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CONTENTS

INSTRUCTION TO AUTHORS ABOUT UNIFORM MANUSCRIPT WRITING

The Chest and Heart Journal is published twice in a year in the months of January and July. The journal publishes original papers, reviews concerned with recent practice and case report of exceptional merits. Papers are accepted for publication with an understanding that they are subject to editorial revision. A covering letter signed by all authors must state that the data have not been published elsewhere in whole or in part and all authors agree their publication in Chest and Heart Journal. All submitted manuscripts are reviewed by the editors and rejected manuscripts will not be returned. Ethical aspects will be considered in the assessment of the paper. Three typed copies of the article and one soft copy in CD or Pen Drive processed all MS Word 6.0 should be submitted to the editor.

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Karalus NC, Cursons RT, Leng RA, et al. Community acquired pneumonia: aetiology and prognostic Index evaluation. Thorax 1991; 46 : 413-12.

c) No author given;
Cancer in South Africa (editorial). S Afr Med J 1994; 84-15.

d) Organization as author
The Cardiac Society of Australia and New Zealand. Clinical exercise stress training. Safety and performance guideline. Med J Aust 1996; 164 : 282-4.

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a) Personal author
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Student's medical dictionary. 26th ed. Baltimore: Williams & Wilkins; 1995. Apraxia; p.119-20.

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a) Journal articles in electronic format
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ORIGINAL ARTICLE

Assessment of Diagnostic Value of C-Reactive Protein in Granulomatous Pleuritis

Saroj Kanti Chowdhury[1], Taposh Bose[1], Md. Khairul Anam[2], Nihar Ranjan Saha[2], Md. Sirajul Islam[3], Bipul Kanti Biswas[3], Mohammed Shahedur Rahaman Khan[4], Biswas Akhtar Hossain[4], K.C Ganguly[4], S.M.Abdur Razzaque[4], AKM Mustafa Hussain[5], Mirza Mohammad Hiron[6]

Abstract:

Background: *Tuberculosis is an ancient human disease. The determination of biological marker levels in pleural effusions has been proposed as an alternative noninvasive means of establishing a diagnosis of granulomatous pleuritis . A variety of biological markers have been proposed to facilitate differential diagnosis of granulomatous pleuritis, C-reactive protein (CRP) is one of them. The present study was conducted to find out the sensitivity, specificity, accuracy, positive and negative predictive value of CRP for diagnosis of granulomatous pleuritis.*

Method: *The study was a cross sectional study conducted for one year from January 2010 to December 2010 in the Department of Respiratory Medicine, National Institute of Diseases of the Chest and Hospital, Mohakhali, Dhaka. Total 103 patients with clinically and radiologically diagnosed pleural effusion were included in the study. All patients of 18-80 years of age and both sexes with pleural effusion who were admitted during the study period in NIDCH and subsequently undergone thoracentesis were included in the present study through purposive sampling following inclusion and exclusion criteria.*

Result: *In the present study mean ± SD of age of the patient was 50.24 ± 18.38 years. Out of 103 cases of pleural effusion cases 50 (48.5%) were granulomatous pleuritis 8 them 46 were CRP +ve & 4 were CRP -ve and another 50 (48.5%) cases were caused by malignancy. Rheumatoid arthritis, nephrotic syndrome and congestive cardiac failure were found as the causes of pleural effusion one in each (1.0%) at cut of value 8.48. Sensitivity, specificity, PPV, NPV and accuracy of C - reactive protein in the diagnosis of granulomatous pleuritis were 92.0%, 90.6%, 90.2%, 92.3% and 91.3% respectively. Positive Likelihood Ratio was 9.752 and Negative Likelihood Ratio was 0.088.*

Conclusion: *The result indicated that the analysis of C - reactive protein levels in pleural fluid constitute a very useful marker for the diagnosis of granulomatous pleuritis which, in addition, can be made quickly and cheaply. So, we may conclude that overall this test is a very helpful adjunct test for the diagnosis of granulomatous pleuritis.*

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Introduction

In many areas of the world tuberculosis remains the most common causes of pleural effusion with granulomatous pleuritis in the absence of demonstrable pulmonary diseases[1]. About 95% cases of granulomatous pleuritis are due to infection by *M. tuberculosis* [2]. Tuberculosis is an ancient human disease. Today pulmonary tuberculosis has become the most important communicable disease in the world which is responsible for more morbidity and mortality than any other bacterial infections. Around one-third of the world's population has latent tuberculosis and that between 2002 and 2020, an estimate of 1000 million people will become newly infected, 150 million people will contact disease and 36 million will die [3]. Bangladesh ranks 6ththth in the world of tuberculosis disease burden with an estimated over 3,00,000 new cases each year of whom 70,000 die per year [4].

There is no prevalence study on granulomatous pleuritis in Bangladesh till now. A study in a small scale was done in 2002, where prevalence of EPTB was only 4.8% [5]. Incidence and prevalence of pulmonary TB in Bangladesh are 225/100000 population per year and 391/100000 population per year respectively [6].

Though pulmonary TB is the most common presentation of *M. tuberculosis* infection, extra pulmonary TB also constitute a frequent problem, particularly due to advancing immunosuppression in the era of HIV infection [7,8]. Tuberculous pleural effusion (TPE) which usually causes grnulomatous pleuritis is the second most common form of extra pulmonary tuberculosis. Granulomatous pleuritis results from a hypersensitivity reaction to tuberculin protein [9]. The incidence of granulomatous pleuritis is linked to the local prevalence of TB in general. In India EPTB constitutes about 15-20% of all cases of tuberculosis in immunocompitent adult, among them tuberculous pleural effusion occurs in 20% cases [7]. In many areas of the world tuberculosis remains the most common causes of pleural effusion with granulomatous pleuritis in the absence of demonstrable pulmonary diseases [1]. It has been a major health problem in the developing country like Bangladesh.

A wide range of diseases may be the cause of an accumulation of fluid in the pleural space but granulomatous pleuritis is almost always caused by infection with *M. tuberculosis* leading to pleural effusion [10]. Pleural effusion is a major diagnostic problem, time may be wasted before an accurate diagnosis is made in patients with pleural effusion, as the pleura is an inner cavity with no direct access, adding some difficulty to the diagnosis [11].

