, moderate in intensity, lasting for 2 to 3 hours and was relieved with analgesics. He developed altered sensorium 5 days prior to admission which was insidious and progressive. He was not able to recognize his relatives. The patient had had 3 episodes of generalized tonic-clonic seizures over 2 days prior to admission. He also had a sudden painless loss of vision of the right eye a week before admission. He had lost vision in the left eye in childhood due to trauma. There was history of loss of appetite and significant loss of weight (77 to 64 Kg over 4 months). He was diagnosed to be HIV positive in August 2011. At that time his CD 4 count was 39 and he was started on zidovudine, lamivudine and nevirapine at ARTC, Patiala. His wife had died 6 years back, was emaciated with a possibility of being HIV positive. He was a farmer and continued to attend his work till a month back. There was no history of cough, expectoration, shortness of breath, pain abdomen or any vomiting. There was no past history of DM/HT/TB/seizure disorder. He was a reformed alcohol consumer.

On examination: The pulse was 98/min, regular and all peripheral pulses were palpable. The blood pressure was 180/130 mm Hg and later came down to 140/90 mm Hg. He had a poor oral hygiene and seborrhea over the beard. Chest examination revealed bilateral normal vesicular breath sounds and there were no added sounds. The cardiovascular system was within normal limits. The abdomen was soft with diffuse tenderness but there was no organomegaly or any free fluid. CNS examination assessment revealed E4M5V3 status. He was conscious, irritable, neck rigidity was present and the Brudzinsky's sign was positive. The right pupil was 4 mm and sluggish. Fundus examination revealed papilledema on the right side. He was moving all four limbs, deep tendon reflexes on both sides were 2+and plantars revealed bilateral withdrawal.

Laboratory investigations: Hemoglobin 11.4 mg%, TLC 3000/ul, serum Na 132 mEq/L, serum K 4.2 mEq/L, blood urea 26 mg%, screatinine 0.5 mg% and bilirubin of 0.9 mg%. CSF examination: TLC–no cells; sugar 10 (CBS 112 mg%); protein 44 mg% ; Gram stain/India ink—budding and capsulated yeast, C/S of the CSF showed growth of yeast species; ABG- pH 7.45 PaO_2- 63.6, $PaCO_2$ 36.3, bicarbonate-24.9 SaO_2-93.2%. Coagulogram:-PT 15", aPTT 40" (range: 25-32"), PTI 93%.

Radiology (Dr Vivek Gupta): Chest X-ray (Fig. 1) showed mediastinal widening with lobulated contour possibly due to lymphadenopathy. Upper zone vascular prominence is also seen.

NCCT and CECT head showed prominent sulci and cisterns suggesting cerebral atrophy. No definite focal lesion or meningeal enhancement seen.

Course in hospital: This 42 years gentleman who was detected to have AIDS in August 2011 and was started on ART presented in December 2011 with fever, headache, altered sensorium, high blood pressure and was started on antihypertensives, antiedema measures and antibiotics. Blood pressure was controlled but the sensorium worsened and he developed cardiorespiratory arrest from which he could not be revived and was declared dead on 4/12/11.

The unit's final diagnosis was- HIV+/AIDS, cryptococcal meningitis, seborrheic dermatitis, hypertension with hypertensive emergency.

Clinical Discussion

In the database, we have a 42-year-old gentleman, who was started on ART at ARTC Patiala 4 months before his demise, presented with fever, headache, altered sensorium with loss of appetite and weight (13 Kg over 4 months). He had high blood pressure, signs of meningeal irritation and CSF examination documented branching and encapsulated budding yeast, low sugars and growth of yeast species. CXR showed mediastinal lymphadenopathy. His CD4 count at the time of presentation were only 39, which means that he presented late in the course of HIV infection as it would take up to 10 years from the time of acquisition of HIV infection for CD4 count to fall to that level in usual progressors. At that low CD4 level, there is risk of various opportunistic infections like *Cryptococcus*, toxoplasmosis, atypical mycobacteria, PMLE and CMV. There are various neurological conditions seen in HIV patients dependent upon the level of CD4 counts. When the CD4 count is more than 500, headache, aseptic meningitis, meningo-encephalitis, peripheral neuropathy, radiculopathy, brachial neuritis and LGBS can occur. When CD4 count is between 200 and 500, LGBS, CIDP, polymyositis, Bell's palsy, TB meningitis and PMLE can occur. At CD4 less than 200, opportunistic infections like cryptococcal meningitis, toxoplasmosis, TBM, PMLE, HSV encephalitis, CMV encephalitis, AIDS dementia complex, nocardia brain abscess, painful peripheral neuropathy, CNS vasculitis, autonomic neuropathy and myelopathy are reported.

