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Extrapulmonary Tuberculosis: A Sleeping Giant !! A Case Series of EPTB Diagnosed Microbiologically by CBNAAT Technique.

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Abstract
TB manifests clinically as pulmonary or extrapulmonary tuberculosis (EPTB), with the former being more common. In India, 10 to 15% of TB cases are estimated to be cases of EPTB. Here we report the analysis of four EPTB cases which had been diagnosed on the basis of CBNAAT and other diagnostic evidences. ***Methodology:*** *The collected specimens received at Pravara Rural Hospital, Loni were subjected to CBNAAT (GeneXpert) testing. The case information (history, records and investigations) was accessed through the 'Medical record department'.* ***Case series: Case1*** *was a case of Pulmonary Tuberculosis with mediastenal lymph node enlargement with MTBC found in a pus sample.* ***Case 2*** *was a case of TB meningitis with metabolic encephalopathy diagnosed microbiologically by CBNAAT technique.* ***Case 3*** *was a case of Multidrug resistant TB meningitis and* ***case 4*** *was a case of abdominal TB with pulmonary focus positive.* ***Conclusion:*** *CBNAAT testing is proving to be a game changer for making a rapid diagnosis of Multidrug Resistant Extrapulmonary tuberculosis.*

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I. Introduction

In an ancient texts from India, tuberculosis (TB) is referred to as '*Rogaraj*- the king of disease and '*Rajayakshma*- the disease of kings'. John Bunyan's 'Captain of all of these men of Death' emphasizes that this disease was and in many countries still is, foremost among the cause of morbidity and mortality.[1] TB manifests clinically as pulmonary or extrapulmonary tuberculosis (EPTB), with the former being more common. In India, 10 to 15% of TB cases are estimated to be cases of EPTB.[2]

Extra pulmonary tuberculosis (EPTB) comprises a wide disease spectrum affecting most of the organs of the body except lungs. It commonly affects the sites which are lymph nodes, pleura, urogenital tract, bones and joints, meninges, intestines, peritoneum, pericardium and skin. Although morbidity, mortality and complications due to EPTB are common, it is generally underestimated as contagious potential of this form is negligible.[3] In this scenario, not only rapid detection of tuberculosis, but also the early determination of MDR status is important. Diagnosis of EPTB is challenging due to its myriad clinical presentation, difficulty in collection of the specimen from deep-seated tissue, paucibacillary nature of the disease.[4]

Various techniques are employed for the laboratory diagnosis of EPTB such as Acid fast staining, Fluorescent staining, culture, histopathology, tuberculin skin test (TST), serological assays, interferon-gamma release assays (IGRAs).[5] However, sensitivities & specificities of these tests are variable. Also, some of these tests require a number of manual steps, and some having relatively long turnaround time. A rapid diagnosis of *Mycobacterium tuberculosis* complex (MTBC) is crucial for prevention and treatment of tuberculosis and to break the chain of transmission. CBNAAT is cartridge-based nucleic acid amplification test/ (MTB/RIF) which detects the presence of tubercular bacilli and also check susceptibility to rifampicin within 2 hours. CBNAAT is likely to revolutionize the diagnosis and treatment of EPTB, as it is a very cost-effective and rapid test. World Health Organization formulated new guidelines in 2013, advocating the use of Xpert MTB/Rif assay for the diagnosis of EPTB.[6]

The CBNAAT laboratory in our tertiary care center receives pulmonary as well as extrapulmonary samples from the rural area around Loni. Here we report the analysis of four EPTB cases which had been diagnosed on the basis of CBNAAT and other diagnostic evidences.

II. Methodology

The specimens received or collected at our tertiary care center were subjected to CBNAAT (GeneXpert) testing. These specimen were processed according to manufacturer's instructions, standard operating procedure guidelines given by WHO & '**Technical & operational guidelines for Tuberculosis control in India (2016)'.**[7] The case information (clinical history, records and investigations) was accessed through the 'Medical record department' of Rural Medical College, Loni. Ethical approval was taken for the study from Institutional ethical committee

