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Role of Sputum Examination in Tuberculous Pleural Effusion Patients at Zagazig University Hospitals

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Abstract:

Background: *Tuberculous pleuritis should be considered in any patient with an exudative pleural effusion. In most cases of tuberculous pleural effusion there are no apparent parenchymal lung lesions on chest radiography. Spontaneous or induced sputum specimen examination for acid fast bacilli (AFB) is important for such cases. Parenchymal lesion including focal areas of subpleural cavitation and lymphadenopathy can be visualized with the use of computed tomography, which is not apparent on routine chest radiography. In such cases there is possibility of isolation of tubercle bacilli in sputum which can be indicator of infectiousness.*

Aim of the Study: *To evaluate the role of sputum examination for (AFB) in tuberculous pleural effusion cases as indicator for infectiousness.*

Subjects and Methods: *The present study included 64 Patients proved to have tuberculous pleural effusions, 32 patients with parenchymal lesions and 32 patients without parenchymal lesion. The diagnosis of tuberculous pleural effusion is performed by direct smear examination and culture of pleural fluid, pleural biopsy examination for AFB, culture and presence of caseating granuloma, and ADA examination of pleural fluid.*

Sputum examination for AFB was done by: *Direct smear examination on 3 consecutive days and Culture of on Lowenstein-Jensen (L-J) media.*

Results: *Sputum Z-N examination for AFB was positive for tuberculosis in 7 out of 32 cases (21.9%) in patients with apparent radiologic parenchymal infiltrate and in 2 out of 32 cases (6.3%) in patients with no apparent radiologic parenchymal infiltrate in which tuberculous etiology was confirmed by histology and /or bacteriology. Sputum culture on L-J media was positive for mycobacterium tuberculosis growth in 13 out of 32 cases (40.6 %) in patients with apparent radiologic parenchymal infiltrate and in 7 out of 32 cases (21.9%) in patients with no apparent radiologic parenchymal infiltrate.*

Conclusion: *Sputum examination for AFB in cases of tuberculous pleural effusion remain doubtful but it may show important benefit in diagnosis, treatment of such cases and as an indicator of infectiousness*

Keywords: ***Tuberculosis, pleural effusion and sputum induction.***

Introduction

The possibility of tuberculous pleuritis should be considered in every patient with an undiagnosed exudative pleural effusion.[1]

Pleura are main site of extrapulmonary TB and only second to lymphatic involvement.[2] 3% to 25% of patients with TB will be susceptible to have tuberculous pleural affection. The percentage is higher in HIV positive patients.[3]

Pleural effusion in TB patients occurs when subpleural caseous focus ruptures into the pleural space so TB antigen enters the pleural space; this is followed by a local, delayed hypersensitivity reaction. This is mediated by CD4+ T lymphocyte cells.[1],[2]

Tuberculous effusion may occur during primary or reactivation of TB and may involve viable, dead or no bacilli. When tuberculous protein enters the pleural space, the delayed hypersensitivity reaction is initiated increasing the pleural capillaries permeability to protein leading to higher rate of pleural fluid formation.[1]

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Also pleural fluid clearance is decreased due to lymphatics obstruction caused by lymphocytic pleuritis.[2]

Objectives:

1. Detect the probable infectiousness of sputum in TB effusion patients.
2. Evaluate the role of sputum examination as a method of diagnosis of TB effusion patients.

Patients and Methods

This was Comparative Cross sectional study and was conducted at Chest Department, Zagazig University Hospital during the study period from November 2015 to November 2016.

Study population

(64 cases) proved to have tuberculous pleural effusion. The patients were divided into two groups according to presence or absence of parenchymal lung lesion, depending on the chest radiographic findings.

- **(Group A)** Patient have radiological finding of pleural effusion with presence of parenchymal lesions (32 cases).
- **(Group B)** having radiological findings of pleural effusion without an apparent parenchymal lesions (32 cases).