As this patient had fever, headache, altered sensorium, with signs of meningeal irritation with CSF abnormalities, he definitely had meningoencephalitis. The causes of meningitis in chronic HIV infection can be fungal like cryptococcal, histoplasmosis, coccidioides; Viral like CMV, HSV and EBV; bacterial like Listeria, *T. pallidum*, pyogenic, atypical or conventional mycobacterial and neoplastic like lymphoma. The CSF examination gives us the clue as the low CSF sugars point toward bacterial, fungal or parasitic infection but with smear showing encapsulated budding yeast with growth of yeast species, we cannot go away from cryptococcal meningitis. It occurs when the CD4 count is less than 200/µl. They present with headache in 90%, fever in 75% and papilledema in a third of patients at diagnosis. The poor prognostic markers for cryptococcal meningitis are abnormal mental status, CSF opening pressure >250 mm, CSF cryptococcal antigen titer >1:1024, CSF white cell count <20 cells/ml and extraneural culture of *Cryptococcus*. According to our own data of 91 cryptococcal meningitis patients of the 6900 HIV patients registered at PGIMER, Chandigarh, 91% had fever, 90% had headache, 13% had blurring of vision and 9.9% had seizures.

Pulmonary manifestations of cryptococcal infection are in the form of chest pain in 40%, and cough in only 20% but the CXR findings are in form of diffuse infiltrates. Hilar adenopathy, effusions and cavity formation are very uncommon. Histoplasmosis mimicks tuberculosis but the usual presentation is in form of fever, emaciation, hepatosplenomegaly and lymphadenopathy. One-fourth of these patients may have focal presentation and meningitis can be one of them but histoplasmosis does not have encapsulated forms on smear though tissue forms may occasionally have pseudocapsule. So this is unlikely. CMV infection presents with fever, leukopenia, esophagitis and retinitis. Two types of CMV encephalitis are reported, one mimicking HIV encephalopathy which presents with progressive dementia and the other as ventriculoencephalitis which presents as cranial nerve palsies, disorientation and ventriculomegaly. Due to absence of retinitis and the classical CMV encephalitis, this seems unlikely.

Mycobacterial infections especially atypical myco-bacterial infections can occur in advanced immuno-suppression. Dual infections with *Cryptococcus* and mycobacteria have been reported from India previously. Dual infections of CMV and EBV have been reported from HIV patients with pyogenic meningitis from Africa. We have seen chronic meningitis due to dual infection of MTB and *M. avium* in patients with CD4 less than 50. These infections are being increasingly recognized by us lately due to the facility of multiplex PCR. Toxoplasmosis, PMLE and CNS lymphoma are unlikely as these usually present with focal signs, which were missing in this patient. AIDS dementia complex presents with 3 to 6 months history of cognitive decline and behavioral disturbances and so is unlikely in this patient. This patient was started on ART 4 months before this presentation so this could be 'unmasking' of occult opportunistic infection. This occurs due to reconstitution of antigen specific T cell mediated immunity with activation of immune system against intact or dead organisms after starting ART. Practical NACO definition of IRIS is occurrence of new opportunistic infections within 6 weeks to 6 months after initiating ART with increase in CD4 count. As we do not have a repeat CD4 count, we cannot go any further on this. Seizures in HIV can be due to various causes like HIV encephalopathy, toxoplasmosis, cryptococcal meningitis, primary CNS lymphoma and PMLE. This patient could have had seizures due to cryptococcal meningitis. There are various causes of cytopenias in HIV, HIV infection *per se*, drugs like zidovudine, co-trimoxazole, infections like fungal, mycobacterial and parvo virus.

