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RESEARCH ARTICLE

YIELD OF INDUCED SPUTUM IN CLINICALLY DIAGNOSED PULMONARY TUBERCULOSIS

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Abstract

Background:- Direct sputum smear examination is used as a basic diagnostic tool in the diagnosis of pulmonary tuberculosis. But difficulty in obtaining adequate sputum is the main cause of failure of bacteriological diagnosis. Alternative methods of obtaining sputum specimens are frequently needed in those patients with clinicoradiological suspicion of TB who are unable to expectorate or are smear negative.

Aim:- To assess the yield of smear positivity of induced sputum (SI) with hypertonic saline nebulisation, broncho-alveolar lavage fluid and Rifampicin resistance in clinically diagnosed pulmonary TB who have no/inadequate sputum.

Materials and Methods:- In the present cross-sectional observational study, we analysed the smear positivity of AFB in induced sputum of patients at Veer Surendra Sai Institute of Medical Science & Research, Burla, Odisha, India, from November 2018 to October 2020. A total of 100 patients with clinically diagnosed pulmonary TB who were unable to produce adequate sputum fulfilling inclusion criteria were included in the study. 10 ml of 3% hypertonic saline was injected into the nebulizer reservoir device. The assembly was connected to the jet nebulizer. The patients were instructed to inhale and exhale the mist of the nebulized solution through the mouth piece only. The inhalation of hypertonic saline was interrupted every 5 min, so that the patient could expectorate the sputum into a clean sterile sputum container. The sputum samples were stained with auramin-rhodamin stain and examined for AFB, also same samples were subjected for CBNAAT(cartridge based nucleic acid amplification test). Selected sputum induction(SI) CBNAAT negative patients are also underwent bronchoscopy and BAL fluid was subjected for CBNAAT.

Results:- Out of 100 clinically diagnosed tuberculosis patients, 49% of cases had only infiltration, 45% cases had consolidation,17% had infiltration with fibrosis,16% cases with cavitary lesions and 6% had miliary lesions in chest X -ray. 17(17%) cases became induced sputum smear positive, 30(30%) cases became induced sputum CBNAAT

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positive. All induced sputum CBNAAT positive cases were rifampicin sensitive. No rifampicin resistance was detected. Out of 70 patients with SI CBNAAT negative, 21 patients with highly clinico radiological suspicion underwent bronchoscopy and 4(19%) patients become post bronchoscopy sputum AFB positive. Out 21 patients 8(38%) patients become BAL CBNAAT positive and all are rifampicine sensitive.

Conclusion:- Induced sputum and BAL fluid are better alternative methods for diagnosis in patients with clinico-radiological suspicion of TB who are unable to expectorate or are smear negative and no rifampicin resistant cases were detected throughout this study.

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Introduction:-

Tuberculosis (TB) is one of the significant infectious disease which can be avoided and treated, yet causing death and is a critical health problem around the world. Finding patients who are infected with "pulmonary tuberculosis" (PTB) disease is a vital part of "THE TUBERCULOSIS CONTROL PROGRAMME" and timely introducing of treatment provides patients to be non-infectious, stops the chain of transmission of TB resulting in decreases the pool of infection[1]. For many decades smear microscopy has been the main diagnostic tool for testing "pulmonary tuberculosis". Nonetheless, in patients with consistent clinical picture of pulmonary tuberculosis "sputum smears" do not diagnose "acid fast bacilli" (AFB) in all cases, "smear negative- culture positive" state is noticed in "22% to 61%" of the cases[1]. "Direct sputum smear microscopy" continues to be crucial tool of diagnosis, but might turn out negative up to 50% case of active pulmonary TB[2]. Although "smear microscopy" possess low "sensitivity" and high "specificity", matters in relation with "quality control", traditional "solid culture" techniques have certain limitations of long duration of many weeks for producing result. "Liquid culture" techniques were refined for timely diagnosis of "MTB" growth, but the average time taken for 21 days to produce result is still a long time period for a "diagnostic test" to be effective in preventing passing on of the disease[3]. Such delays in diagnosis increases "morbidity" and "mortality" and increase in the load of infectious patient leading to "secondary resistance" leading to passing on of "resistant strains" in the community. Above tests have its personal benefit and harm and diagnostic outcome differs in terms of "safety", "tolerability", and "feasibility" in various set-ups[4]. A peculiar test "Gene Xpert MTB/RIF", which was in recent times approved by the 'world health organization"(WHO), has the capacity to lead a transformation in the diagnosis of "active TB disease" plus "multidrug-resistant". That's why it is of great value in detection of both "drug susceptible" as well as "drug resistant" cases. "Gene Xpert" test can be done on "sputum samples" or "bronchoalveolar lavage (BAL)" samples. CBNAAT (cartridge based nucleic acid amplification test), a programmed instrument that has certain fundamental properties i.e., "sample processing", "nucleic acid amplification", and "detection of the target sequences in sample or complex samples using real time PCR and reverse transcriptase PCR". Results are accessible in below two hours. The accelerated finding of "Mycobacterium tuberculosis" and "resistance" to 1st line drug "Rifampicin" (RIF) facilitates the treating doctor to take important decisions in the treatment of patient in regards to treatment in the same visit.

