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COMPARATIVE STUDY OF SMEAR POSITIVITY AND CBNAAT AFTER ADMINISTRATION OF PEANUTS AS ADJUVANT THERAPY IN PATIENTS OF TUBERCULOSIS

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Abstract

Background: As per the World Health Organization, Cartridge based nucleic acid amplification test (CBNAAT) has been approved as a rapid molecular diagnostic test for detection of tuberculosis, rifampicin resistance and smear examination is the readily available source for detection of pulmonary tuberculosis. Aim: In this present study, the smear positivity results were compared with CBNAAT for evaluating the outcome of arginine rich food peanut supplementation as adjuvant therapy in confirmed smear positive pulmonary tuberculosis cases as the literature is not fully available. Methods: The effect of arginine on smear positive tuberculosis patients was compared to acid-fast microscopic examination using Ziehl-Neelsen (ZN) stain and CBNAAT. A total of 196 specimens of smear positive pulmonary tuberculosis of age groups between 16 to 60 years were included in the study with no co morbidities. The study was done from October 2018 to October 2022. Results: After the administration of Arginine in the form of boiled and soaked peanuts in smear positive pulmonary tuberculosis patients, there was a significant decrease in the bacillary counts when subjected to CBNAAT. The smear conversion rate was also high after one month of administration of peanuts. The arginine content in the peanuts was analysed before giving as adjuvant therapy in the patients. Conclusion: In a high TB burden country like India, arginine supplementation can be a landmark innovation for early recovery of disease as the smear conversion rate was high and also the tubercle bacillary detection in CBNAAT has marked reduction after two weeks of the DOTS therapy along with peanut supplementation. This therapy may aid in early recovery of the pulmonary tuberculosis patients which will help in the control of Tuberculosis in developing and underdeveloped countries.

Keywords: Arginine, CBNAAT, Peanuts, Smear Conversion, Mycobacterium Tuberculosis.

INTRODUCTION

WHO global TB report discloses that the estimated incidence of TB is 2.2 million with a prevalence of 2.5 million and a mortality of 0.22 million [1-3]. India stands prominently among countries grappling with a significant burden of TB. A study highlights that the estimated incidence of TB is 2% among new cases and a notably higher 15% among cases necessitating re-treatment. Moreover, India annually bears one fourth of the global burden of TB underscoring the magnitude of the challenge within the country [4,5].

The early and precise diagnosis serves as the pivotal first step in managing TB effectively. However, the control of TB faces obstacles due to diagnostic techniques with subpar sensitivity, especially in detecting drug resistant strains and in individuals coinfected with human immunodeficiency virus (HIV). Timely detection is imperative to interrupt transmission and mitigate mortality rates, nonetheless the intricate nature of diagnostic procedure and the requisite infrastructure demand highly sensitive methods there by constraining their accessibility and efficacy [2,3]. The diagnosis of pulmonary tuberculosis (PTB) primarily hinges on identifying acid-fast bacilli (AFB) in

sputum smear samples; however this method is hampered by its low sensitivity, which limits its effectiveness in accurately detecting the presence of TB [2,3]. Conventional mycobacterial cultures such as Solid culture in Lowenstein-Jensen medium typically require 6-8 weeks for results to be obtained. Conversely newer liquid culture methods like Mycobacterial growth indicator tube (MGIT) or BACTEC offer relatively quicker result. However these methods are more expensive compared to conventional cultures [4,5]. The mainstay of diagnosis is culture, but relying on it often leads to delay, compromising patient care and outcome [5-8]. To mitigate these delays, the WHO swiftly sanctioned the use of the Xpert MTB/RIF a cartridge based nucleic acid amplification test (CBNAAT) WHO in December 2010 for TB diagnosis. This innovative assay is specifically tailored to target drug-resistant strains, human immuno-deficiency virus (HIV) and TB co-infection, paediatric tuberculosis, extrapulmonary tuberculosis and smear negative pulmonary tuberculosis developed by (Cepheid Inc., CA, USA), the XPERT MTB RIF assay stands as a pivotal milestone in the realm of rapid molecular TB diagnostics, significantly improving diagnostic capabilities [9-11]. This versatile diagnostic platform operates as an automated closed system, conducting real-time PCR. Its user – friendly design allows operators with minimal technical expertise to utilise it effectively. Remarkably, this system facilitates the diagnosis of TB and simultaneous assessment of rifampicin resistance within a mere 2-hour time frame [11]. The endorsement from the WHO for investigating pulmonary TB with CBNAAT underscores its significance in diagnosing this form of the disease. Furthermore, recent evaluations have expanded to include a diverse range of non-respiratory clinical samples from individuals presenting with Extra pulmonary tuberculosis EPTB highlighting the assay's versatility and potential in diagnosing TB beyond the lungs [12]. Hence, the objective of this study was to identify an early diagnostic marker for assessing the recovery of tuberculosis patients both with and without arginine rich peanut adjuvant therapy. The study aimed to compare the outcome of CBNAAT and Smear conversion at specified intervals to evaluate their efficacy in monitoring patient recovery.