Inclusion Criteria[4]:

Patients proved to have tuberculous pleural effusion. The diagnosis was reached through:

1. The clinical findings included[1,4,5,6,7,8]: History of contact to known tuberculous case, general manifestation of toxemia (as fever, night sweating, anorexia and weight loss), local chest signs revealed mainly findings related to pleural involvement including (decrease breath sounds and stony dullness on percussion overlying the effusion).
2. The radiographic findings included[3,9,10,11]:
    - Classical Radiological presentation of pleural effusion whether free or encysted (by plain CXR and CT).
    - Other radiographic features e.g cavitation or apical opacities.
3. Tuberculine skin tests (Manatoux Technique)[1,9]
4. Pleural fluid examination. (gross appearance, biochemical tests, total and differential leukocyte, smear Z-N for AFB, culture on L-J media)[12,13]
5. Pleural tissue biopsy Via Abram's for Z-N, Culture and histopathological examination for caseating granuloma.[3,11,13,24,25]
6. Sputum examination for AFB For all selected cases was done by:
    a. Direct smear examination by Z-N stain on 3 consecutive days.
    b. Culture for mycobacterium tuberculosis growth on L-J media.

Sputum specimen are often not evaluated because many of these patients are not able to produce sputum spontaneously, sputum induction is a safe procedure with a high diagnostic yield for pulmonary tuberculosis.[26,27,28]

- **Sputum induction[26]:**

It is advisable that bronchodilators medication, oxygen supply and resuscitation equipment should be available at procedure place. Although experienced technician can conduct sputum induction, a physician should supervise the procedure. Two methods are commonly used. In one of them the inhalation of the same concentration of hypertonic saline (4.5%) for increasing time interval is used while in the other inhalation for the same period of increasing concentration of hypertonic saline (3%, 4%, 5%) is used.

The ultrasonic nebulizer produces a mist of hypertonic saline droplets. The smaller droplets are deposited peripherally in the lung. It is suggested that the hypertonicity of the deposited saline will draws interstitial fluid into the lower airways by osmosis. The hypertonic fluid also causes bronchial irritation and this stimulates bronchial secretions. After 10-20 minutes of nebulization the fluid produced mobilize the material in the lower airways. Repeated coughing by the patient help in movement of this material into trachea to facilitate expectoration.[27]

Exclusion Criteria[5]:

1. Patients had received Anti-tuberculous therapy during the three months before enrollment.
2. Patients on anticoagulant therapy as thoracentesis and pleural biopsy were contraindicated.
3. Patients proved to have a diagnosis of another disease as:
    - Congestive heart failure.
    - Liver cell failure.
    - Renal failure.
    - Para pneumonic effusion.

Statistical Analyses

All statistical analyses were carried out using the SPSS (statistical package for the social science software) statistical package version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows. Quantitative data were expressed as mean and standard deviation (X+SD) and analyzed by applying student t-test for comparison of two groups of normally

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distributed variables. The results of the "t"- value is then checked on student's "t"-table to find out the significance level (p-value) according to the degree of freedom. All these tests were used as tests of significance at $P < 0.05$.

Results

Table (1): Comparison between Group A and Group B as regard Basic characteristics:

Characters	Group A (N=32)		Group B (N=32)		P-value
	No	%	No	%	
Gender					
Male	24	75%	20	62.5%	0.281
Female	8	25%	12	37.5%	
Age					
Mean ± SD	40 ± 15.68		40.84 ± 14.79		0.826 0.
Smoking					
No	8	25%	10	31.25%	0.139
Mild	12	37.5%	18	56.25%	
Moderate	4	12.5%	1	3.1%	
Severe	8	25%	3	9.4 %	

Table (2): Comparison between Group A and Group B as regard clinical presentation:

Characters	Group A (N=32)		Group B (N=32)		P-value
	No	%	No	%	
Clinical picture					
Fever	28	87.5 %	25	78.1%	0.509
Sweating	26	81.25 %	19	59.4%	0.099
Anorexia	16	50%	15	46.9%	1
Weight loss	17	53.1%	11	34.4%	0.207
Cough	30	93.75%	25	78.1%	0.148
Expectoration	22	68.8%	2	6.3%	< 0.001
Dyspnea	20	62.5%	7	21.9%	0.002
Pain	17	53.1%	28	87.5%	0.005
Tuberculin test					
Positive	24	75%	28	87.5%	0.337
Negative	12	25 %	4	12.5%	