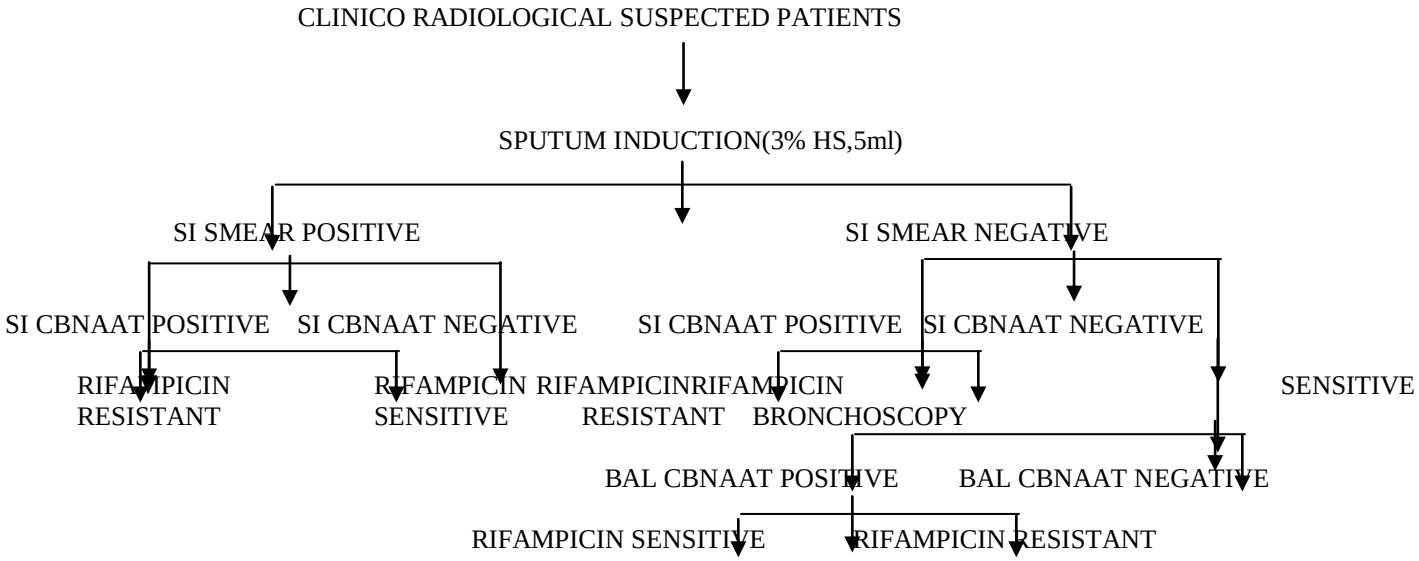
The purpose of this study is to assess the yield of smear positivity of induced sputum with hypertonic saline nebulisation, broncho-alveolar lavage fluid and Rifampicin resistance in clinically diagnosed pulmonary TB who have no/inadequate sputum.