MATERIAL AND METHODS

This observational comparative study enrolled individuals diagnosed with smear positive TB and presenting clinical symptoms indicative of active pulmonary tuberculosis (n=196). Among these, 98 patients were administered peanuts in addition to Directly observed Therapy (DOTs) as the study group, while the remaining 98 served as controls, receiving only DOTs therapy. Sputum samples were collected at baseline, two weeks, one month and two months after treatment initiation for smear microscopy Ziehl Neelsen (ZN) and CBNAAT analysis. Approval for the study was obtained from the Institutional ethics committee (Ref no. and all participating subjects provided informed written consent prior to study commencement.

Inclusion criteria

Patients aged 18 years or older who tested smear positive by microscopy according to the guidelines of World Health Organisation and Ministry of Health and family welfare, Government of India, were included in the study for treatment.

Exclusion criteria

Additional exclusion criteria comprised patients age over 60 years, individuals with smear graded as scanty as per guidelines, those with peanut allergy, individuals

requiring hospitalisation, pregnant women, individuals diagnosed with HIV, individuals with a history of previous tuberculosis, patients currently undergoing medical treatment for other conditions and individuals who discontinued participation in the study.

During sample processing, smears were stained with. Zeihl Nelson staining method and graded in accordance with the guidelines of the Revised national tuberculosis control program (RNTCP) currently known as National Tuberculosis Elimination Program (NTEP). Smear grading compromised Scanty (1-9AFB/100 fields), 1+ (10-99AFB/100 fields), 2+ (1-10AFB/ fields) and 3+ (>10AFB/field). A person was classified as smear positive if at least one of the smears was graded 1+ or higher.

Samples were subjected to analysis using the Xpert MTB/RIF assay with version 4 cartridges strictly following the manufacturer's instructions. The process began by adding a sample reagent (containing NaOH and isopropyl alcohol) to clinical specimen at a 2:1 ratio designed to eliminate the Mycobacteria and liquefy the samples. After thorough shaking and a 10 minute incubation period two ml of the digested material was transferred to the cartridge. Subsequently, the cartridge was inserted into the GeneXpert instrument, where all subsequent steps. This process was iterated at intervals of baseline, two weeks, one month and two months subsequent to commencing the treatment regimen. Upon detection of MTB target DNA results were categorised as high, medium, low or very low, contingent on the Ct value of MTB target present in sputum sample. A chart was meticulously prepared in accordance with laboratory standard operating procedures, where a ct value less than 16 denoted high, 16-22 ct value was indicated as medium, 20-28 as low and values exceeding 28 as very low were carried out automatically, leaving to the display of results.

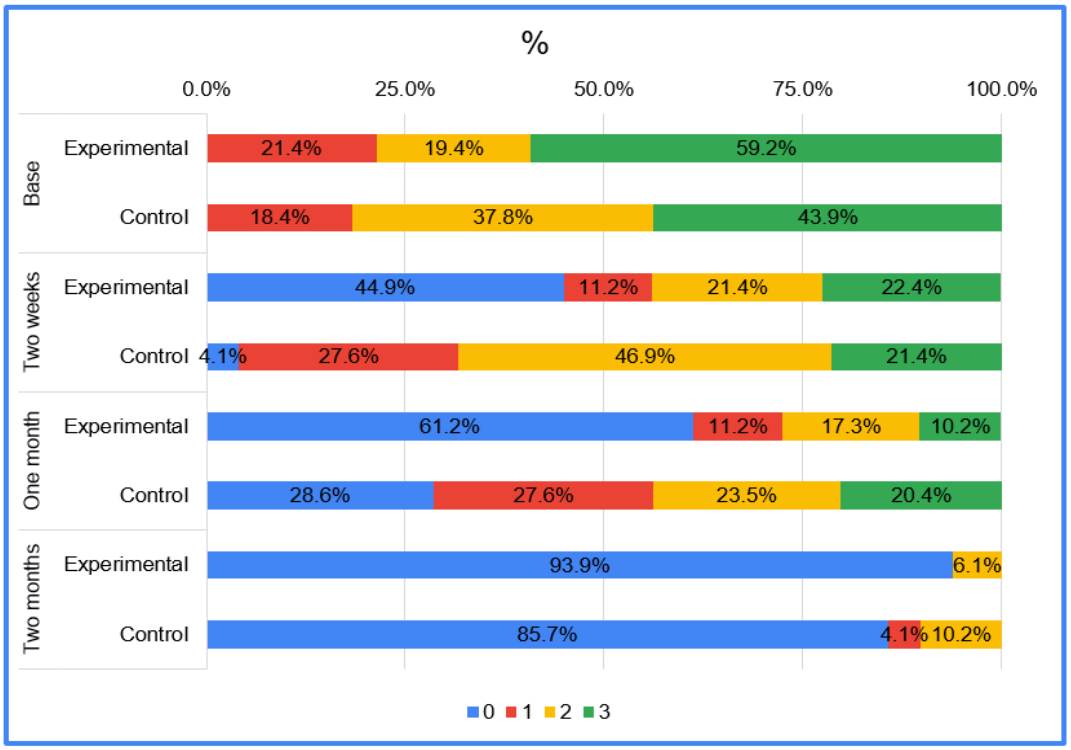
Data Collection: The collected data encompassed various aspects including the patients' demographics, semi quantitative bacillary load determined by AFB microscopy and their past history of TB treatment.