Table (3): Comparison between Group A and Group B as regard Pleural fluid bacteriology, Pleural biopsies, and ADA:

Characters	All patients (N=64)		Group A (N=32)		Group B (N=32)		p-value
	No	%	No	%	No	%	
Pleural fluid ZN / culture							
Positive	5	7.8	3	9.4	2	6.3	1
Negative	59	92.2	29	90.6	30	93.8	
Abram biopsy granuloma							
Positive	33	51.6	17	53.1	16	50	0.83
Negative	26	40.6	12	37.5	14	43.8	
Failed	5	7.8	3	9.4	2	6.3	
Abram biopsy ZN							
Positive	7	10.9	3	9.4	4	12.5	0.84
Negative	52	81.3	26	81.3	26	81.3	

DOI: 10.23958/ijirms/vol02-i08/17

Failed	5	7.8	3	9.4	2	6.3	
Abram biopsy culture							
Positive	27	42.2	14	43.8	13	40.6	0.83
Negative	32	50	15	46.9	17	53.1	
Failed	5	7.8	3	9.4	2	6.3	
ADA							
Positive	31	48.4	15	46.9	16	50	0.802
Negative	33	51.6	17	53.1	16	50	

Table (4): Comparison between Group A and Group B as regard sputum induction

Characters	**Group A (N=32)**		**Group B (N=32)**		**P-value**
	No	%	No	%	
Sputum induction					
Failed	4	12.5%	5	15.6%	1
Success	28	87.5%	27	84.4%	

Table (5): Comparison between Group A and Group B as result of sputum examination

Characters	All patients **(N=64)**		Group A **(N=32)**		**Group B (N=32)**		**p-value**
	No	%	No	%	No	%	
Sputum ZN							
Positive	9	14.1%	7	21.9%	2	6.3%	0.198
Negative	46	68.75%	21	65.6%	25	78.1%	
Failed	9	14.1%	4	12.5%	5	15.6%	
Sputum culture							
Positive	20	31.3%	13	40.6%	7	21.9%	0.269
Negative	35	54.69%	15	46.9%	20	62.5%	
Failed	9	14.1%	4	12.5%	5	15.6%	

Table (6): Results of sputum examination among patients with tuberculous pleural effusion cases diagnosed by different methods in group A:

Method of etiologic diagnosis	**Sputum smear/ culture positive Group A**			**P value**	**Signi-ficance**
	Negative(N=15)	**Positive (N=13)**	**Failed(N=4)**		
Diagnosed by pleural fluid ZN / culture					
Negative (n=29)	15 (100%)	10 (76.9%)	4 (100%)	0.08	NS
Positive (n=3)	0	3(23.1%)	0		
Diagnosed by pleural biopsy ZN					
Negative (N=26)	12 (80%)	10 (76.9)	4 (100%)	0.092	NS
Positive (N=3)	3 (20%)	0	0		
Not done (N=3)	0	3 (233.1%)	0		
Diagnosed by pleural biopsy culture					
Negative (N=15)	9 (60%)	2 (15.4%)	4 (100%)		S

DOI: 10.23958/ijirms/vol02-i08/17

Positive (N=14)	6 (40%)	8 (61.5%)	0	0.012	
Not done (N=3)	0	3 (23.1)	0		
Diagnosed by pleural biopsy granuloma					
Negative (N=12)	6 (40%)	6 (46.2%)	0	0.484	NS
Positive (N=17)	8 (53.3%)	6 (46.2%)	3 (75%)		
Not done (N=3)	1 (6.7%)	1 (7.7%)	1 (25%)		
Diagnosed by ADA					
Negative (N=17)	9 (60%)	8 (61.5%)	0	0.075	NS
Positive (N=15)	6 (40%)	5 (38.5%)	4 (100%)		