Pleural effusion can occur as a complication of many different diseases. It is a common clinical problem and it has been estimated that there are >800,000 cases/year in the USA. The diagnosis of granulomatous pleuritis remains a controversial issue in terms of cost to both patients and the healthcare system. Conventional methods are not always capable of establishing the cause of pleural effusion, and alternative tests permitting rapid and accurate diagnosis are greatly needed. Malignant disease involving the pleura and parapneumonic effusion are the leading causes of exudative pleural effusions [12]. Fluid collection within the pleural cavity can be assessed with clinical and radiological means. When pleural effusion is detected, the characteristics of the fluid (exudate or transudate) must be revealed using thoracocentesis [13].

The determination of the aetiology of the pleural effusion with granulomatous pleuritis was based on the clinical presentation, appropriate diagnostic test results and response to treatment of each patient [12]. Granulomatous pleuritis is thought to be the result of a delayed hypersensitivity reaction in response to the presence of mycobacterial antigens in the pleural tissue. This immunologic reaction causes the stimulation and differentiation of lymphocyte which release lymphokines which in turn activate macrophages for an enhanced bactericidal effect [14]. Despite the introduction and popularity of the new methods of *Mycobacterium tuberculosis* detection and identification, the diagnosis of some cases of tuberculosis, continues to pose considerable difficulty. The definitive diagnosis of granulomatous pleuritis is difficult because of the low sensitivity and specificity of non invasive traditional diagnostic tools, the result of pleural fluid staining for acid fast bacilli (AFB) is virtually negative and pleural fluid culture for AFB is positive in only <25% cases [15].

The determination of biological marker levels in pleural effusions has been proposed as an alternative noninvasive means of establishing a diagnosis of pleural effusion [12]. A variety of biological markers have been proposed to facilitate diagnosis of granulomatous pleuritis, including pleural fluid concentrations of adenosine deaminase (ADA), interferon (IFN)-ã, a variety of tumour markers and cytokines. Although there is a large body of literature on these biological markers and their utility in the diagnosis of pleural effusion, to date diagnosis is usually based on each individual marker separately [12,16,17]. But these tests need specific and/or expensive equipment that is not available in most laboratories. So alternative tests permitting rapid and accurate diagnosis is greatly needed for diagnosis of granulomatous pleuritis. Measurement of C - reactive protein (CRP) level in pleural fluid is such an inexpensive, rapid and accurate diagnostic tool. CRP is an acute phase protein widely used as a marker of inflammation and tissue damage. Its determination is simple, quick, and inexpensive. CRP has been found higher in granulomatous pleuritis and pneumonic pleural effusion. Several report suggest that in patient with lymphocytic pleural effusion an elevated pleural fluid CRP level of e"50 mg/L predicts granulomatous pleuritis with sensitivity of 45% (CI= 23-68),and specificity of 95% (CI=89-98), LR+ 9.3 (CI=3.71-23.30), LR- 0.57 (CI=0.38-0.86) [18]. On the contrary CRP of <30 mg/L virtually exclude this possibility. Although CRP in pleural fluid increases in many non tubercular diseases but it is higher in granulomatous pleuritis. In developing countries where TB is endemic an ideal test for patient of granulomatous pleuritis should be economic, minimally invasive, highly accurate and quick to perform. Pleural fluid CRP is such an investigation [19]. So the aim of this study is to analyze whether CRP may be a diagnostic aid in clinically suspected patient of pleural effusion with grnulomatous pleuritis. The determination of this biological marker level in pleural fluid has been proposed as an alternative noninvasive means of establishing a diagnosis of granulomatous pleuritis.

Materials and Methods:

This cross sectional study was conducted for one year from January 2010 to December 2010 in the Department of Respiratory Medicine, National Institute of Diseases of the Chest and Hospital, Mohakhali, Dhaka. Total 103 patients, 18-80 years of age and both sexes with pleural effusion who were admitted during the study period in NIDCH and subsequently undergone thoracentesis were included in the present study by purposive sampling following inclusion and exclusion criteria.

Inclusion Criteria: Clinically and Radiologically suspected cases of tuberculous pleural effusion patients who underwent thoracentesis and pleural biopsy, having granulomatous pleuritis in biopsized tissue.

Exclusion criteria:

1. Haemothorax
2. Patients with other systemic diseases like CLD, CRF etc
3. Patient unwilling to be involved in the study.
4. Pregnancy.

Sampling method: Purposive sampling.

Data collection tool: Data were collected through semi structure questionnaire.

The accumulated questionnaire was analyzed to find out the patients who meet the clinical inclusion and exclusion criteria to be cases. This patients were evaluated properly by taking medical history and clinical examination to determine whether they are eligible for inclusion in the study and conventional investigations like full blood count, X-ray chest and other investigation were done and recorded. Aspirated fluid was taken for cytology, microscopy, biochemical examination, malignant cells, CRP and ADA assay.

Data were recorded systematically in a preformed data collection sheet and analyzed using appropriate software (SPSS 15). Statistical analysis was made by two-tailed unpaired t test for the differences between CRP levels in pleural fluid of granulomatous and non granulomatous pleuritis.

Result and observations:

The present cross sectional study was conducted between the period of January 2010 to December 2010 in the Department of Respiratory Medicine, National Institute of Diseases of The Chest and Hospital, Mohakhali, Dhaka. Total 103 cases were enrolled in the study. Out of 103 cases 50 were

granulomatous pleuritis and 53 were non granulomatous pleuritis. Among non granulomatous cases 50 were due to malignancy and rest 3cases were due to nephritic syndrome, congestive cardiac failure and rheumatoid arthritis one in each.

