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Case Report

Cartridge based nucleic acid amplification test negative in highly suspected case of tuberculosis: a case report

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ABSTRACT

Diagnosis of pulmonary tuberculosis is challenging till today. Smear microscopy is the easiest, commonest and widely employed tool for confirmatory diagnosis of pulmonary tuberculosis, but it has low sensitivity and specificity. Sputum culture can increase the diagnostic yield by 20-40%, but it takes long duration of 2-8 weeks to give result. The role of newly introduced cartridge based nucleic acid amplification test (CBNAAT) in the revised national TB control program (RNTCP) is highly promising with a higher yield of bacteriological diagnosis in sputum negative pulmonary tuberculosis patients with detection of rifampicin resistance rapidly. However, it also has some limitations which may result in false negative results. Case of a 50- year-old-male was reported who was initially managed for community-acquired pneumonia in view of negative sputum and CBNAAT but was later confirmed to have TB but by then he had developed cavities in lung and had transmitted the infection to his son.

Keywords: Cartridge based nucleic acid amplification test, Diagnosis, Pulmonary tuberculosis

INTRODUCTION

Tuberculosis (TB) remains a major global health problem. In 2015, 10.4 million new TB cases were detected worldwide. Of these, 5.9 million (56%) were men, 3.5 million (34%) were women and 1.0 million (10%) were children.[1] India accounts for one fourth of the global TB burden. In 2015, an estimated 2,800,000 cases occurred in India and 480,000 people died due to TB. India has the highest burden of both TB and MDR-TB, based on estimates reported in Global TB Report 2016 (0.13 million of 0.48 million of global MDR cases).[2]

In December 2010, WHO endorsed CBNAAT/GeneXpert MTB/RIF1(Cepheid, USA) for diagnosis of TB. CBNAAT was adopted in India by RNTCP in 2012. It first started as a pilot project in Maharashtra state, India.[3] Revised National TB Control Programme (RNTCP) also currently recommends use of CBNAAT to diagnose pulmonary TB, pediatric TB, extrapulmonary TB and rifampicin resistant and Multi Drug Resistant Tuberculosis in high risk populations like HIV positive as recommended by WHO under 2013 policy recommendations.[4]

The CBNAAT assay consists of a closed system that is based on real-time polymerase chain reaction (PCR) which requires minimal technical expertise in the diagnosis of TB and rifampicin resistance within two hours.[5] It has been validated and optimized for sputum samples to diagnose HIV associated TB and multidrug-resistant TB also. WHO strongly recommends widespread use of CBNAAT for these groups of patients.[6]

However, CBNAAT also has its own set of limitations which may result in false negative reports leading to delay in starting AKT and spread of the infection to

family members. Authors present one such report where negative CBNAAT report proved to be detrimental.

CASE REPORT

A 50-year-old-male, chronic alcoholic and smoker, vendor by occupation and a known case of ischemic dilated cardiomyopathy (DCM) since two years came to Medicine OPD on 29th July 2019 with chief complaints of shortness of breath and cough since one and half year, fever and weight loss since six months.

The patient was apparently alright before one and half year when he developed shortness of breath. Initially, he was breathless while walking uphill which progressed gradually over one and half year to breathlessness even at rest. Orthopnea and paroxysmal nocturnal dyspnea were also present. There was no seasonal or diurnal variation. He also had cough with expectoration which was whitish in color, mucoid, non-blood stained, non-foul smelling and with no diurnal, seasonal or postural variation. He also complained of atypical chest pain.

Table 1: Investigation reports of the patient.