III. Cases

Case 1: 40 year/ female hailing from Sanagamner, was referred (as suspected case of pulmonary Koch by private practitioner) to the casualty department of Pravara Rural Hospital, Loni with chief complains of mild grade fever (on & off), generalized weakness & loss of appetite since one month. There was no history of cough, cold, throat pain, chest pain, palpitation, abdominal pain. On examination, patient's general condition was normal with pulse (70/ min), BP (100/60 mm Hg). Pallor was present. On systemic examination, air entry was decreased in right upper lobe, crackles were present in right middle & left upper lobe. There were no other abnormal findings detected in the systemic examination. On High resolution computed tomography thorax minimal pleural effusion was observed in right side of the lung and mediastinal lymph nodes were enlarged. On Ultrasonography there was minimal pleural effusion on right side. Laboratory Investigations in which haemogram revealed decreased Hb (9.2 g/dl). Her ESR was raised (40mm/1h). Patient's SGOT (55IU/ L) & LDH (357 U/L) were raised.. Her sputum culture revealed growth of *Klebsiella pneumoniae & candida* spp. Blood culture report was sterile. Patient's Rapid ICT test for Dengue & Malaria was negative. Serological tests (Weil felix & Widal) didn't show any abnormal values. Sputum sample was sent to CBNAAT & AFB staining was found to be negative. The patient's serum sample was negative for HIV antibody test. The patient was diagnosed as case of Pulmonary Tuberculosis with mediastenal lymph node enlargement and was started on Anti-tubercular therapy. After two months, patient presented in the Surgery OPD with neck swelling since 15 days (insidious in onset & gradual in progress). Pus was aspirated from Lymph node was sent for bacterial culture. Culture report was found to be sterile after 24 hours. On advice of microbiologist, this pus sample was subjected to Acid fast stain on which acid fast bacilli were found, later the same was sent for CBNAAT testing. It was found to be positive on GeneXpert (MTB DETECTED MEDIUM, Rif Resistance NOT DETECTED). Currently the patient is on treatment of EPTB and showing improvement.

Case 2-

43 year old male was visited to the local practitioner with chief complains of fever & cough since 2 days. He was treated symptomatically by the practitioner. After two weeks of asymptomatic period, he suddenly developed tonic flexed posture with up rolling of eyes with frothing from mouth at home. He also had one episode of vomiting. Personal history revealed that the patient was regular 'Tobacco (Gutka) chewer'. Patient sleep, bowel & bladder habits were normal but he was complaining of decreased appetite. Past & family history revealed no significant findings. On examination BP was 150/90 mm Hg and Pulse rate was 100/min. CNS examination revealed, patient was conscious, oriented with no neurological deficit. Rest of the systemic examination revealed no abnormal findings. On laboratory investigation, patient's haemogram revealed Hb- 9.9, TLC- 11,810, (N-83, L-11, M-6, E- 0, B-0). CSF microscopy showed high white cell count with predominant Lymphocyte. Patients' ESR was raised. Patients' biochemistry parameter showed serum raised LDH (283IU/L). Serum Na was found to be decreased (118 mEq/L). CSF biochemistry showed raised Protein (300 mg/dl), glucose- 73 mg/dl and raised LDH- 150IU/L. Renal function test revealed decreased sodium (122 mEq/L). CSF was found to be negative for Cryptococcus on Negative staining. CSF Adenosine deaminase assay was normal (9.11 U/L). ZN stain showed presence of scanty acid fast bacilli. On culture of CSF showed growth of *Stenotrophomonas maltophilia.* Patient was found to be 'Reactive' for HIV antibodies. CBNAAT testing was done on CSF which revealed 'MTB DETECTED VERY LOW and Rifa resistance NOT DETECTED. Patient was diagnosed as a case of TB meningitis with metabolic encephalopathy. He was treated for encephalopathy in the hospital. He was also referred to ART center for starting antiretroviral therapy and also initiated on the anti tubercular drugs. Patient showed the improvement due to which he was discharged with advice of continuing ART & AKT.