Table-I

Distribution of age by sex of the respondents

Age (in year)	Sex		Total
	Male	Female	
<20	08 (10.8)	0 (0.0)	08 (07.8)
20-30	06 (08.1)	07 (24.1)	13 (12.6)
31-40	09 (12.2)	02 (06.9)	11 (10.7)
41-50	08 (10.8)	07 (24.1)	15 (14.6)
51-60	22 (29.7)	08 (27.6)	30 (29.1)
61-70	14 (18.9)	03 (10.3)	17 (16.5)
>70	07 (09.5)	02 (06.9)	09 (08.7)
Total	74 (100.0)	29 (100.0)	103 (100.0)
Mean ± SD	50.73 ± 19.27	49.00 ± 16.12	50.24 ± 18.38

Table I shows the distribution of age by sex of the respondents. Mean ± SD of age of the patient was 50.24 ± 18.38 years. Out of 103 patients with pleural effusion 74 were male and 29 were female. Male, Female ratio was 1.9: 1. Among the male highest number of patients were in the age group of 51 to 60 years (29.1%) followed by 61 to 70 years (18.9%). Mean ± SD of age of male was 50.73 ± 19.27 years. Among the female highest number of patients were in the age group of 51 to 60 years (27.6%) followed by 41 to 50 years (24.1%) and 20 to 30 years (24.1%). Mean ± SD of age of female was 49.00 ± 16.12 years.

Table II

Distribution of previous history of TB or contact with TB

Previous history of TB or contact with TB	Frequency	Percent
Present	05	04.8
Absent	98	95.2
Total	103	100.0

Table II shows the distribution of previous history of TB or contact with TB. Most of the patients had no history of TB or contact with TB (95.2%). Only 5 (4.8%) had history of TB or contact with TB.

Table-III

Distribution of presenting complaints of the respondents

Presenting complaints	Frequency*	Percent
Fever	99	96.1
Cough	98	95.1
Chest pain	48	46.6
Weight loss	43	41.7
Dyspnoea	22	21.4
Heamoptysis	09	08.7
Loss of appetite	03	02.9
Night sweats	03	02.9
Others	02	01.9

*Multiple responses

Table III shows the distribution of presenting complaints of the respondents. Common clinical presentations of patients were fever (96.1%), cough (95.1%), chest pain (45.6%) and weight loss (41.7%). Other clinical presentations were dyspnoea (21.4%), heamoptysis (8.7%), loss of appetite (2.9%) and night sweats (2.9%).

Table-IV

Distribution of pleural effusion in X-ray

Pleural effusion in X-ray	Frequency	Percent
Location		
• Right	78	75.7
• Left	25	24.3
Volume		
• Moderate	74	71.8
• Large	07	06.8
• Massive	17	16.5

Table IV shows the distribution of pleural effusion in X-ray. Out of 103 cases of pleural effusion 78 (75.7%) had right sided and 25 (24.3%) left sided pleural effusion. Highest number of cases presented with moderate pleural effusion (71.8%) followed by massive pleural effusion (16.5%). Five (4.9%) presented with small pleural effusion and 7 (6.8%) presented with large pleural effusion on X-ray findings.

Table-V

Distribution of histopathological diagnosis of causes of pleural effusion of the respondents

Diagnosis	Frequency	Percent
Granulomatous pleuritis	50	48.5
Non Granulomatous pleuritis		
• Malignancy	50	48.5
• Rheumatoid arthritis	01	1.0
• Nephrotic syndrome	01	1.0
• Congestive Cardiac Failure	01	1.0
Total	103	100.0

Table V shows the distribution of histopathological diagnosis of causes of pleural effusion of the respondents. Out of 103 cases 50 (48.5%) were caused by Granulomatous pleuritis, 53 (51.5%) were caused by non- Granulomatous pleuritis. Of non- Granulomatous cases malignancy were 50(48.5%) and rheumatoid arthritis, nephrotic syndrome and congestive cardiac failure were found the causes of pleural effusion one in each (1.0%). Commonest causes of malignancies were adenocarcinoma 36, squamous cell carcinoma 4, small cell carcinoma 6, lymphoma 4.

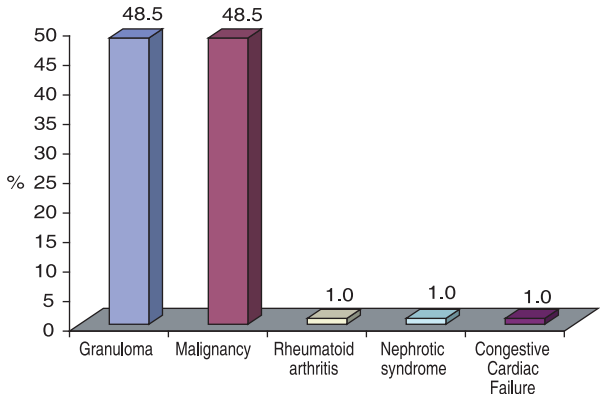

Fig.-1: Bar diagram of diagnosis of causes of pleural effusion of the respondents

Table-VI

Mean ± SD of C - reactive protein value among the respondents

Diagnosis	Mean ± SD	Range
Granulomatous Pleuritis	45.84 ± 11.48	6-96
Malignancy	16.44 ± 12.82	6-48
Rheumatoid arthritis	12.00	
Nephrotic syndrome	12.00	
Congestive Cardiac Failure	6.00	
Total	30.52 ± 19.16	6-96

Table VI shows the mean ± SD of C - reactive protein value among the respondents. Mean ± SD of C - reactive protein value was 30.52 ± 19.16 with a range of 6 to 96. Mean ± SD of C - reactive protein value among the cases of granulomatous pleuritis were 45.84 ± 11.48 and among the cases of pleural effusion due to malignancy were 16.44 ± 12.82.

The horizontal line shows the cut-off level of 48mg/L. Mean ± SD of C - reactive protein value among the cases of pleural effusion due to granulomatous pleuritis was 45.84 ± 11.48 and among the cases of non granulomatous pleuritis group was 16.44 ± 12.82.