Table (7): Results of sputum examination among patients with tuberculous pleural effusion cases diagnosed by different methods in group B:

Method of etiologic diagnosis	**Sputum smear/ culture positive Group B**			**P value**	**Signi-ficance**
	Negative(N=20)	**Positive (N=7)**	**Failed(N=5)**		
Diagnosed by pleural fluid ZN / culture					
Negative (n=29)	20 (100%)	5 (71.4%)	5 (100%)	0.022	S
Positive (n=3)	0	2 (28.6%)	0		
Diagnosed by pleural biopsy ZN					
Negative (N=26)	19 (95%)	3 (42.9)	4 (80%)	0.022	S
Positive (N=4)	1 (5%)	2 (28.6%)	1 (20%)		
Not done (N=2)	0	2 (28.6%)	0		
Diagnosed by pleural biopsy culture)					
Negative (N=17)	11 (55%)	2 (28.6%)	4 (80%)	0.055	S
Positive (N=13)	9 (45%)	3 (42.9%)	1 (20%)		
Not done (N=2)	0	2 (28.6%)	0		
Diagnosed by pleural biopsy granuloma					
Negative (N=12)	8 (40%)	4 (57.1%)	2 (40%)	0.620	NS
Positive (N=17)	11 (55%)	3 (42.9%)	2 (40%)		
Not done (N=3)	1 (5%)	0	1 (20%)		
Diagnosed by ADA					
Negative (N=16)	9 (45%)	5 (71.4%)	2 (40%)	0.430	NS
Positive (N=16)	11 (55%)	2 (28.6%)	3 (60%)		

DOI: 10.23958/ijirms/vol02-i08/17

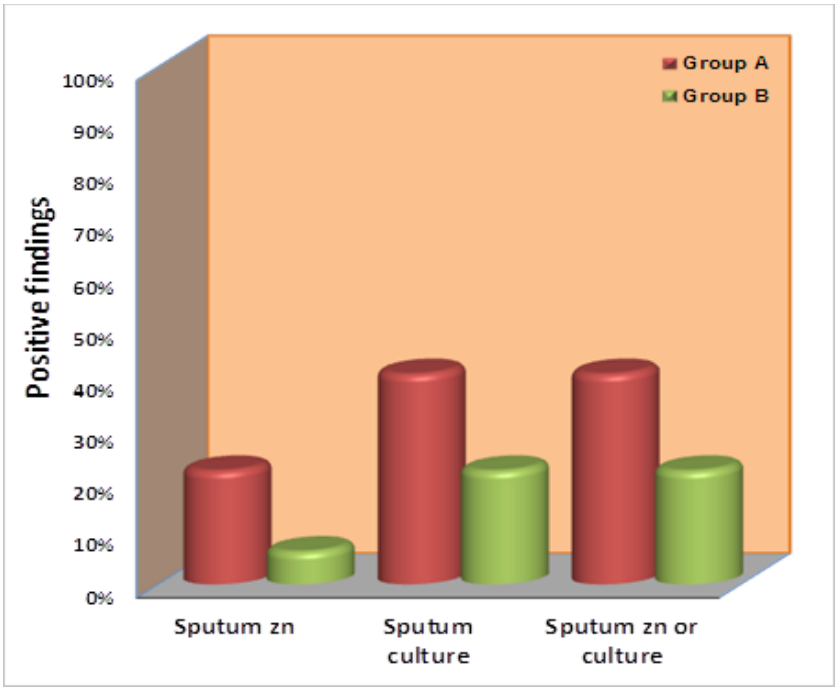

Figure (1) Shows the relationship between group A and group B as regard sputum examination.

