



Original Research Article

Comparative study of detection of tuberculosis by sputum microscopy versus cartridge based nucleic acid amplification test



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ABSTRACT

Introduction: Tuberculosis kills more people than any other communicable disease. Good diagnosis is key to its prevention and control. While AFS is cost effective and widely used, CB-NAAT is costly and its usage is increasing in recent times.

Objectives: to quantify the diagnostic capabilities of CB-NAAT and AFS in sputum samples suspected pulmonary tuberculosis patients. To know the efficacy of AFS as a screening test against CB-NAAT as the standard test.

Materials and Methods: Our cross sectional study analysed the results of 1210 sputum samples among suspected pulmonary tuberculosis patients in whom both CB-NAAT and AFS was done during 2017-18. Descriptive statistics with McNemar test ($p < 0.05$), sensitivity, specificity, positive value and negative predictive value for the tests have been used.

Results: Out of 1210 samples tested, AFS detected positive in 13(1.07%) samples and CBNAAT was positive in 173(14.29%) samples. There was statistically significant difference. Concordance in both the tests positive in 13(1.07%) samples and negative in 1037(85.70%) sample was seen respectively. CB-NAAT as standard, sensitivity of AFS was 7.51%. Specificity was 100%. positive predictive value of AFS was 100% and negative predictive value of AFS was 86.63%. AFS failed to detect 92.48% of the cases that were diagnosed by CB-NAAT.

Conclusion: AFS was detected positive results in 1% of samples while CB-NAAT was positive results in 14% of samples. CB-NAAT was superior test compared to AFS. Concordance was 1% and 86% in positive and negative results respectively. Sensitivity of AFS was very low while its specificity, positive predictive value and negative predictive value was very superior.

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1. Introduction

Tuberculosis (TB) is one among the top 10 causes of death worldwide. Every year, more than 10 million fall ill with TB and nearly 1.7 million of deaths. About 95% of TB deaths are most commonly seen in low- and middle socio-economic countries.¹

Tuberculosis disease is diagnosed by detecting *Mycobacterium tuberculosis* bacteria in sputum sample taken from the patient. This is done commonly by Acid Fast Staining of sputum smear (AFS). Clinically history, general physical and radiological examination which strongly

suggest tuberculosis but does not confirm it.²

Early diagnosis is needed for early patient management and successful patient results. False-negative results and misdiagnosis of TB suspects are commonly seen in developing countries, as most TB control programmes are based on Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and needs multiple visits by the patients that leads to higher default. Mycobacterial culture is considered as the gold standard test but it is slow and usually takes 2-6 weeks time to yield a final result and it requires proper infrastructure and technical expertise.³

Each year, one among three people who are affected with TB are left undiagnosed or not noticed by health systems. These underdiagnosed or un-diagnosed 3.6 million people

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are main cause for high rates of TB transmission. The increase and more efficient execution of existing diagnostic tools will help countries to find and treat these millions of people. However, reaching the End TB Strategy targets needs a drastic increase in case detection which can be achieved only by major advances in novel diagnostic test technologies.⁴

Cartridge based nucleic acid amplification test (CB NAAT) is a novel technique based on polymerase chain reaction (PCR) for detection of tuberculosis. It is a MTB-specific automated, cartridge based nucleic acid amplification assay, having fully incorporated and automated amplification and detection using real-time PCR, results are obtained within 100 minutes. It is a highly specific test as it includes 3 specific primers and 5 unique molecular probes.⁵

While it is proven that CB- NAAT is superior to AFS, CB-NAAT is not available in all places and is still used widely. Our study mainly aims to quantify the difference between the diagnostic capability between CBNAAT and AFS, both of which are included under Revised National Tuberculosis Control Program (RNTCP) in mandya Institute of Medical Sciences (MIMS), Mandya

2. Objective

To quantify the difference between the diagnostic capability between CB-NAAT and AFS in sputum samples of suspected pulmonary tuberculosis patients to the RNTCP centre.

To evaluate efficacy of AFS as a screening test against CB-NAAT as the standard test.

To determine the number of cases being missed due to the non-usage of CB-NAAT

3. Materials and Methods

Our cross sectional hospital based study analysed the results of all the sputum samples from July 2017 and December 2018 in suspected pulmonary tuberculosis patients. Both CB-NAAT and AFS was done at the Designated Microscopy Center (DMC) at MIMS Mandya was done for all these 1210 patients.

McNemar test has been used to calculate the statistical significance for the difference between the proportions. P value of less than 0.05 has been considered significant. Descriptive statistics is based on proportion and percentages

Sensitivity, specificity, positive and negative predictive value for the tests have been calculated to validate AFS against CB-NAAT as the standard.

4. Results

A total of 1210 sputum samples from suspected PTB patients who were referred to DMC, MIMS, Mandya was analysed. Both AFS and CB-NAAT diagnostic tests were

performed on all these patients and their results were taken for analysis.

Among these 1210 samples tested, AFS was positive for 13(1.07%) samples for tuberculosis whereas CB-NAAT showed *Mycobacterium tuberculosis* in 173(14.29%) in suspected pulmonary tuberculosis patients.