Table-VII

Distribution of C - reactive protein by histopathology findings

C-Reactive protein	Histopathology findings		Total
	Granuloma	Non granuloma	
Positive	46 (TP)	05 (FP)	51
Negative	04 (FN)	48 (TN)	52
Total	50	53	103

Table VII shows the distribution of C - reactive protein by histopathology findings. Among the cases of granulomatous pleuritis 46 were positive for the C - reactive protein and 4 were negative for the C - reactive protein. Among the cases of non granulomatous pleuritis 5 were positive for the C - reactive protein and 48 were negative for the C –reactive protein.

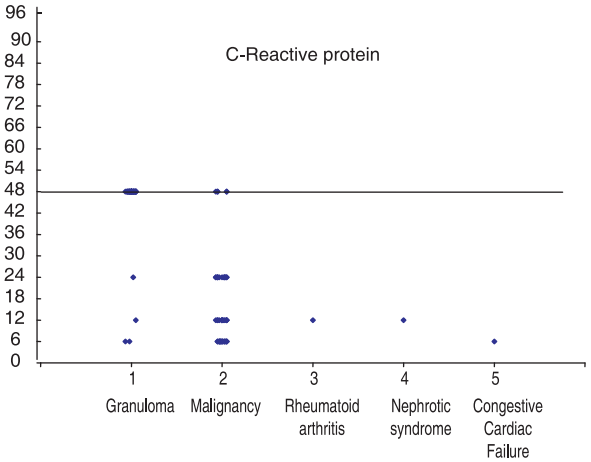

Fig.-2: *C - reactive protein value among the respondents*

Table-VIII
Validity test

Validity test	Value (%)	95% CI
Sensitivity	92.0	84.8-96.1
Specificity	90.6	83.7-94.5
PPV#	90.2	83.1-94.2
NPV*	92.3	85.3-96.3
Accuracy	91.3	84.2-95.3
Positive Likelihood Ratio	9.752	
Negative Likelihood Ratio	0.088	
Pre-test probability	48.5%	
Post test probability (LR +ve)	90.5%	
Post test probability (LR -ve)	7.5%	

#PPV = Positive Predictive Value
*NPV = Negative Predictive Value

Table VIII shows the validity test of C - reactive protein in the diagnosis of granulomatous pleuritis. Sensitivity, specificity, PPV, NPV and accuracy were 92.0%, 90.6%, 90.2%, 92.3% and 91.3% respectively. Positive Likelihood Ratio was 9.752 which is much more away from 1.0 the base line to determine the sensitivity. The likelihood ratio for negative result was 0.088 which indicate that a negative result has less chance to have the disease which intern increases the specificity. At cut off value (48mg/l), the pre-test probability of CRP value for the diagnosis of granulomatous pleuritis is 48.5% but post test probability at the same cut off value (48 mg/l) is 90.5% which indicate that the test is applicable for the diagnosis of granulomatous pleuritis.

Table-IX
Distribution of CRP with ADA results and biopsied pleural tissue (granuloma positive)

CRP	ADA positive	Biopsy for Granuloma positive
CRP Positive n=51	46 (69.7)	46 (92.0)
CRP Negative n=52	20 (30.3)	04 (08.0)
Total n=103	66 (100.0)	50 (100.0)

Number within parenthesis indicate percentage

Table IX shows the distribution of CRP with ADA results and biopsied pleural tissue granuloma positive. Among the 103 patients with pleural effusion 51 were CRP positive and 52 CRP negative. Among the 66 patients with ADA positive 46 were CRP positive and 20 CRP negative. Among the 50 patients with granulomatous pleuritis positive 46 were CRP positive and 4 CRP negative. All the CRP positive cases were positive for both ADA and granuloma.

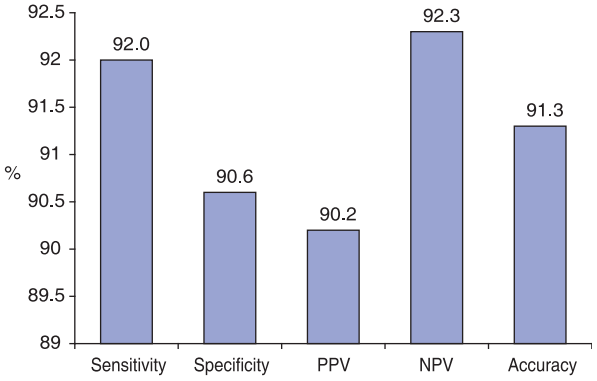

Fig.-3: *Validity test*

Discussion:

C-reactive protein (CRP) in pleural fluid have been found to be higher in tuberculosis and parapneumonic effusions than in other causes of pleural effusion [18]. The present cross sectional study was conducted between the period of January 2010 to December 2010 in the Department of Respiratory Medicine, National Institute of Diseases of the Chest and Hospital, Mohakhali, Dhaka. The present study was conducted with the aim to find out that the C - reactive protein is a sensitive and specific marker for the diagnosis of patients with granulomatous pleuritis. Total 103 patients with clinically and radiologically diagnosed pleural effusion were included in the study.

In the present study out of 103 patients with pleural effusion 74 were male and 29 were female. Male and female ratio was 1.9:1. Mean ± SD of age of the patient was 50.24 ± 18.38 years. Among the male highest number of patients were in the age group of 51 to 60 years (29.1%) followed by 61 to 70 years (18.9%). Other age groups were d"20 years, 20-30 years, 31-40 years, 41-50 years and more than 70 years were 08 (10.8%), 06 (08.1%), 09 (12.2%), 08 (10.8%) and 07 (09.5%) respectively. Mean ± SD of age of male was 50.73 ± 19.27 years. Among the female highest number of patients were in the age group of 51 to 60 years (27.6%) followed by 41 to 50

years (24.1%) and 20 to 30 years (24.1%). Other age groups were 31-40 years, 61-70 years and more than 70 years were 02 (06.9%), 03 (10.3%), 09 (12.2%) and 02 (06.9%) respectively. Mean ± SD of age of female was 49.00 ± 16.12 years (Table I).

Previous history of TB or contact with TB is an important factor for development of TB. In the present study most of the patients had no history of TB or contact with TB (95.2%). Only 5 (4.8%) had history of TB or contact with TB (Table II).