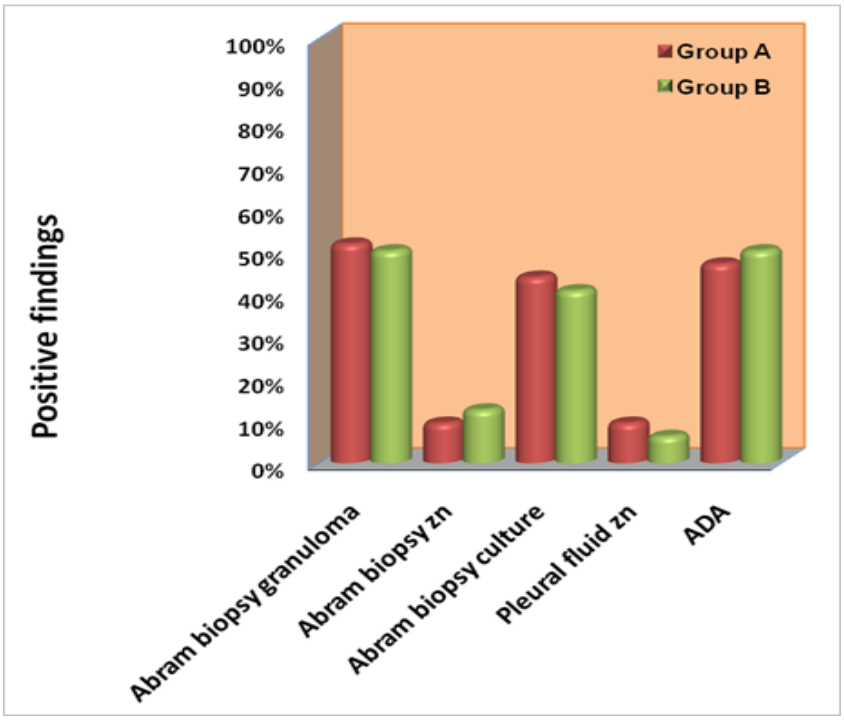

Figure (2) Shows the relationship between group A and group B as regard different methods of diagnosis of tuberculous pleural effusion.

A total of 64 subjects proved to have TB pleural effusion, including 32 case with parenchymal lung lesions & 32 with no parenchymal lung lesions. The mean age of the patients was (40.02 ± 15.13), male: female was 44:20. 18 patients were nonsmoker, 46 patients were smoker (Table 1).

Regarding the clinical picture of the patients, 57 patients have fever, 49 patients presented with sweating, 31 patients with anorexia, 28 patients with weight loss, 57 patients with cough, 24 patients with expectoration, 27 patients with dyspnea and 45 patients with chest pain (Table 2).

Analysis of the pleural fluid showed that it was straw colored in most cases (95%) and hemorrhagic in 5% of patients.

Pleural fluid was exudative in all patients according to Light's criteria with predominant lymphocytes in 85% of

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cases and in 15% the predominant cells were neutrophils. No malignant cell was found in any case.

All cases are proved to have TB pleural effusion by different diagnostic procedures. Searching for AFB in pleural fluid deposit was done by Z-N staining. It was positive in 3 cases of group A and 2 cases of group B. pleural fluid culture was positive in 5 cases of group A and 3 cases of group B. no bacteria could be detected by gram's stain of pleural fluid.

Abram pleural biopsy was done in 59 patients and failed in 5 patients. 33 cases (17 in group A and 16 in groups B) showed caseating epitheloid granuloma with giant cell.26 cases showed chronic nonspecific inflammation.

Examination of the pleural tissue for AFB by Z-N method was done. It was positive in 7 cases. 27 cases (42.2%) showed mycobacterium TB growth on culture of pleural tissue in L-J media.

ADA was done to confirm the diagnosis of tuberculous pleural fluid and to diagnose cases which not diagnosed by bacteriology or histology.

ADA level of more than 40IU/l considered positive for tuberculous etiology of exudative lymphocytic pleural fluid. ADA was positive in 31 cases (48.4%) (Table 3), (Figure 2).