Investigation		29/6/19	1/7/19	2/7/19	3/7/19	4/7/19	5/7/19	15/7/19 (on follow up)
Haemoglobin (gm %)		10.2	11.1	10.4	11.1	11.0	11.4	12.2
Total WBC count (per cumm)		9700	10700	1110	10300	8000	8800	6300
Differential Leucocyte Counts (%)	Neutrophils	77	79	76	84	76	78	70
	Lymphocytes	14	12	16	16	14	15	22
	Eosinophils	4	4	4	3	5	3	4
	Monocytes	5	5	4	3	5	4	4
	Basophil	0	0	0	0	0	0	0
Platelets (lacs/cumm)		5.3	5.0	4.29	4.4	3.69	3.47	3.5
ESR (mm/hour)		70						34
Total Billirubin (mg%)		0.6					2.6	1.1
Direct Billirubin (mg%)		0.3					1.4	0.5
Indirect Billirubin (mg%)		0.3					1.2	0.6
SGPT (IU/L)		23					156	49
SGOT(IU/L)		35					351	27
Urea (mg%)		32	42	64	58	50	39	17
S. Creatinine (mg%)		0.8	0.9	1.1	1.0	0.9	0.9	0.8
Sputum Gm Zn stain		Negative						
Sputum AFB		Negative						
Chest X ray		Multiple pocket of Multiple air fluid levels noted in Right lower zone along with thickening of transverse fissure obscuration of right costo-phrenic angle. s/o Lamellar effusion.						
CT Scan Thorax		HRCT Thorax: 21/8/2018 Consolidation in Right Lung Field in Perihilar Region with internal Air Brochogram. Small Cavitation in Antero-basal segment in Right Lower lobe. Right Moderate Pleural Effusion and Left Sided Minimal Pleural Effusion.			CECT Thorax: 1/7/2019 Irregular heterogeneously enhancing soft tissue density lesion with internal area of cavitation and air bronchogram within it involving right upper lobe. Moderate pleural effusion on right side with multiple internal air foci within passive collapse associated with mild pleural thickening and enhancement on right side. Multiple tiny nodular opacities in right upper lobe with few of them showing tree in bud pattern with patchy area of consolidation in left upper lobe with multiple enlarged non calcified lymphnodes in pretracheal, precranial and AP window. Left sided minimal pleural effusion also present			
2-D Echo		All cardiac chamber dilated sized. No LVH. LVEF <25%. Grade IV MR, Severe TR, Severe PAH. No AR. Dilated Cardiomyopathy with severe LV systolic and Diastolic Dysfunction.						

With these complaints he visited some local hospital where some blood investigations, sputum analysis, chest x-ray and CT thorax were done. Blood investigations were grossly within normal range while sputum analysis showed presence of gram-positive cocci. No Acid-Fast bacilli were seen. Chest X- ray showed heterogenous opacities in right upper and lower zones of lung with

pleural effusion. CT thorax also revealed right upper and lower zone consolidation with right sided pleural effusion (Table 1). The patient was treated for the same with intravenous antibiotics, bronchodilator and other symptomatic treatment. With the treatment he improved symptomatically and was discharged after 10 days on some oral medication.

However, within few weeks after discharge, he again developed similar complaints following which he was admitted multiple times in various hospitals. HRCT thorax was repeated which again showed consolidation in right lower zone with right sided pleural effusion but this time, hilar lymphadenopathy was also seen (Table 1), and a possibility of neoplasm was raised for which he was advised to undergo lung biopsy. But the patient and relatives denied. He was discharged on symptomatic improvement on oral medicines. For the next six months, he had complaints off and on for which he seeked symptomatic treatment locally, but no investigations were done.

After six months, he started having low-grade evening rise fever associated with chills and noticed loss of weight also. However, again he took only symptomatic treatment and denied any investigations.

In May 2019, that is after four months of fever, in view of persistent symptoms and ten kg weight loss in six months, he went to a tertiary care hospital, where respiratory examination revealed reduced air entry on right side with coarse crepitations. Here again routine blood investigations, sputum analysis and chest x-ray were done. Thoracocentesis was done and fluid was investigated for tuberculosis, but all turned out to be negative for tuberculosis. Pleural fluid CBNAAT was also negative. He was diagnosed and treated for lower respiratory tract infection and was discharged after ten days on oral medications as he showed improvement in shortness of breath and fever.

After 15 - 20 days, patient again developed same complaints and came to the hospital on 29th July 2019. On examination, he was febrile. Pulse rate was 110 beats per minute, with blood pressure of 90/60 mmHg in right cubital fossa in supine position, respiratory rate was 22 per minute with abdomino-thoracic type of breathing. He had pallor and grade II clubbing while icterus, lymphadenopathy and edema were absent.