Symptoms and signs of pleurisy often precede the development of an effusion. However, the onset may be insidious. Breathlessness is the only symptom related to the effusion and its severity depends on the size and rate of accumulation [20]. In the present study among the respondents common clinical presentations of patients were fever (96.1%), cough (95.1%), chest pain (45.6%) and weight loss (41.7%). Other clinical presentations were dyspnoea (21.4%), heamoptysis (8.7%), loss of appetite (2.9%) and night sweats (2.9%) (Table III).

The classical appearance of pleural fluid on the erect PA chest film is of a curved shadow at the lung base, blunting the costophrenic angle and ascending towards the axilla. Fluid appears to track up the lateral chest wall. Around 200 ml of fluid is required to be detectable on a PA chest X-ray, but smaller effusions can be identified by ultrasound or CT scanning [20]. Out of 103 cases of pleural effusion 78 (75.7%) had right sided and 25 (24.3%) left sided pleural effusion. Highest number of cases presented with moderate pleural effusion (71.8%) followed by massive pleural effusion (16.5%). Five (4.9%) presented with small pleural effusion and 7 (6.8%) presented with large pleural effusion on X-ray findings (Table IV).

The cause of the majority of pleural effusions can usually be identified through a thorough history, examination and relevant investigations. Particular attention should be paid to a recent history of respiratory infection, the presence of heart, liver or renal disease, occupation (e.g. exposure to asbestos), contact with tuberculosis, and risk factors for thromboembolism [20]. Out of 103 cases of pleural effusion histopathological diagnosis of 50 (48.5%) were granulomatous pleuritis and another 50 (48.5%) cases were caused by malignancy. Rheumatoid arthritis, nephrotic syndrome and congestive cardiac failure were found the causes of pleural effusion one in each (1.0%) (Table V).

In general, a WBC count of >1,000 cells/cumm suggests an exudates, while most transudates have WBC counts of <1,000 cells/cumm. Parapneumonic effusions usually have WBC counts of >10,000 cells/cumm. However, similar WBC counts are also seen in pleural effusions that are related to pancreatitis, collagen vuscular diseases, malignancy and tuberculosis . In this study, WBC count was above 1000/cumm in 44(70%) cases of TB effusion. Berger and Mejia (1973) showed count above 1000/cumm in 56% cases of TB effusion. Total leukocyte count in pleural fluid was valuable only in distinguishing transudative from exudative effusions [21] and comparison of total count of WBC in exudative pleural effusion of different etiologies revealed no significances. Differential leukocyte counts were of no value in indicating the cause of a pleural effusion [22].

The lymphocytosis was found in 59(95%) cases of TB pleural effusion in this study. Similar findings of TB pleural effusion was reported inseveral studies [21,23]. Similar to our findings, others also noted lymphocytosis in pleural fluid in malignancy, rheumatoid arthritis, nephrotic syndrome and congestive cardiac failure [21].The mean percentage of lymphocytes in tuberculous effusion did not differ significantly from that in transudate and in effusion caused by malignant diseases as evidenced by Pettersson and Riska [21].

In the present study most of the patients with granulomatous pleuritis had lymphocytosis in pleural fluid more than 50% and only one had less than 50%. Mean ± SD of lymphocyte count among the cases of granulomatous pleuritis was 78.30±9.77. Among the patients with malignancy 37 had lymphocytosis more than 50% and 13 had less than 50%. Mean ± SD of lymphocyte count among the cases of pleural effusion due to malignancy was 59.08±17.04.

Mean ± SD of C - reactive protein value was 30.52 ± 19.16 with a range of 6 to 96. Mean ± SD of C - reactive protein value among the cases of pleural effusion with granulomatous pleuritis was 45.84 ± 11.48 and among the cases of pleural effusion due to malignancy was 16.44 ± 12.82 (Table VI).

Kapisyzi et al. [24] studied to assess whether CRP in pleural fluid is a sensitive marker for discriminating transudative from exudative pleural effusions. They found that in neoplastic effusion CRP levels (11.7±9 mg/L) were significantly lower than parapneumonic tuberculous and chronic non-specific pleurisy. In six cases, with neoplastic effusions they found lower liquid CPR (6.28 ± 4.4 mg/L). Garcia-Pachon [18] studied to analyze whether CRP (a simple and inexpensive test) may be a diagnostic aid for granulomatous pleuritis in lymphocytic pleural effusions. In their study one hundred and forty-four patients were included and they found that the CRP pleural fluid level was higher in granulomatous pleuritis (54 ± 24 mg/l) than in lymphocytic effusions of other origin (21 ± 16 mg/l; $p < 0.001$).

The combination of adenosine deaminase and C-reactive protein levels might be sufficient for discriminating between the three different groups of exudative pleural effusion: malignant, tuberculous and parapneumonic [12]. CRP levels may have a role in identifying the advanced and extensive disease patients thereby indirectly helping the health workers to pick up delayed convertors/potential defaulters, so as to guide them to put in extra efforts on these groups, in tuberculosis control programs [25]. Porcel[19] in a study concluded that elevated pleural fluid levels of CRP, sTREM and LBP identify patients with infectious effusions, particularly those with CPPE . In the present study among the cases of granulomatous pleuritis 46 were positive for the C - reactive protein and 4 were negative for the C - reactive protein. Among the cases of non granulomatous pleuritis 5 were positive for the C - reactive protein and 48 were negative for the C - reactive protein (Table VII).