Case 3-

40 years old female patient, housewife by occupation, resident of Nandgaon (Ahmednagar district) brought by relatives to the Pravara Rural Hospital, came with chief complains of headache, slurring of speech, left sided weakness and on & off fever. She was a known case of migraine. There was no history of chest pain, breathlessness, giddiness. There was no history of diabetes, hypertension and asthma. No other significant history was found. On general examination, her overall condition was poor. Her pulse rate was 100/min and blood pressure was 100/60 mm Hg. No pallor, oedema or icterus was noted. On CNS examination, patient was drowsy but responding to deep pressure stimulus, plantar reflexes were decreased. All other reflexes (biceps, triceps, supinator, ankle & knee) were exaggerated with ankle clonus positive. On Cardiovascular system, Respiratory system & Per abdominal examination, no any other abnormal finding was detected. Laboratory investigations revealed: [CSF WBC count was 350/cumm (predominantly Lymphocytes). On haemogram showed decreased haemoglobin. Erythrhocyte sedimentation rate was raised. Patient's Liver function tests enzymes were raised, (SGOT- 92IU/L, Alkaline phosphatase- 359 IU/L), while Renal function testes (Urea- 11mg/dl, creatinin- 0.4 mg/dl, Serum sodium- 127 mEg/L) values were slightly decreased. CSF biochemistry revealed decreased glucose (20 mg/dl), while raised protein (192mg/dl) and raised LDH (153 IU/L). The patient was reactive for anti HIV-1 antibodies by CLIA method. Patients CSF sample was sent for CBNAAT revealed, 'MTB DETECTED VERY LOW' and 'Rifa Resistant DETECTED'.} The patient was diagnosed as a case of Multidrug resistant TB meningitis and was started Antitubercular therapy & Antiretroviral therapy. While treatment, her condition deteriorated, she was shifted on ventilator, later she was shifted to higher medical center for further management.

Case 4-

26 years female, resident of Kumbhephal (Dist- Aurangabad, Maharashtra) presented in antenatal clinic with chief complain of amenorrhea since one and half months, pain in abdomen since 15 days and PV bleeding since 3-4 days. Urine pregnancy test was done before visiting Pravara Rural Hospital, which was negative. Patient was also complaining of cough with expectoration since 5-6 days. There was no history of headache, nausea, giddiness & vomiting. Also there was no history of fever and weight loss. On obstetrics history she was P1L1. There was no history of hypertension, diabetes, tuberculosis in the past. No significant personal & family history was noted. On general examination patient was afebrile. Her pulse rate (98/min) and blood pressure (100/78) were recorded. Pallor was present but there was no sign of icterus or clubbing. On systemic examination, there was tenderness per abdomen. Rest of the systemic examination revealed no abnormality. On USG abdomen revealed left tubal abortion with hemoperitoneum. Patients hemogram revealed decreased haemoglobin (10.8 g/dl). Ascitic fluid was haemorrhagic on gross appearance, its microscopy revealed 3000 cells/cumm with predominant lymphocytes. Biochemistry investigations revealed Plasma glucose (random)- 80 mg/dl, low serum albumin (2.5 gm/dl) while high serum globulin (3.6 gm/dl), high serum LDH (266 IU/ dl), raised ESR (35mm/1h). Sputum fluorescent microscopy was positive for tubercular bacilli. Patient tested negative for Hepatitis B antigen, HIV antibodies, and Syphilis TPA antibodies on CLIA asssay. After obstetric evaluation (plus radiological evidences- which revealed 'left tubal abortion with hemoperitoneum) the patient was diagnosed as a case of ectopic pregnancy with tubular rupture. The patient was operated for the same. Intraoperatively, surgeon noticed few evidences which were suggestive of abdominal tuberculosis (adhesions as well as tubercles on the thickened peritoneum). Ascitic fluid was sent for CBNAAT testing which revealed 'MTB DETECTED VERY LOW, Rif Resistance NOT DETECTED'. Therefore patient was also diagnosed as a case of abdominal TB with pulmonary focus positive. Anti Koch treatment was started after surgery (and postoperative care) and patient is still on the treatment with improvement in the condition.

IV. Discussion

As India has high burden of TB cases, and hence proportionately higher percentage of EPTB cases are also observed.[5] The observed diverse array of manifestations of EPTB often mimics other pathological conditions which results in more diagnostic challenges. This indirectly associated with delayed diagnosis and giving it a greater potential for morbidity and mortality.[8] Thus, EPTB deserves an increasing focus for proper management of TB cases and TB control program. Here we report the four cases of tuberculosis with extrapulmonary organs involvement

In our first case, patient was diagnosed as a case of pulmonary tuberculosis on the basis of clinical & radiological findings as microbiological results were negative for pulmonary TB. Patient was discharged after starting AKT, but developed neck swelling after 2 months (MTBC detected in pus on CBNAAT). **Nwagbara *et al* (2013)** also reported a case of Pulmonary TB with lymph node enlargement.[9] Peripheral tuberculous lymphadenitis is a local manifestation of the systemic disease. It may however occur during primary TB infection, because of reactivation of dormant foci or direct extension from a contiguous focus.[10] The present case was 40 year old female. Tuberculous lymphadenopathy is reported in a studies to be rare above the age of