Materials And Methods:-

The present study was conducted in the Department of Pulmonary Medicine, Veer Surendra Sai Institue of medical Science and Research, Burla, Odisha, India, from November 2018 to October 2020. The research protocol was approved by the institutional Ethics Committee. An informed and written consent was taken from all participants or nearest relative of the participants. A total of 100 patients with clinically diagnosed pulmonary TB who are unable to produce adequate sputum were included in the study. The inclusion criteria were persistent cough for atleast 2 weeks, Sputum either not produced or inadequate for examination (only saliva or sputum quantity < 2ml), Chest radiograph showing changes consistent with active pulmonary TB (Parenchymal consolidation, Lymphadenopathy, Tuberculoma, Miliary TB, Airway Involvement, Pleural effussion, Cavitation) and age more than 18 years. The exclusion criteria were patients with uncontrolled asthma or chronic obstructive pulmonary disease, patients with

active hemoptysis, patients with unstable angina or Arrythmias, patients already on Antitubercular therapy and HIV infected patients.

To avoid contamination, the patients will be asked for rinsing and repeated gargling with tap water until the returned fluid is free from debris. 10 ml of 3% hypertonic saline will inject into the nebulizer reservoir device. The assembly will connect to the jet nebulizer. The procedure will carry out in a well-ventilated room. The patients will be instructed to inhale and exhale the mist of the nebulized solution through the mouth piece only. The inhalation of hypertonic saline will interrupt every 5 min, so that the patient could expectorate the sputum into a clean sterile sputum container. The procedure will be continued until an adequate amount of sputum sample (2ml or more) is obtained or for a maximum of 15 min without success or the patients complains of shortness of breath or wheeze. The patient will be in observation during the procedure and 1 hour post-procedure. The sputum samples will stain with auramin-rodamin stain and examine for AFB also same sample will send for CBNAAT. Selected SI CBNAAT negative patients also will undergo bronchoscopy. Bronchoscopy will undergo with fiberoptic bronchoscope under local anaesthesia with 2% lignocaine and xylocaine jelly and mouth spray bronchial washing and lavage will done by instilling 20ml of normal saline at room temperature upto 100 ml and collected into a sterile suction by aspiration. BAL fluid will send for CBNAAT.



Results:-

A total of 100 cases with clinically diagnosed PTB, 71% were males and 29% were females included in the study. Male to Female ratio was 2.44:1. The most common age group was 55-65 years. The detail characteristics are shown in [Table-1].

Table 1:- Baseline characteristic and demographic profile.

a) Variable		*b) Male (n=71)*	*c) Female (n=29)*
d) Age Group (in years)	15-25	10	3
	25-35	2	4
	35-45	16	5
	45-55	14	8
	55-65	17	6
	65-75	7	3
	75-85	5	0
e) Occupation	Contractor	3	0
	Driver	5	0
	Farmer	25	3

	Housewife	0	26
	Labour	19	0
	Painter	1	0
	Policeman	1	0
	Student	8	0
	Tailor	6	0
	Teacher	3	0
Smoker		17	0

Table 2:- Symptoms.

Symptoms (n=100)	Frequency	Percent
Cough	79	79.0
Fever	75	75.0
Shortness of breath	9	9.0
Hemoptysis	17	17.0
Loss of appetite	11	11.0
Weight loss	26	26.0
Weakness	28	28.0

The most common symptom observed was cough without expectoration(79%), followed by fever(75%),weakness(28%),weight loss(26%) and hemoptysis(17%).

Table 3:- Symptoms – Duration.