RESULT

Among the 210 sputum AFB smear positive samples, Peanut administration was initiated for 105 patients however only 98 patients adhered to the complete drug and nutritional (peanuts) regimen as instructed resulting in a dropout rate of merely 7 (6.6%) and the remaining 105 patients constituted the control group, who took only DOTS therapy.

To ensure comparability with the test group receiving peanut supplementation only 98 results from control group were considered. Throughout the study no side effects or adverse reactions were observed during food supplementation, and the comprehensive results were compiled and tabulated for analysis.

Table 1: Smear Positivity Grading at intervals of baseline, two weeks, one month and two months.



The analysis of smear positivity at baseline, comparing patient data coded with peanuts versus patient data without peanuts, yielded the following results:

1. Smear Positivity Baseline: Among patients, 21.43% had a smear positivity of 1+, 19.39% had a smear positivity of 2+, and 59.18% had a smear positivity of 3+ when with peanut supplementation. In comparison, patients without peanut supplementation had smear positivity rates of 18.37% for 1+, 37.76% for 2+, and 43.88% for 3+.
2. Two Weeks Follow-up: After two weeks, 44.90% of patients with peanut adjuvant therapy showed smear negative, while 11.22%, 21.43%, and 22.45% had smear positivity grades of 1+, 2+ and 3+, respectively. Among patients without peanut supplementation 4.08% showed smear negative, while 27.55%, 46.94%, and 21.43% had smear positivity grades of 1+, 2+ and 3+ respectively.
3. One Month Follow-up: At the one-month mark, 61.22% of patients with peanut adjuvant therapy turned smear negative. Additionally, 11.22%, 17.35%, and 10.20% had smear positivity grades of 1+, 2+ and 3+ respectively. Among patients without peanut adjuvant therapy, 28.57% turned smear negative, while 27.55%, 23.47%, and 20.41% had smear positivity grades of 1+, 2+ and 3+ respectively.
4. Two Months Follow-up: After two months, a significant decrease was observed in smear positivity rates among patients with peanut supplementation, with 93.88% subjects were smear negative. However, 6.12% still were smear positive at grade 3+. In comparison, in control group without peanut supplementation had 85.71% were smear negative, 4.08% with a smear positivity at grade of 2+ and 10.20% with grade of 3+.

These results provide valuable insights into the association between peanut adjuvant therapy and smear positivity over different time intervals, suggesting potential effects on the progression of the condition and the importance of follow-up visits for accurate monitoring. Considering the total number of patients (98 in both groups), it can be observed that the presence of peanut adjuvant therapy correlated with higher smear conversion. These findings shed light on the association between peanut coding and smear positivity, emphasizing the potential impact of peanuts on the observed results.