Sensitivity, specificity, PPV, NPV and accuracy C - reactive protein in the diagnosis of granulomatous pleuritis were 92.0%, 90.6%, 90.2%, 92.3% and 91.3% respectively. Positive Likelihood Ratio was 9.752 and Negative Likelihood Ratio was 0.088 (Table IX). Kapisyzi et al.[24] studied to assess whether C-reactive protein (CRP) in pleural fluid, is a sensitive marker for discriminating transudative from exudative pleural effusions and to evaluate it can be used to distinguish inflammatory pleural effusions from malignant ones. They showed that when the pleural fluid CRP level was < 15 mg/L, the sensitivity was 94.7%, specificity 60.2%, accuracy 68.9% negative predictive value 97.1%. Pleural fluid CRP levels < 20 mg/L had a sensitivity of 78%, specificity 66.6% a positive predictive value of 60.9% negative predictive value 81.8% and accuracy 69% for malignant effusion. They concluded that pleural CRP-levels useful marker in diagnosis of pleural effusion. Lower level of CRP was obtained in the lymphocyte exudates group [24]. Garcia-Pachon[18] studied to analyze whether CRP (a simple and inexpensive test) may be a diagnostic aid for granulomatous pleuritis in lymphocytic pleural effusions. In their study one hundred and forty-four patients with a lymphocytic pleural effusion (more than 50% lymphocytes in the differential white blood cell count) were included. The CRP pleural fluid level was higher in granulomatous pleuritis (54 ± 24 mg/l) than in lymphocytic effusions of other origin (21 ± 16 mg/l; $p < 0.001$). High CRP levels (>or=50 mg/l) have a high specificity for granulomatous pleuritis (95%), and low levels (<30 mg/l) have a high sensitivity (95%) for excluding disease. They concluded from their study that CRP pleural fluid level determination is useful in the diagnostic workup of lymphocytic pleural effusions and high CRP levels are very suggestive of granulomatous pleuritis, and low CRP levels make this diagnosis unlikely [18].

Conclusion:

The present study was conducted in the department of respiratory medicine of NIDCH to find out the C - reactive protein is a sensitive and specific marker for the diagnosis of clinically suspected patient of pleural effusion with granulomatous pleuritis. The study found that C - reactive protein level in patients with granulomatous pleural effusion are more pronounced than the pleural effusion due to other causes. Sensitivity, specificity, and accuracy of C - reactive protein in the diagnosis of granulomatous pleuritis were 92.0%, 90.6% and 91.3% respectively. The result indicated that the analysis of C - reactive protein levels in pleural effusion constitute a very useful marker for the diagnosis of granulomatous pleuritis which, in addition, can be made quickly and cheaply. So, we may conclude that overall this test is very helpful adjunct test for the diagnosis of granulomatous pleuritis.

References:

1. Light RW. Tuberculous pleural effusion; in Light RW: Pleural Diseases, ed 4 Philadelphia, Lippincott, 2001,182-195.
2. Robert H, Poe MD. Sensitivity, specificity and predictive value of closed pleural biopsy. *Arch Int. Med;* 1984; 144 (2): 325-328.
3. Innes JA, Reid PT. Respiratory diseases. In Davidson's Principles and Practice of Medicine. 20th Ed. Philadelphia, Elsevier Science; 2006, 695.
4. National Tuberculosis control program, 'National Guidelines and Operational Manual for Tuberculosis Control, 2004, 13.
5. Talukder MAS and Zakaria MM. Prevalence of Extra-pulmonary tuberculosis in hospital patietns. ASCON; 2002; 10: 117.
6. WHO report Global tuberculosis control, country profile, Bangladesh. Geneva: WHO 2008. Available from www.who.int/ entity/tb/ publications/ global_report/2008/pdf/bgd.pdf.
7. Sharma SK and Mohan A. Extrapulmonary tuberculosis. India J Med Res; 2004; 120: 316-353.
8. Gopi A, Madhavan SM, Sharma SK and Sahn SA. Diagnosis and treatment of tuberculous pleural effusion. Chest; 2007; 131: 880-889.
9. A.Gopi, SM Madhavan, SK Sharma. Diagnosis and treatment of tuberculous pleural effusion in 2006. *Chest;* 2007; 97: 605-610.
10. L Valdes, D Alvarez, E San Jose. Tuberculous pleuritis: a study of 254 patients. *Arch. Int. Med*; 1998; 15: 2017-2021.
11. Froudarakis ME. Diagnostic Work-Up of Pleural Effusions. Respiration; 2008; 75: 4–13.
12. Daniil ZD, Zintzaras E, Kiropoulos T, et al. Discrimination of exudative pleural effusions based on multiple biological parameters. Eur Respir J; 2007; 30: 957–964.
13. Akarsu S, Kurt ANC, Dogan Y, Yilmaz E, Godekmerdan A, Aygun AD. The Differential Diagnostic Values of Cytokine Levels in Pleural Effusions. Mediators of Inflamm; 2005; 1:2–8.
14. Mane P, et al. Role of Pleural fluid ADA estimation in diagnosis of Tuberculosis in Pediatric Surgical Patients with suppurative thorasic lesion. *Journal of Indian Association of Pediatric Surgeons*; 2005; 6(4):125-129.
15. Krenke R, Safianowska A., Paplinska M, et al. Pleural fluid adenosine deaminase and interferongammaas diagnostic tools in tuberculous pleurisy. Journal Of Physiology And Pharmacology; 2008; Vol 59: Suppl 6, 349–360.
16. Ocana I, Martinez- Vazquez JM, Segura RM, Fernandez de Servilla T,Capdevila JA. ADA in pleural fluids: Test for diagnosis of tuberculous pleural effusion. Chest; 1983; 103: 458-465.
17. Valdes L, Alvarez D, San Jose E, et al. Value of ADA in the diagnosis of tuberculous pleural effusion in young patients in a region of high prevalence of tuberculosis. Thorax; 1995; 50: 600-603.
18. Garcia-Pachon E, Soler MJ, Padilla-Navas I, Romero V, Shum C. C-reactive protein in lymphocytic pleural effusions: a diagnostic aid in tuberculous pleuritis. Respiration; 2005; 72(5): 486489.
19. Porcel JM, Vives M Esquerda A. Tumor necrosis factor-a in pleural fluid. A marker of complicated parapneumonic effusions. Chest; 2004; 125: 160–164.
20. Reid PT and Innes JA. In: Respiratory disease. Nicki R. Colledge, Brain R. Walker, Stuart H. Ralston. editors. Davidson's Principles and Practice of Medicine. 21st ed. Edinburgh, Churchill Livingstone, 2010, 641-730.
21. Pettersson T and Riska H. Diagnostic value of total and differential leukocyte counts in pleural effusions. *Acta Med Scand*; 1981; Vol 210: 129-135.
22. Luellen EC, Carr DT. Pleural effusion: a statistical study of 436 patients. *N Engl J Med*; 1955; 252: 79-83.
23. Epstein DM, Kline LR, Albelda SM and Miller WT. Tuberculous pleural effusions. *Chest;* 1987; 91 : 106-109.
24. Kapisyzi P, Argjiri D, Byrazeri G, Mitre A, Beli J, Vakeflliu Y, et al. Use of pleural fluid c-reactive protein level as a diagnostic marker for pleural effusions. Chest; 2009; 136: 30-36.
25. Rao S and Bernhardt V. Serum C Reactive Protein in Pulmonary Tuberculosis: Correlation with Bacteriological Load and Extent of Disease. Infectious Diseases in Clinical Practice; 2009; 17(5): 314-316.