McNemar's test is a statistical test used on paired nominal data. It is applied to 2×2 contingency tables with a dichotomous trait, with matched data of subjects. This test was used to determine the difference in the results when two types of tests were used and to know whether it was due to chance or whether it is statistically significant. As per norm we have taken a probability ('p') value at less than 0.05. The resulting value of the test was 168.0, which interprets that the p value is less than 0.05. Hence it can be concluded that the difference in the results given by the two tests on the same samples are significantly different.

Table 1: Results of AFS as a screening test, CBNAAT being gold standard

AFS	CBNAAT Positive	Negative	Total
Positive	13	0	13
Negative	160	1037	1197
Total	173	1037	1210

Above table shows that, 13 samples were positive by AFS screening test while 173 samples were positive by CB-NAAT. All the samples which were AFS positive were CB-NAAT positive. Only 13(7.51%) of the 173 CB-NAAT positive were AFS positive. Concordance in both the tests being positive was seen in 13(1.07%) sputum samples.

1197 samples were negative for AFS screening test. 1037 samples by CB-NAAT showed negative results. 1037(86.63%) of the 1197 AFS negative were CB-NAAT negative. All CB-NAAT negative were AFS negative. Concordance in both the tests being negative was seen in 1037(85.70%) samples.

CB-NAAT is superior and more sensitive diagnostic tool. To evaluate AFS by taking CB-NAAT as the standard the following statistical tests were done. It means that CB-NAAT positive is taken as diseased and CB-NAAT negative is taken as non-diseased.

Sensitivity of a test is its ability to identify the diseased. Since CB-NAAT was taken as standard, the 173 samples positive by CB-NAAT are taken as diseased. Sensitivity in our study is the proportion of diseased that were tested as positive by AFS. AFS could identify only 13 of the 173 diseased. Hence the sensitivity of AFS was 7.51%.

Specificity of a test is its ability to identify the non-diseased. Since CB-NAAT is taken as standard, the 1037 CB-NAAT negative are considered as non-diseased. Specificity in our study is the proportion of non-diseased that were tested as negative by AFS. AFS could identify all

the 1037 as non-diseased. Hence the specificity of AFS is 100%.

The Positive Predictive Value of a test shows the proportion of the positive result who actually have the disease. All the 13 who were AFS positive had the disease. Hence the positive predictive value of AFS was 100%.

The Negative Predictive Value of a test shows the proportion of the negative result who are actually non-diseased. 1197 were AFS negative. Out of these only 1037 were non-diseased. Hence the negative predictive value of AFS was 86.63%.

Of the 1210 samples tested, AFS showed 13(1.07%) samples as positive for tuberculo sis bacilli while CB-NAAT identified *Mycobacterium tuberculosis* in 173(14.29%) sputum samples among suspected pulmonary tuberculosis patients. This means that 160 of the cases were not diagnosed. That is 92.48% of the cases were not diagnosed by AFS.

5. Discussion

Our study shows that 1.07% samples were positive by AFS and 14.29% samples were positive by CB-NAAT. Results of other studies are in concordance with our study, which showed that these proportion to be 11% & 16.67% for AFS and 40% & 48.68%.^{5,6} The difference was statistically significant in all studies, hence proving that CB- NAAT is a superior test compared to AFS.

Our study showed that sensitivity of AFS was 7.51% and specificity was 100%. This was different in other studies which showed it to be 22.2%, % and 78.5% respectively.³ Another study showed that sensitivity was 16.67% and negative predictive value was 20.83% for AFS.⁶ Various studies have shown that sensitivity of AFS is between 14% to 85% depending upon the slight differences in methods of staining used.⁷

In a prospective autopsy study published in The Lancet of patients who died at a hospital in Lu saka, Zambia, researchers identified that a large percentage of patients were infected with tuberculosis but were gone undiagnosed and untreated, and 17 percent of patients infected with tuberculosis were in fact infected with multidrug-resistant TB but also remained undiagnosed.⁸

Even though sputum smear microscopy is inexpensive and simple, its sensitivity is only about 50-60%. In countries with a high prevalence of both pulmonary TB and HIV infection, the detection rate can be even lower, as many people with HIV and TB co-infection have very low levels of TB bacteria in their sputum, and are therefore recorded as sputum negative.⁹

Our findings reiterate the statement given in Standards for TB care in India that CB-NAAT (cartridge-based nucleic-acid amplification test) is the preferred first diagnostic test in children and PLHIV. CB-NAAT is a key diagnostic stool in the National Strategic Plan (NSP) 2017-

2025. The main objective of NSP is to detect all TB patients and especially those with drug sensitivity. CB-NAAT has the additional advantage that it can also detect TB in children and HIV positive people, where AFS will usually be negative. CB-NAAT will also increase the confidence of private practitioners to refer suspected TB cases for correct diagnosis. NSP also proposes to use CB-NAAT to screen high risk populations.¹⁰

6. Conclusion

Of the 1210 samples tested, AFS was positive in 13(1.07%) samples and CB-NAAT was positive in 173(14.29%) samples. The difference was statistically significant. Concordance in both the tests being positive was seen in 13(1.07%) samples. Concordance in both the tests being negative was seen in 1037(85.70%) samples. When CB-NAAT is taken as standard, sensitivity of AFS was 7.51%. Specificity was 100%. Positive predictive value of AFS was 100% and negative predictive value of AFS was 86.63%. Records show that AFS could not detect 92.48% of the cases that were diagnosed by CB-NAAT.

7. Source of Funding

None.

8. Conflict of Interest

None.

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