Glomerulonephritis, IgA nephropathy and TTP have been associated with malignant hypertension but we do not

have any clues to any of these. Low PaO_2 in this patient may be due to lung involvement due to opportunistic infections. Finally lupus anticoagulant without any clinical significance but with prolongation of pTTK has been reported in HIV patients and the same may explain the abnormality in this patient.

So my final diagnosis is HIV/AIDS with cryptococcal infection which definitely is causing meningoencephalitis, but may be disseminated. There may also be other dual/polymicrobial infections especially atypical mycobacterial infections and the cause of death seems to be due to raised intracranial pressure.

Open House Discussion

Dr Sakhuja opened the case for discussion. Dr Lal disagreed with the conclusion of raised intracranial pressure as the CT scan done a day before demise was normal with no evidence of hydrocephalus. Further, he stated that papilledema which means disk edema may be due to raised ICP. However, all patients of disk edema do not have papilledema. The sudden vision loss is very odd as papilledema does not cause a sudden visual loss; rather it causes visual loss very late in the course of the disease due to optic atrophy. He opined that the patient had optic neuritis due to direct infiltration by *Cryptococcus* and that the cause of death was not raised ICP but disseminated cryptococcosis with probably other added infections. Dr Sharma felt that cryptococcosis causing death without a raised ICP is difficult to explain. Professor Sakhuja wondered if the papilledema was linked to hypertension rather than raised ICP and also said that the cause of hypertension was not determined. Professor Verma commented that acute ischemic anterior neuropathy in malignant hypertension can mimic papilledema and may cause visual loss. Raised ICP seems unlikely as the pulse rate was high, i.e. 98/min. The mediastinal lymphadenopathy could be due to infections like *Cryptococcus*/TB or in the present setting even an extranodal lymphoma. Dr Sakhuja concluded the clinical discussion and invited Dr Swapnil Rane to demonstrate the pathology.

Pathology Discussion (Dr Swapnil U Rane)

A complete autopsy was performed in this 42-year-old HIV positive male. The prosector noted the patient to be well built with seborrhea on the scalp. The serous cavities were within normal limits.

- *Central nervous system:* External examination showed the brain to be mildly edematous, overweight (1554 gm) with uncal and tonsillar notching. Meninges showed slimy white exudates over both cerebral convexities, while the basal meninges were dull. Large cerebral vessels did not show any thrombus. On coronal slicing, mild ventricular dilatation was noted. A single lacunar infarct was seen in the subcortical white matter in the right frontal lobe. Histological examination (Figs 2A and B) showed meningeal expansion of the entire brain and cord, more in the cerebral hemispheres packed with abundant yeasts with narrow based budding and thick capsule which was highlighted by mucicarmine and *alcian* blue stains, consistent with the morphology of *Cryptococcus*. Meningeal reaction was variable with areas of no reaction to areas showing macrophages and multinucleated giant cells and an occasional focus showing epithelioid cell granuloma with Langhan's giant cell. No necrosis was seen. Cryptococci were identified within the granulomas as well as within the multinucleated giant cells. The meningeal inflammation was extending into the Virchow Robbin's spaces surrounding the blood vessels in the brain parenchyma leading to their expansion and formation of microcyst. Focally there was a breach of the blood brain barrier with extension of the cryptococci into the brain parenchyma. Ventricles and choroid plexus also showed similar giant cell reaction laden with cryptococci (Fig. 2B). Lacunar infarcts seen grossly were confirmed. Stain for AFB was negative. A single focus of microglial nodule was seen.
- *Lungs:* Pleural surface was dull with few tags. Lungs weighed 750 gm, were subcrepitant with patchy areas of consolidation. Left upper lobe shows parahilar consolidation with white discoloration of the lung parenchyma. Adjacent parahilar lymph nodes show areas of caseation. No infarct or thromboembolism is noted. Histological examination (Figs 3A to D) from the left upper lobe showed many airspace epithelioid cell granulomas with central caseation, surrounded by lymphocytes and Langhan's type of giant cells. Similar granulomas were also seen in the parahilar lymph nodes. Ziehl- Neelsen's stain and auramine-rhodamine stain identified many mycobacteria in the lung as well as the lymph node. In addition, the same lymph node also showed the presence of cryptococccci at the edge of the caseation and within the giant cells. Rest of the lung parenchyma showed interstitial expansion with presence of ill-defined interstitial granulomas with many macrophages and multinucleated giant cells loaded with cryptococci. Carinal lymph nodes also showed abundant cryptococci replacing the lymph node parenchyma, imparting a foamy appearance (Figs 4A and B). No thrombi or emboli are noted. Many circulating cryptococci are also seen in the capillary vessels. Ultrastructural examination did not reveal any other organism or viral particle.