After confirming tuberculous etiology of pleural effusion examination of the sputum for AFB by ZN staining and culture in L-J media. 9 patients failed to induce sputum. AFB by Z-N was positive in 9 cases, 7 in group A and 2 in group B. 20 cases showed mycobacterium tuberculosis growth on culture, 13 in group A and 7 in group B (Table 4,5), (figure 1).

The relationship between different methods of diagnosis of tuberculous pleural effusion (pleural fluid ZN / culture, pleural biopsy ZN, culture, granuloma, ADA) and sputum examination for AFB by ZN and culture showed no statistical significant difference in group A except for pleural fluid culture (Table 6). In group B it showed significant difference for pleural fluid ZN / culture and pleural biopsy ZN, culture, but no statistical significant difference for pleural biopsy granuloma, ADA (Table 7).

Discussion

This study included 64 patients with proved tuberculous pleural effusion etiology. The gross appearance of pleural fluid was straw colored in most cases (95%) and hemorrhagic in 5% of patients. Biochemical examination shows that it was exudative in all patients according to Light's criteria with predominant lymphocytes in 85% of cases and in 15% the predominant cells were neutrophils.

In agreement with this study, in a retrospective study including 214 patients proved to have tuberculous pleural effusion, there were predominant polymorphonuclear cells in only 11% of cases. Patients with lymphocytic pleural fluid, showed a higher incidence of positive pleural fluid culture for mycobacterium TB compared with those who have predominant polymorphonuclear cells (50% vs. 10%).[29]

The patients in the current series study were classified into two groups: group A with radiologic parenchymal infiltrate and group B with no apparent radiological parenchymal infiltrate.

This is consistent with various studies which reported that from 20% to 50% of cases showed coexisting parenchymal lesions.[30]

CT Chest improves the diagnostic accuracy by confirming the presence of associated parenchymal lesions and lymphadenopathy. In a prospective study in patients with tuberculous pleurisy, coexisting parenchymal lesions were observed in 86% of patients when using chest CT.[31]

In this study AFB was found in smear of pleural fluid in 3 cases (9.4%) of group A and in 2 (6.3%) cases of group B. This is consistent with **Chaudhuri AD et al.,**[32] who did their study on 45 cases with no apparent parenchymal infiltrate. They found that AFB in pleural fluid smear in 4 cases (8.88%). Unfortunately **Antoniskis et al.,**[33] found AFB in 27% of cases with tuberculous pleural effusion having pulmonary infiltrate and in 7% of cases of tuberculous pleural effusion with no apparent radiological parenchymal infiltrate.

In this study we did Abram's biopsy for 59 cases, 5 cases failed biopsy, 3 in group A and 2 in group B.

AFB was found in smear of pleural tissue in 3 cases (9.4%) of group A and in 4 cases (12.5%) of group B. This finding is consistent with **Chaudhuri AD et al.,**[32] who found AFB in pleural tissue in (16.21%) of cases. In **Antoniskis et al.,**[33] AFB was found in pleural tissue in (17%) of cases. **Bueno et al.,**[34] studied the cytologic and bacteriologic examination of pleural fluid and pleural biopsy specimens with Abram's needle. Their study included 414 patients with undiagnosed pleural effusion. The diagnostic yield of tuberculous effusion by finding AFB in pleural tissue was (10%).

The yield of pleural tissue culture on L-J media in this study was 14 (43.8%) in group A and 13 (40.6%) in group B. This result is comparable to **Chaudhuri AD et al.,**[32] study, where 21 cases out of 37 (56.75%) showed growth of mycobacterium tuberculosis on culture of pleural tissue. Also **Gopi et al.,**[13] showed positive pleural tissue culture in

DOI: 10.23958/ijirms/vol02-i08/17

39% of cases. In contrast, **Valdes et al.,**[9] who studied the case histories of 254 patients in whom TB pleural effusion were diagnosed. They compared the sensitivity of various diagnostic tests. They showed positive culture of pleural tissue in 140 out of 248 cases (56%). This higher percentage may be due to larger number of cases.