Duration of Symptoms (Days)	Mean (SD)	Median (IQR)	Minimum	Maximum
Cough (n=79)	37.56(32.9)	30.0 (18.0 – 45.0)	10	180
Fever (n=75)	20.43(15.9)	15.0(12.0-25.0)	4	90
Shortness of breath (n=9)	17.44(28.5)	5.0(4.0-20.0)	2	90
Hemoptysis (n=17)	7.76(9.31)	4.0(2.0-10.0)	1	30
Loss of appetite (n=11)	27.82(12.62)	30.0(16.0-30.0)	15	60
Weight loss (n=26)	45.35(28.01)	30.0(30.0-60.0)	15	120
Weakness (n=28)	51.43(57.65)	30.0(15.0-60.0)	15	300

Table 4:- Chest X-Ray and HRCT Scan Thorax findings.

	Variables	Frequency	Percentage(%)
X-Ray findings (n=100)	Infiltration	49	49.0
	Consolidation	45	45.0
	Cavity	16	16.0
	Fibrosis & Infiltration	17	17.0
	Miliary	6	6.0
CT Scan Thorax – findings (n=21)	Necrotizing pneumonia	5	23.5
	Consolidation	8	38.5
	Cavitary lesion	4	19
	Ground glass capacity & infiltration	4	19

Most common associated radiological lesion was infiltration(49%) followed by consolidation(45%),fibrosis+infiltration(17%), cavity(16%) and miliary(6%). Out of 21 patients who underwent bronchoscopy, most common HRCT chest finding was consolidation(38.5%), followed by necrotizing pneumonia(23.5%), cavitory lesion(19%) and ground glass opacity & infiltration(19%).

Table 5:- Induced sputum results.

Ivestigations		Frequency	Percentage (%)
Induced Sputum-AFB (n=100)	Positive	17	17
	Negative	83	83
Induced Sputum-CBNAAT (n=100)	MTB detected	30	30
	MTB not detected	70	70

Rifampicin sensitive (n=100)		30	30

Out of 100 cases, 17 cases become induced sputum smear positive(17%), 30 cases become induced sputum CBNAAT positive(30%) and all induced sputum CBNAAT cases were rifampicin sensitive. No rifampicin resistance were detected.

Table 5:- Broncho alveolar lavage (BAL) results.

Investigations	Post bronchoscopy	Frequency	Percentage (%)
BAL – AFB (n=21)	sputum AFB positive	4	19
	sputum AFB negative	17	81
BAL – CBNAAT (n=21)	MTB detected	8	38
	MTB not detected	13	62
Rifampicin sensitive (n=21)		8	38

Out of 70 patients with SI CBNAAT negative, 21 patients with highly clinic-radiological suspicion underwent bronchoscopy, 4 (19%) patients become post bronchoscopy sputum AFB positive, 8 (38%) patients become BAL CBNAAT positive and all are rifampicine sensitive.

Discussion:-

Direct sputum smear examination is used as a basic diagnostic tool in the diagnosis of pulmonary tuberculosis. But difficulty in obtaining adequate sputum is the main cause of failure of bacteriological diagnosis. Alternative methods of obtaining sputum specimens are frequently needed in those patients with clinic-radiological suspicion of TB who are unable to expectorate or are smear negative. Induced sputum CBNAAT, Broncho alveolar lavage (BAL) fluid CBNAAT is some alternative methods.