45 years with women more affected than men.[11,12] Tuberculous (TB) lymphadenitis is the most common presentation of EPTB and has been shown in about 35% of EPTB cases.[10]

Second case was TB meninigitis (TBM) with metabolic encephalopathy. TB meningitis is the most devastating form of meningitis and occurs in 7–12% of TB patients in developing countries.[13] The case fatality rate for untreated patients is almost 100% and delayed treatment often leads to permanent neurological damage.[14,15] Therefore, the prompt diagnosis of a case of TB meningitis is crucial for successful clinical outcome. TB meningitis can also cause metabolic complications. The commonest metabolic complication associated with TBM is hyponatraemia which affects >50% of patients with the disease.[16] Similar observation was found in the present (second) case at our center. CSF biochemistry revealed raised Protein but normal glucose. Similar observation was also found by **Jawad N et al (2017).**[17] CSF microscopy revealed high white cell count with predominant Lymphocytes and raised ESR. Typically, the CSF in TB meningitis reveals a high CSF white cell count, which is predominantly lymphocytic, with a high protein and low CSF to blood glucose ratio. The present patient was also 'tobacco (Gutka) chewer'. There is strong association between tobacco use and TB treatment outcomes.[18] Among other risk factors, patient was also infected with HIV. The risk of developing tuberculosis (TB) is estimated to be between 16-27 times greater in people living with HIV than among those without HIV infection.[19]

Our third case was also Multidrug resistant TB meninigitis. The patient was known case of migraine. Similar case was also reported by **Germa F *et al* (2002)**.[20] This patient was also infected with HIV. The incidence of CSF multidrug resistance in tuberculosis is higher in children, young adults, patients in developing countries and patients infected with HIV.[21] Patient's CSF WBC count was high (predominantly L-70%, N-30%). Erythrhocyte sedimentation rate was raised. Patient's Liver function tests enzymes were raised, while Renal function testes values were slightly decreased. CSF biochemistry revealed decreased glucose, while raised protein. Most of these values match with typical presentation of TB meningitis.

Fourth case discussed here was a case of abdominal tuberculosis, signs of which were an accidental finding during a surgery for tubal abortion (a case of ectopic pregnancy). In a study done by **Sarala K *et al* (2017)** on ectopic pregnancy, 4 % cases were associated with history of tuberculosis.[22] Abdominal TB comprises tuberculosis of gastrointestinal tract, peritoneum, mesentery and other intra abdominal organs such as liver, spleen and pancreas.[5] Intraoperatively adhesions as well as tubercles on the thickened peritoneum were observed by surgeon intraoperatively which were suggestive of abdominal tuberculosis. Similar findings were also found by **Turkdogan *et al* (1998).**[23] Sputum fluorescent microscopy was positive for tubercular bacilli and later on ascitic fluid was also positive on CBNAAT testing for tuberculosis. Extrapulmonary tuberculosis (e.g abdominal TB) often results from hematogenous or lymphogenous dissemination. Infection may directly extends from an adjacent organ.[24] The use of molecular techniques like PCR for the diagnosis of abdominal TB has been explored as there is a diagnostic dilemma in histopathology, and PCR can further help in ruling out the malignancy.

All the above cases of tuberculosis were microbiologically diagnosed on the MTB/RIF (CBNAAT) rapidly (turnaround time- 2 hrs), because of which they received timely Antitubercular therapy. Because of simultaneous susceptibility testing, the treatment was also given according to the Susceptibility report (rather than empirically).

Most of the laboratory techniques for lab diagnosis of EPTB are time consuming (including culture which is 'Gold stndard'), biohzardous, labor intensive, having variable sensitivities & specificities. Xpert MTB/RIF endorsed by WHO in 2013 for initial diagnosis of EPTB.[6] Both International Standards for TB care and Standards of TB care in India (2014), recommended Xpert assay for EPTB.[25] [26] It simultaneously detects MTB and Rifampicin resistance within 2 hrs. As the limit of detection of the assay is 131 CFU/ml of the specimen, it is expected to have better accuracy in paucibacillary diseases like EPTB.

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