Table 2: CBNAAT results at intervals of baseline, two weeks, one month and two months

CBNAAT	Experimental				Control				P-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR	
Baseline	16.2	5.3	14.0	8.0	17.4	5.0	18.0	10.0	.090
Two Weeks	21.6	4.6	20.0	6.3	19.2	5.5	20.0	10.0	**.026**
One Month	26.3	3.7	26.0	5.0	20.3	8.0	23.0	10.0	**<0.001**
Two Months	26.9	9.1	30.0	6.0	6.8	10.0	0.0	16.5	**<0.001**
P-value (Base vs Two months)	**<0.001**				**<0.001**				

The analysis of the provided data on CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) reveals the following findings:

1. CBNAAT at Baseline: Among patients with peanuts supplementation, 78.57% had CBNAAT values below 20, while 21.43% fell in the range of 20-30. Patients without peanut supplementation had percentages of 69.39% and 30.61% for values below 20 and in the range of 20-30, respectively.
2. CBNAAT at Two Weeks: After two weeks, 56.12% of patients with peanut administration had CBNAAT values below 20, while 41.84% fell in the range of 20-30. Patients without peanuts administration had percentages of 51.02% and 48.98% for values below 20 and in the range of 20-30, respectively.
3. CBNAAT at One Month: At the one-month mark, 2.04% of patients with peanut supplementation had CBNAAT values below 20, while 80.61% fell in the range of 20-30. Patients without peanut supplementation had percentages of 41.84% and 58.16% for values below 20 and in the range of 20-30, respectively.
4. CBNAAT at Two Months: After two months, 9.18% of patients with peanut adjuvant therapy had CBNAAT values below 20, while 58.16% fell in the range of 20-30. Patients without peanut adjuvant therapy had percentages of 78.57% and 21.43% for values below 20 and in the range of 20-30, respectively.

These results provide insights into the distribution of CBNAAT values among patients with peanuts supplementation and those without peanuts over different time intervals. They suggest variations in CBNAAT levels based on peanut administration, which may be indicative of potential associations between peanuts and CBNAAT test results.

DISCUSSION

The present study findings reveal that CBNAAT is an efficient diagnostic test with higher sensitivity for diagnosing pulmonary tuberculosis cases [18]. In 2012 WHO recommended the use of CBNAAT for routine use under government program [19]. In this present study, a total of 196 specimens were tested using CBNAAT; 98 patients were with peanut supplementation and 98 subjects were without peanut administration

that was tested at intervals labelled as baseline, two weeks, one month and two months after therapy with peanut adjuvant and without peanut adjuvant.

The findings of the study suggest a potential role for arginine rich peanut supplementation as an adjuvant therapy in treatment of smear positive pulmonary tuberculosis (TB). The comparison of smear positivity with CBNAAT results before and after arginine supplementation revealed promising outcome, indicating significant reduction in bacillary counts and an increase in smear conversion rates among patients receiving arginine rich peanuts. The observed decrease in bacillary counts following arginine supplementation aligns with existing knowledge regarding the immunomodulatory effects of arginine. [20]

A study performed by Fasil Wagnew [21], to understand the effect of under nutrition on sputum culture conversion and treatment outcomes among people with multidrug-resistant tuberculosis: a systematic review and meta-analysis stated that the treatment in undernourished had significantly unsuccessful treatment outcomes, including mortality and longer time to sputum culture conversion among people with MDR-TB. The present study findings suggest that by supporting targeted nutritional interventions alongside standardized TB drugs there was an early recovery indicated by faster sputum conversion and reduced CTvalues in CBNAAT.

Another study performed by Sinclair D et al [22], to study the nutritional supplements for people being treated for active tuberculosis no proper conclusion was drawn to prove the nutritional supplementation in tuberculosis outcome. But a study by K.B. Gupta et al [23], revealed that patients on supplementation with nutritional supplementation had an early recovery from tuberculosis. Peanuts are the richest source of all the nutrients and the present study was with supplementation of nutritionally rich food that had a positive outcome and early recovery from disease with higher smear conversion [24].

A true experimental study by Isa Ma'rufi et al [25], on *Channa striata* (Ikan Gabus) Extracts showed that there was an increased acceleration in recovery of tuberculosis patients due to the abundance of micro nutrients. The results are in concordance with the present study that there was an early recovery in patients with arginine supplementation in the form of peanuts.

This analysis showed that the presence of peanuts in the regimen correlated with higher smear conversion. Patients with peanut adjuvant therapy had higher percentages of smear conversion in all grading categories (1, 2, and 3) compared to patients without peanut adjuvant therapy. This indicates a potential association between peanuts and smear conversion, suggesting that peanuts may lead to higher smear conversion which may be considered as an early recovery marker upon peanut supplementation.

CBNAAT values also varied based on peanut administration over different time intervals. Patients with peanuts supplementation had different percentages across the CBNAAT categories compared to those without peanuts supplementation. There were no previous studies supporting the present findings. This suggests a potential association between peanuts and CBNAAT test results, indicating that peanut supplementation may affect CBNAAT values.

Though the diagnostic efficacy of CBNAAT is high when compared with AFB smear [18]. The present study shows that the AFB smear is a good marker to show the recovery of tuberculosis patients as CBNAAT was positive for longer duration in both control and study groups.

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