Of the 100 cases included in this study with clinically diagnosed PTB, predominantly males(71%) compared to female(29%) also there was predominance of males in previous study conducted by various authors [5,6,7]. The most common symptom observed in this study was cough without expectoration (79%) followed by fever (75%) which was noticed in a similar study conducted by Boyilla N et al[6] which showed 74% cough followed by fever(52%) and Avashia S et al[8] reported cough in 72.2% and fever in 69.9% of patients. Other symptoms observed was hemoptysis(17%) which was noticed in a similar study conducted by Boyilla et al[6] 16% and Avashia S et al[8] 9.7%. In a study by Woodering et al[9], Krysyl et al[10] observed that pulmonary infiltration is 80% of cases and tubercular cavity is present in between 19-50% of all tuberculosis patients. In this study 49% of cases had only infiltration, 45% cases had consolidation,17% had infiltration with fibrosis,16% cases with cavitory lesions and 6% had miliary lesions. In this study 16% of patients had cavitory lesion which is similar to study conducted by Avashia S et al[8] reported cavity in 14% of cases. 33% of cases had consolidation in a study conducted by Avashia S et al[8], whereas in our study there were 45% patients with consolidation. It was observed that induced sputum smear positivity rate is highest with chest X-Ray having infiltrative lesion(28.6%) followed by consolidation(17.2%). Cavitatory lesions had 12.5% SI smear positivity rate. None of the miliary shadow came sputum smear positive. Parry CM et al[11] by using hypertonic saline found that sputum was successfully induced from 73 of the 82 patients (26 previously smear negative and 47 previously unproductive). The induced sputum was smear-positive in 18 patients, i.e.21.95% positivity. Toubes ME et al[12] in their study comparing the utilities of sputum induction by hypertonic saline using two different type of nebulizers,for diagnosis of tuberculosis, found that induced sputum samples were positive by culture in 90.6% cases and by direct smear microscopy in 20.5% cases. Anderson C et al[13] compared sputum induction to fibreoptic bronchoscopy in the diagnosis of pulmonary tuberculosis and found that sputum induction yielded a positive smear result in 19% cases. In this study, by hypertonic saline induction was found that 17% of smear positivity which is similar to results of Parry CM et al[11], Toubes ME et al[12], Anderson C et al[13]. Out of 100 cases induced sputum CBNAAT was turned out to be positive in 30% cases with mean age of 48.27 years. Out of total female patients 31% were SI CBNAAT positive and in male patients 29.6% cases were SI CBNAAT positive. No rifampicin resistant cases were detected. It was observed that induced sputum CBNAAT is highest with chest X-Ray having infiltrative and fibrotic lesions (47.1%) followed by consolidation (26.7%). Cavitatory lesions had 25% SI CBNAAT positivity rate. One of the miliary shadow came SI CBNAAT positive(16%). Dewan R et al[14], in their study found that mean age of patients was 35±9 years; 69% of were in 20-40 years age group and 76% were males. Sensitivity of conventional sputum smear microscopy by Ziehl-Neelsen staining was very low (10.68%). Geleta DA et al[15] have also found a very low sensitivity (9.3%) of sputum smear for AFB. In this study, overall

CBNAAT was positive in 38.31% PTB cases. Sensitivity of CBNAAT varied significantly between 100% in sputum smear-positive PTB and 15.38% in sputum smear-negative PTB. In studies conducted by Mukherjee S et al[16] and Geleta DA et al[15] showed similar results of very high sensitivity of CBNAAT in smear positive cases have been reported. Mohanty T et al[15], and Dewan R et al[14], reported sensitivity of 32% and 32.58% of CBNAAT in smear negative PTB, which correlates with present study. Sreekanth B et al[18],studied the effectiveness of CBNAAT in smear negative PTB, Samples from 107 cases with high clinico-radiological presumption were subjected to CBNAAT examination, out of which 41 (38.31%) were confirmed positive microbiologically. The mean age was 45±18.30 years, 24% were females and 76% were males. So in this study SI CBNAAT positivity rate almost similar to results of Dewan R et al[14], Sreekanth B et.al[18], Mohanty T et al[17]. Out of 21 patients who underwent bronchoscopy with highly clinic-radiological suspicion 8 cases (38%) become BAL CBNAAT positive and all were Rifampicin sensitive. Post bronchoscopy sputum AFB become positive in 4 cases (19%). This correlate similar study conducted by Mahavarkar R et.al[19] Out of 100 patients who were clinico radiologically suspected for PTB who underwent bronchoscopy 45 were diagnosed PTB out of which 32 tested positive for CBNAAT, no rifampicin resistance was detected in those cases.

Limitation Of Study

The study population is small, so for definite conclusion more number of cases to be included and difficulty to do bronchoscopy in all SI CBNAAT negative patients.

Conclusion:-

Induced sputum and BAL fluid are better alternative methods for diagnosis in patients with clinico-radiological suspicion of TB who are unable to expectorate or are smear negative and no rifampicin resistant cases were detected throughout this study.

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