Heart: 360 gm, concentric left ventricular hypertrophy (LV wall thickness 2 cm) with papillary muscle hypertrophy, borderline right ventricular hypertrophy (RV wall thickness 0.6 cm) and dilatation was seen. Aorta showed grade II atherosclerosis, while coronaries show calcified plaques. Coronary lumina were not significantly occluded. Left ventricular wall is mildly discolored and showed myocyte hypertrophy, atrophy and many foci of myocyte necrosis with interstitial inflammation composed of lymphocytes and occasional multinucleated giant cell. No organisms were identified in any section on PAS-AB, Grocott and ZN stain. In addition, myocytes show variable degenerative changes, with increase in perivascular and interstitial fibrosis. Mild endocardial sclerosis and thickening is also noted. Features are of myocarditis with a possibility of HIV cardiomyopathy.

Liver: Liver was normal except for scattered foci of lobular inflammation, and focal loose epithelioid cell granuloma with multinucleated giant cells with cryptococci.

Spleen: 150 gm, depletion of T-zone area with relatively preserved B-zone area seen. Subcapsular region and sinuses showed foamy macrophages with occasional multinucleated giant cells with *Cryptococcus* in their cytoplasm. Stain for AFB is negative.

Gastrointestinal tract: Grossly and microscopically within normal limits. Appendix and ileum showed depletion of lymphoid tissue.

Mesenteric lymph nodes: All lymph nodes were small with depletion of the parafollicular lymphoid cells, while follicular areas were relatively preserved. Occassional multinucleated giant cell as well as ill-formed granuloma are seen with presence of cryptococci within them. Stain for AFB is negative.

Kidneys: Glomeruli show presence of cryptococci within the capillary loops distending them and in the urinary space. Focal granulomatous reaction, extending into the adjacent interstitum noted. AFB was negative. Arteries showed intimal thickening. Arterioles were within normal limits. Immunofluorescence showed complement activation surrounding the cryptococci. Glomeruli were negative for all immunoglobulins and complement. Ultrastructural examination did not reveal any viral particle.

Urinary bladder and prostate: Urinary bladder was normal grossly and microscopically, while prostate shows presence of nodular aggregates of cryptococci walled off by fibrosis.

Adrenals: Both adrenals show presence of cyrptococcal microcysts as well as focal lymphoid aggregates and loose granulomas surrounding cryptococci. In addition, sinusoids also show cryptococci.

Testis: Interstital inflammation and an occasional multinucleated giant cell. No definite fungal profile or AFB is seen.

Bone marrow: Bone marrow was normocellular and shows adequate megakaryocytic and erythroid series. Circulating cryptococci were noted within the capillaries.

PCR: Dr Kusum Sharma (Dept of Microbiology)—Postmortem lung was subjected to multiplex PCR by using primers specific for *Mycobacterium tuberculosis* and *M. avium* and showed positivity for both (Fig. 5).