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ORIGINAL ARTICLE

Study of Direct Antiglobulin Test among the Patients Receiving Antitubercular Chemotheraphy

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Abstract:

This cross-sectional study was carried out in the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, with collaboration 0f National Institute of Diseases of the Chest and Hospital, Mohakhali, Dhaka. Bangladesh from July 2009 to June 2010 for a period of one year. The aim of the present study is to identify drug induced antibody by direct antigobulin test among tubercular patients taking antitubercular drugs. These drug induced antibodies are diagnosed by positive direct antiglobulin test.

A total number of 100 patients who were diagnosed as cases of tuberculosis between age of 5 years to 70 years of both sexes and who were admitted in NIDCH under antitubercular chemotherapy were included in this study. Maximum age group was 21-30 years 35(35.0%) followed by 10-20 years of age group 20(20.0%) and 31-40 years group 16(16.0%). In this study male is predominant than female which is 62(62.0%) cases and 38(38.0%) cases respectively with a ratio of 1.63: 1. Pulmonary tuberculosis is found in 96(96.0%) cases and extrapulmonary tuberculosis in 04% cases. Smear positive and negative tuberculosis are found in 78.0% cases and 22.0% cases respectively. According to degree of anaemia maximum cases are moderately anaemic 76(76%) followed by severely anaemic 18(18.0%) and mildly anaemic 6(6%). Fever was found in 88(88.0%) followed by cough 89(89.0%), haemoptysis 33(33.0%), chest pain 60(60.0%) cases respectively.

Rifampicin is used in all 100(100.0%) cases with Streptomycin 35(35.0%), Isoniazide (INH) 98(98.0%), Pyrazinamide 92(92.0%) and Ethambutal 99(99.0%). Nausea, vomiting and high coloured urine are complained by 94(94.0%) cases, 71(71.0%) cases and 22(22.0%) cases respectively. The most common blood group is O group which is 37(37.0%) cases followed by B group and A group which are 28(28.0%) cases and 26(26.0%) cases respectively. The AB group is found in only 9(9.0%) cases. Mostly are Rh positive blood group 97(97.0%) & 03(3%) are Rh negative. Chest X-ray finding like cavity, patchy opacities are found in 85(85%) and rest of 15(15%) cases show normal finding.

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Introduction:

Drug induced antibody may coat red cell surface which may cause premature destruction of red blood cells, resulting anaemia. Drug induced immune haemolytic anaemia is an acquired form of immune haemolytic anaemia caused by the interaction of certain medications with patients immune system[1]. This interaction induced the production of antibodies that damage red cells and results in premature red cell destruction. Drug induced immune hemolytic anaemia is apart of a larger group of disorders characterized by antibody production against red cells[2]. The red cells may be coated with antibody, antibody and complement, or complement alone. The mechanism of red cell damage associated with drug induced immune hemolytic anaemia depends largely on whether or not activate complement typically result in extravascular hemolysis. Those processes that activate complement result in red cell lysis and intravascular hemolysis[3].

There are four mechanisms by which drugs are believed to induce a positive DAT[1]. Three are immune mediated. The fourth is a non-immune mechanism that results in the development of a positive DAT, but not associated with hemolysis[1]. The presence of antibody on the red cells does not necessarily indicate red cell destruction[4]. Many drugs can cause a positive DAT however, only some of these will cause hemolytic anaemia[1]. As such the serologic data must be correlated with the patient's clinical history and drug history. The clinical evidence like fever, anaemia, jaundice that would support the diagnosis of drug induced immune hemolytic anaemia includes the association of hemolysis with the initiation of drug therapy and resolution with discontinuation of the drug[3]. The three immune mechanisms are drug adsorption, drug dependents antibody and autoimmune haemolysis[5].

Antibodies that are directed only against the drug bound to the surface of the red cell in characteristic of a drug adsorption reaction. Antibodies directed against a combination of the drug and red cell membrane components are characteristic of a drug dependent or immune complex mechanism. Autoantibody production occurs when the drug stimulates production of antibodies that are primarily directed against intrinsic red cell membrane component[6].

Tuberculosis is a disease which needs long term chemotheraphy with multiple drugs having more or less side effects including hemolysis. Bangladesh is a high burden Tubercular country with a ranking of 6th among 22 high burden countries of the world[7]. DOTS is the most effective strategy available for controlling tuberculosis epidemic. The overall goal of tuberculosis control is to reduce morbidity, mortality and transmission of tuberculosis until it is no longer a public health problem[6]. For medical treatment of tuberculosis, problem of drug induced hemolysis may cause discontinuation of antitubercular drugs. Rifampicm, isoniazid, streptomycin may cause immune hemolytic anaemia during chemotheraphy of tuberculosis[7]. Rifampicm has been implicated as the causative drug for immune hemolysis. The onset of hemolysis was not abrupt and the DAT was strongly positive not only or predominantly for Cad, but also for IgG[6,7]. Antitubercular drugs causing immune hemolytic anaemia and positive DATs include Rifampicin, Isoniazid and Streptomycin[6]. DAT positive antitubercular drugs with immune complex mechanism include Rifampicin, Isoniazid and Streptomycin which cause intravascular hemolysis. The direct antiglobulin test (DAT) also known as the direct coomb's test demonstrate the presence of antibodies or compliment on the surface of red cells and the hallmark of autoimmune hemolysis[9]. The evidence of hemolysis due to drugs depends on detection of antibody by direct antiglobulin test (DAT). Other subsidiary evidence include anaemia, hyperbilirubinaemia, hemoglobinuria and renal failure[6]. Rifampicin dependent antibodies should be suspected in a patient with haemolycis and or renal failure taking rifampicin. This reaction has been observed in patients taking drug intermittently or irregularly[8].