Regarding the pleural tissue histology, tuberculosis was evidenced by the presence of caseating epithelioid granuloma in 17 cases (53.1%) in group A and in 16 cases (50%) in group B. This is consistent with the result of **Gopi et al.,**[13] study, who found caseating epithelioid granuloma in (50-97%) of cases, and in (51.11%) of cases in **Chaudhuri et al.,**[32] study.

ADA was done to confirm the diagnosis of TB pleural effusion. It was positive in 46.9% of patients in group A and 50% of patients in group B. In **Chaudhuri et al.,**[32] study, 15 cases out of 45 were ADA positive.

After confirming the diagnosis of tuberculous pleural effusion sputum examination was done for all cases. 9 cases showed failed sputum induction.

From all cases 9 cases (14.1) showed positive sputum Z-N for AFB, 7 patients (21.9%) in group A and 2 patients (6.3) in group B. Sputum culture by L-J media was positive in 20 cases (31.3%), 13 cases (40.6%) in group A and 7 cases (21.9%) in group B.

In agreement with our study, **Antoniskis et al.,**[33] evaluated 59 confirmed cases of tuberculous pleural effusion, 27 patients had radiological parenchymal infiltrate and 32 patients had no radiological parenchymal infiltrate. Sputum culture for mycobacterium tuberculosis was positive in 60% of cases with parenchymal lesion and 23% of cases with no parenchymal lesion.

This is also consistent with **Chaudhuri AD et al.,**[32] study, who stated that 5 out of 45 (11.11%) cases showed positive sputum smear for AFB and 10 out of 45 (22.22%) of cases showed growth of mycobacterium tuberculosis in sputum culture.

Also **Ghosal et al.,**[35] studied 30 diagnosed cases of tuberculous pleuritis having no apparent lung lesion on routine chest radiography, 3 cases (10%) showed positive sputum smear and 11 cases (27%) showed positive sputum culture.

Unfortunately, in **Kim et al.,**[31] study, 106 cases with tuberculous pleural involvement were included (range of age, from 16 to 89 years). 33 casess (31%) had positive sputum or bronchial lavage findings for AFB smears or culture. With the use of CT chest, parenchymal lesions were found in 91 patients (86%). From them 39 patients (37%), showed radiographic characteristics of active pulmonary tuberculosis. This higher percentage may be due to higher numbers of cases and using of bronchial wash.

In contrast with our study, prospective study of **Conde et al.,**[27] did their study in 113 patients with suspected pleural tuberculosis who were unable to produce sputum spontaneously. They evaluated the diagnostic yield of acid-fast bacilli smear and culture for *Mycobacterium tuberculosis* using sputum induction (SI). A final diagnosis of pleural TB was made in 84 patients; from them 71 were HIV seronegative and 13 patients were HIV seropositive, and a final diagnosis of another disease in 29 patients. The yield of SI culture for *M. tuberculosis* was 55% in patients with no parenchymal radiographic lesions and 45% in those with evidence of parenchymal disease suggestive of pulmonary TB (p = 0.6). This may be due to including patients who were HIV seropositive**.**

From sputum smear examination for AFB and mycobacterium growth in L-J media results of this study it is evident that all patients of tuberculous pleural effusion, it is advisable to do careful sputum examination for AFB smear and culture.

Patients with no apparent radiological parenchymal lesion are usually considered sputum negative and noninfectious. However, sputum examination may reveal the presence of tubercle bacilli so these cases are infectious. The needs for contact examination of all cases of tuberculous pleural effusion is important even in those having no apparent parenchymal lesion on chest X-ray.

Tuberculous pleural effusion is considered extra-pulmonary and they are treated with category III regimen under Revised National Tuberculosis Programme (RNTCP). If sputum examination is positive in some patients with tuberculous pleural effusion, this should change therapeutic approach to category I regimen. Therefore, careful sputum examination has significant therapeutic implication as well.

Conclusion

The role of sputum examination for AFB in patients with tuberculous pleural effusion remain doubtful. It is simple and inexpensive methods and may show important benefit in diagnosis, treatment of such cases and as indicator of infectiousness.

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