Final Autopsy Diagnosis

A 42-year-old male, HIV positive with AIDS on ART, with

- Disseminated cryptococcal infection involving:
  - Central nervous system—causing cryptococcal meningoencephalitis, ventriculitis, choroid plexitis, cerebral edema, uncal and tonsillar herniation
  - Lungs, kidneys, adrenals, spleen, liver, lymph nodes and bone marrow
- Pulmonary tuberculosis with caseous pneumonia in left upper lobe and tubercular lymphadenitis in parahilar lymph nodes—mutiplex PCR positive for both *Mycobacterium tuberculosis* and *Mycobacterium avium*
- Myocarditis—possibly HIV cardiomyopathy, along with concentric left ventricular hypertrophy.

Open House Discussion

Dr Sakhuja opened the case for discussion with the comment aprt from *Cryptococcus*, the additional infection was

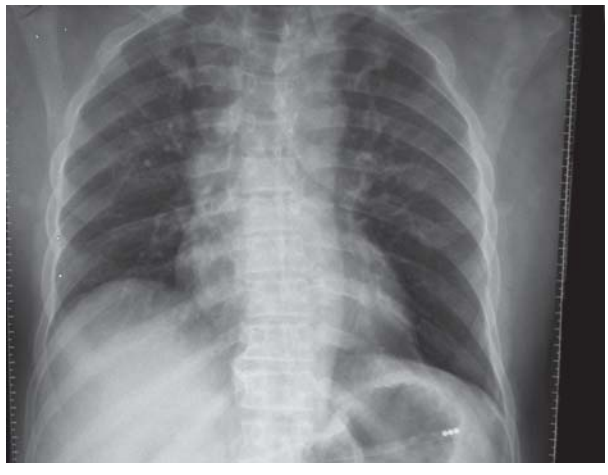
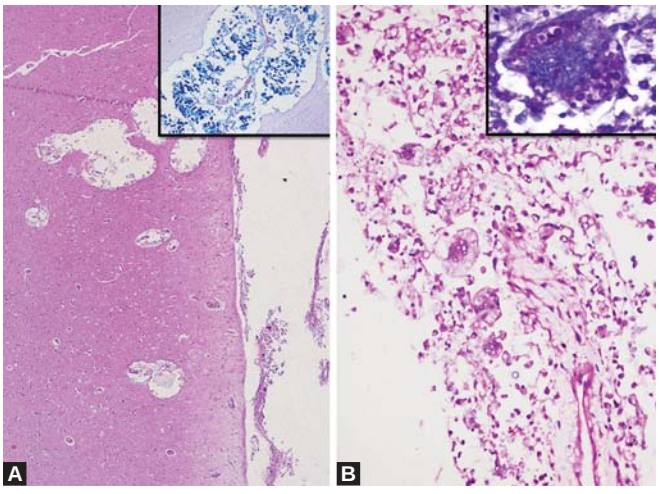
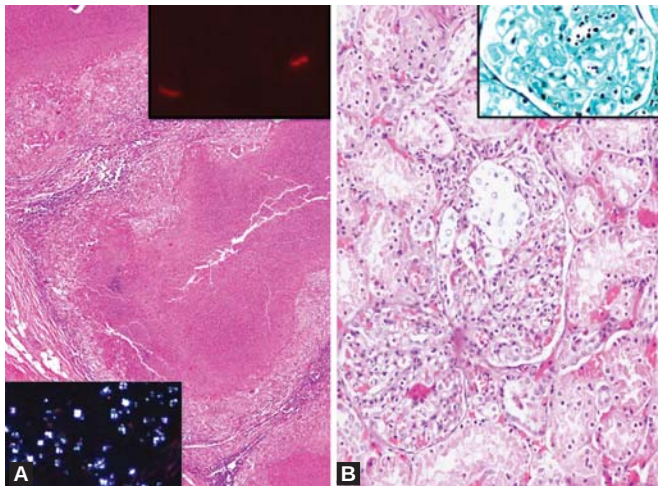