On respiratory examination, shape of the chest was ellipsoid with no scar marks, sinuses, dilated veins or visible pulsations. Trachea was shifted to left side, apex beat was in 6th intercostal space one cm medial to midclavicular line, with decreased chest expansion on right side and increased Tactile vocal fremitus in right middle and lower zone. There was a stony dull note on percussion in right lower zone and resonant note over rest of lung fields with normal cardiac and liver dullness. On auscultation, there was decreased air entry on right side with coarse crepitations with bronchophony and egophony in right middle and lower zones.

Complete blood count was suggestive of anemia with raised ESR. RFT and LFT were within normal limit (Table 1). Sputum routine microscopy was negative for Acid Fast bacilli but gram-positive cocci in pairs and chains were seen with few gram-negative bacilli. His chest x-ray again showed right lower zone consolidation with right sided pleural effusion but this time it also showed multiple fluid filled pockets (Figure 1).CECT thorax was suggestive of infective etiology in right lower zone with mediastinal lymphadenopathy with right sided moderate pleural effusion and minimal left sided pleural effusion. Pleural fluid was exudative with no acid-fast bacilli or malignant cells (Table 1 and Figure 2).

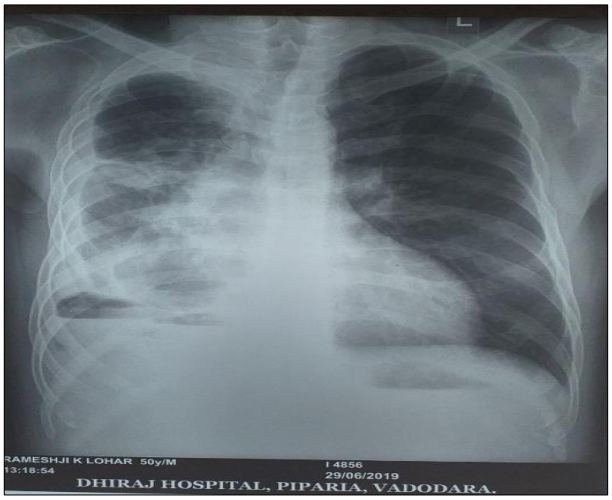

Figure 1: Multiple air fluid pockets in right lower zone with pleural effusion.

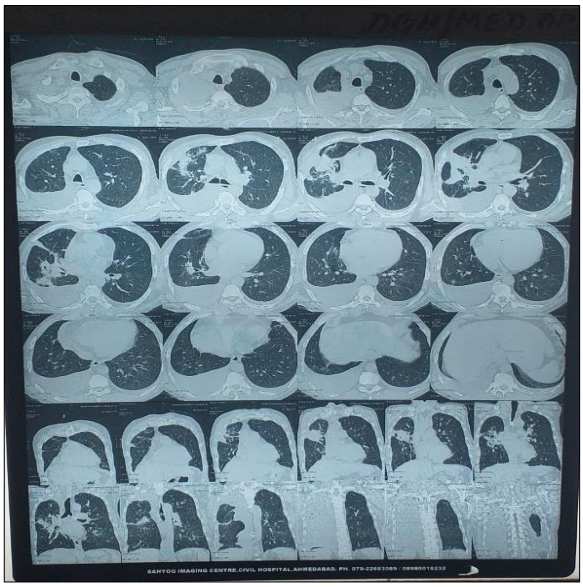

Figure 2: CECT Thorax showing multiple tiny nodular opacities in right lower lobe and pleural effusion.

In view of strong suspicion of tuberculosis, persistent symptoms sputum analysis was repeated and was also sent for CBNAAT and bronchoscopy was done. Bronchoalveolar lavage (BAL) fluid was negative for Acid fast bacilli as well as CBNAAT. However, pleural fluid analysis showed predominant lymphocytosis with ADA being 14 IU/L (Table 2) and following that, sputum CBNAAT turned out to be positive for Acid fast bacilli this time. Thus, diagnosis of pulmonary tuberculosis was established and Anti-Koch's therapy (AKT) was started while continuing treatment for ischemic dilated cardiomyopathy. Later, the sputum culture also confirmed tuberculosis.