Fig. 1: Chest X-ray showed mediastinal widening with lobulated contour possibly due to lymphadenopathy. Upper zone vascular prominance also seen

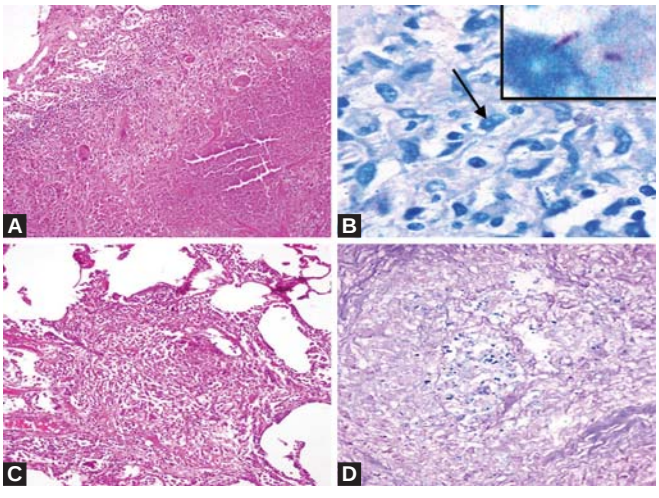
tuberculosis. The involvement of kidneys was unusual. The cause for hypertension remained unexplained. He also wanted the pathologist like to comment as to how frequently cryptococci are seen within the glomeruli. Dr Rane mentioned that in immunocompromised patients, cryptococci within the glomerular capillary loops as well as in the urinary space have been noted a couple of times but in autopsies. A resident mentioned that molecular techniques like PCR are required for demonstration of *M. tuberculosis* and atypical mycobacteria and that the treatment for the two infections was different. Dr Aman Sharma reiterated that these two infections can coexist and molecular diagnosis is important. Dr Yash Pal Sharma opined that the cause of hypertension in the present case seems to be essential. Accelerated hypetension, atherosclerosis, endocarditis and myocarditis are known to



Figs 2A and B: (A) Histopathological section (H and E stain, 100x total magnification) of cerebral cortex showing meningitis with perivascular extension into the brain forming perivascular pseudocysts which are filled with cryptococcal spores (inset-PAS-AB stain) (B) Choroid plexitis with presence of numerous giant cells with a foamy cytoplasm which are filled with numerous crytococcal spores (inset: PAS stain)



Figs 4A and B: (A) Lymph node (H and E stain, original magnification 100x) showed central caseation surrounded by foamy macrophage collections. The central caseation showed many bacilli on Auramine Rhodamine stain(Upper inset) consistent with *M. tuberculosis*. The foamy macrophages on the other hand were filled with cryptococci which showed the Maltese cross bireferengence on polarised light (lower inset) (B) H and E stained photomicrograph (original magnification 200x) of kidney showed circulating cryptococci within the glomerular capillary loops leading to their distension. The cryptococci are better appreciated on the Grocott stain (upper right inset)



Figs 3A to D: Panel of lung photomicrographs showing co-infection with tuberculosis and cryptococcus (A) Low power photomicrograph (H and E stain, Original magnification 100x) showing airspace epithelioid cell granulomas with caseation and langhans type of giant cells. (B) Ziehl-Neelsen stain (1000x original magnification) showed acid fast bacilli consistent with *M. tuberculosis* (C) Lung also showed interstitial epithelioid cell granulomas (H and E stain, 200x original magnification) which show cryptococci on (D) PAS-AB stain

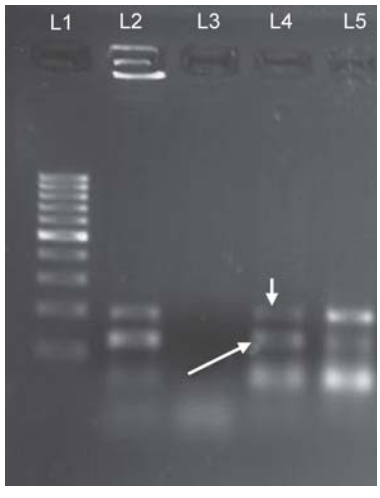


Fig. 5: Multiplex PCR done from the DNA extracted from the formalin fixed tissue. Lane 1—L1-molecular markers, L2—positive control. L3—negative control, L4—DNA from the index case, L5—positive control. In L4—the upper band is 187 bp—*M. avium* (small arrow) and the second band is 123 bp—*M. tuberculosis* (long arrow)

occur in HIV positive patients. In HIV patients, myocarditis may be subclinical; myocardial hypertrophy could have be picked up by the ECG. Dr Aman Sharma mentioned that as far as treatment protocols are concerned in HIV positive patients, the threshold for treatment used to be a CD4 count of 200/µl, but now it is CD4 count of 350/µl. In India, treatment is initiated at a CD4 count of 350/µl. However, there is no consensus or any robust evidence for initiating treatment with a CD4 count of >500/µl. Dr Sanjay Jain opined that it is practically impossible to label whether this patient had essential hypertension, because the brunt of the hypertensive disease is born by the blood vessels, and there have been no significant changes in the blood vessels as shown in the pathology presentation. However, as was shown in this case, many of the afferent arterioles in the kidney were damaged by the cryptococcal infection, and that by itself could also result in renin mediated hypertension. There is evidence of raised intracranial pressure as evidenced by uncal herniation. In cryptococcal infection, it is not necessary to have ventricular dilatation or any sign of hydrocephalus. Typically, there are two patterns are seen, one is a compressed ventricle due to cerebral edema and other is dilated ventricles. And documentation of raised ICP in cryptococcal meningitis is based on direct measurement of the intracranial pressure. Dr Aman Sharma concurred with Dr Jain. Dr Radhika Srinivasan said that that CD4 count of this patient at the time of admission to PGI was not available. The fact that this patient had caseous necrosis in the lung, walled off by a granulomatous response along with a histiocytic and giant cell response in the CNS, indicates that his CD4 count may not be very low. She asked the pathologist to clarify about the status of the mucosa associated lymphoid tissue in this patient. Dr Rane said that the GIT, especially the ileum and appendix were depleted of the normal lymphoid tissue. There were hardly any lymph nodes identifiable in the entire body. The only preserved and demonstrable lymph node which he could identify, were in the mesentery, which on histological examination showed relatively preserved follicles with parafollicular atrophy. The mediastinal lymph nodes were enlarged, but that was not due to hyperplasia of the lymphoid tissue, but due to involvement by either cryptococcomas or by the caseation caused by tuberculosis. Prof Sakhuja mentioned that although this patient was on triple drug HAART, we were not sure about his compliance. Dr Aman said that since the patient had mounted a granulomatous response, there might be an unmasking of the disease due to immune reconstitution syndrome (IRIS), once the immunity had improved. Dr Rane countered that immune reconstitution syndrome was considered as a possibility, but, due to the lack of information on the CD4 count of this patient, we cannot label this patient to have IRIS. Dr Verma opined that the high load of *Cryptococcus* in this patient and the low CSF cell count was low pointed to severe disseminated cryptococcal disease and so, to say that this patient was actually mounting an immune response or having IRIS is purely presumptive. Dr Rane agreed that a diagnosis of IRIS cannot be made in this patient. Pathologically, patients with IRIS tend to have a very localized disease, with smears and cultures being negative, due to the infection being walled off by the immune response. On the other hand, this patient had a disseminated disease. Prof V Sakhuja concluded the case discussion.

COMMENTS

This case is unique due to the coexistence of *Mycobacterium tuberculosis* and nontubercular Mycobacterium (NTM) infections in HIV positive patients which is now being documented worldwide. Polymerase chain reaction (PCR) is the most rapid and sensitive method for diagnosing mycobacterial infections and identifying the etiological mycobacterial species in order to administer the appropriate therapy for better patient management.

NCCT/CECT are very poor modalities for detecting cryptococcal meningitis/meningoencephalitis. This was however, proven in this case. An MRI would have clinched the diagnosis.