Table 2: Reports of BAL and pleural fluid analysis.

	BAL analysis	Pleural fluid analysis
PH	7.0	7.0
Sugar (mg/dl)	25	73
Protein (gm/dl)	0.8	2.0
Albumin (gm/dl)	0.4	1.0
ADA (IU/L)	5	14
LDH (IU/L)	139	300
Lymphocyte (%)	10	85
Polymorphs (%)	25	10
Sqammous cell/ Mesothelial cell (%)	5/0	0/5
Respiratory epithelial cell (%)	60	-
Cobweb	Absent	Absent
Cytology	Predominantly respiratory lining epithelium, macrophage and abundant neutrophils. No Atypia.	Abundant of Macrophage and Lymphocytes are seen in Pink Proteinaceous Background.
AFB under RNTCP	Negative	Negative
Gram stain	Few Pus cell seen	Few Pus cell seen
ZN stain	AFB not seen	AFB not seen
Culture	No organism isolated	No organism isolated

Looking at the long untreated course of illness, the patient's wife and son were also tested. The son was found to be sputum positive and AKT was started for him also.

Symptomatically the patient improved, fever disappeared, and cough also reduced. He was discharged in stable condition after about fifteen days of hospital stay. He came for follow up twice after every month, each time for breathlessness related to DCM. However, after about four months he succumbed to his illness.

DISCUSSION

The cartridge-based nucleic acid amplification test or CBNAAT was developed in 2009 and is considered an important breakthrough in the fight against TB. It was the first molecular test which was simple and robust and can be easily introduced and used. It is more sensitive than the smear microscopy both for pulmonary and extrapulmonary TB and can detect rpo B gene mutations responsible for rifampicin-resistance (RR-TB) also in less than two hours.[7,8]

WHO recommended the use of the CBNAAT for diagnosis of pulmonary and extrapulmonary TB in December 2010,which was followed by an unprecedented uptake of this new technology, so much so that in excess of 2000 instruments of CBNAAT and 5 million cartridges were procured by the end of December 2013 in 98 countries.[9]

Specimens that can be sent for testing include respiratory specimens such as sputum, bronchial or tracheal aspirates, broncho-alveolar lavage and gastric lavage as well as extra pulmonary specimens like tissue biopsy including lymph node, pus from abscess, CSF, ascitic fluid, pericardial fluid and pleural fluid.[10] However, CB-NAAT testing for TB on other samples such as stool, urine and blood is not recommended.[9]

The sensitivity and specificity of CBNAAT for detecting MTB in pulmonary samples of patients with TB has been reported to be 95.7% and 99.3% respectively. However, the researchers have also observed that in smear-positive culture-positive samples, it was 99.2% while in smear-negative culture positive samples, the sensitivity was 77.7%. For detecting resistance to rifampicin, the sensitivity and specificity were 94.5% and 97.7% respectively with respect to culture as gold standard.[11] A few studies also reported poor sensitivity of CBNAAT in detecting TB in gastric aspirate and tubercular pleural effusion.

Limitations of CB-NAAT test include a requirement of stable electrical power supply, temperature control and annual calibration of instrument. Sufficient measures must be taken so that power supply remains uninterrupted (with additional batteries, a generator or solar panels), cartridges must be stored at the recommended temperature range (2-28°C), and the equipment itself between 15°C and 30°C.[9]

This case had clinical features suggestive of tuberculosis. The clinicians suspected and tested also, but since all tests including CBNAAT were negative, antitubercular therapy was not offered. The CBNAAT report sent third time came out to be positive but by then patient had already developed tubercular cavities in lung and had transmitted the tubercular bacillus to his son.

Thus, despite the obvious advantages, CBNAAT has its own set of disadvantages and limitations, so that it may at times give false negative result. Denying AKT to such cases who have been falsely reported negative in CBNAAT may prove to be detrimental. Culture remains the gold standard for diagnosis of tuberculosis but takes a lot of time. Hence, in cases where CBNAAT is negative but there is strong suspicion of tuberculosis, probably giving AKT empirically till the culture report arrives would be a better alternative.

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