In this study patients taking antitubercular drugs are included to find the evidence of hemolysis which may be due to drug induced antibody by brief clinical history, drug history and by direct antiglobulin test.

In this study patients taking antitubercular drugs are included to find the presense of autoantibody by DAT. A brief clinical history and drug history with positive DAT is used to diagnose cases of drug induced antibody production.

Rationale of the study:

Antibodies develop in patients receiving antitubercular drugs rarely.But these antibodies may cause immune haemolysis, which cause positive DAT. To prevent interruption of treatment of tuberculosis due to anaemia or high coloured urine, it is essential to find out the presence of antibody by DAT. In this study DAT is performed to observe wheather the drug is responsible for anaemia which cause discontinuation of therapy. In the study ABO & Rh grouping is also done to observe the association between ABO & Rh group with positive DAT. Patients of tuberculosis often take antitubercular drug irregularly due to either high cloured urine or different degree of anaemia or weakness. To continue further treatment it is essential to find out the cause of high coloured urine or anaemia. In this study DAT is performed to observe weather the drug is responsible for anaemia and discontinuation of therapy.

Aims and Objectives:

General Objective

To observe weather the antitubercular drug is responsible for positive DAT among patients taking antitubercular drugs.

Specific Objectives

- To detect drug dependent antibody by direct antiglobulin test (DAT) among patients receiving antitubercular drugs.
- To determine the degree of anaemia among drug induced haemolytic anaemia in tuberculous patients.

Table

Serologic Characteristics of Drug-Induced Hemolytic Anemias

	AutoAB formation	Drug adsorption	Neoantigen formation
DAT			
Polyspecific	+	+	+
IgG	+	+	Usually –
C3	Usually -	Usually -	+
Serum Ab			
Routine	±	-	-
Soluble drug	±	-	+
Drug-treated RBC's	±	+	+
RBC eluate Ab			
Routine	+	-	-
Soluble drug	+	-	-
Drug-treated RBC's	+	+	-

*Ab, antibody; DAT, direct antiglobulin test; RBC, red blood cell.

Materials and Methods:

It was cross-sectional study. The study was carried out in the Department of Transfusion Medicine, BSMMU, Dhaka, with collaboration with NIDCH, Mohakhali, Dhaka. Bangladesh. This study was conducted from July 2009 to June 2010 for a period of one (1) year. Patients who were diagnosed as cases of tuberculosis of both sexes and who were admitted in NIDCH and under antitubercular chemotherapy were enrolled in this study.

Selection criteria of subjects:

Inclusion criteria:

- Diagnosed tubercular patients who were admitted in NIDCH and taking anti-tubercular chemotherapy.
- History of anaemia and Jaundice.
- Patients between 5-70 years of both sexes.
- Participants, who gave consent and willing to comply with the study procedure

Exclusion criteria:

- Diagnosed tuberculous patient who are not admitted in NIDCH.
- MDR and X-DR patient.
- Patients who had already positive DAT.
- Patients or attendants unwilling to take part in the study

Sample size was calculated by using appropriate formula. Approximate sample size was 100 of both sexes after fulfilling the inclusion and exclusion criteria. Purposive sampling technique was used

Demographic variables:

- Age
- Sex
- Marital status
- Religion
- Family Member
- Monthly income
- Level of occupation
- Level of education

Residential condition:

- Place of residence
- Type of house
- Type of toilet
- Source of water
- Overcrowding

Illness characteristics:

- Duration of tuberculosis

Research Tool:

A questionnaire was filled up among tuberculous patients taking antitubercular chemotherapy. 5ml clotted blood and 3ml of EDTA blood were taken from each patient after fulfilling data sheet. A DAT was performed from clotted blood & other blood parameters were performed from EDTA blood.

Data collections were conducted by researcher herself. All data were compiled and edited meticulously by thorough checking and rechecking. All omissions and inconsistencies were corrected and were removed methodically. All data were recorded systematically in preformed data collection form (questionnaire) and quantitative data was expressed as mean and standard deviation and qualitative data was expressed as frequency distribution and percentage. Statistical analysis was performed by using SPSS for windows version 12.0. 95% confidence limit was taken. Probability value <0.05 was considered as level of significance.

Result and observations:

A total number of 100 patients who were diagnosed as cases of tuberculosis at any age of both sexes and who were admitted in NIDCH under anti-tubercular chemotherapy were enrolled in this study.

Table I

Distribution of study population according to age

Age (in year)	Frequency	Percent
5-10 yrs	4	4.0
11-20 yrs	20	20.0
21-30 yrs	35	35.0
31-40 yrs	16	16.0
41-50 yrs	13	13.0
51-60 yrs	11	11.0
61-70 yrs	1	1.0
Total	100	100.0
Mean ± SD (Range)	31.76 ± 14.39	4.6-70

Table I shows the distribution of study population according to age. Among 100 patients maximum are in the age group of 21-30 years which is 35(35.0%) cases followed by 11-20 years of age group, 31-40 years group, 41-50 years group and 51-60 years which are 20(20.0%) cases, 16(16.0%) years, 13(13.0%) cases and 11(11.0%) cases respectively. in less than or equl to 10 years age group and more than 60 years group are in only 4(4.0%) cases and 1(1.0%) case.

Table-II

Distribution of the study population according to sex

Sex	Frequency	Percent
Male	62	62.0
Female	38	38.0
Total	100	100.0

Male : Female = 1.63 : 1

Table II shows the distribution of the study population according to sex. In this study male is predominant than female which is 62(62.0%) cases and 38(38.0%) cases respectively. the male and female ratio is 1.63: 1.

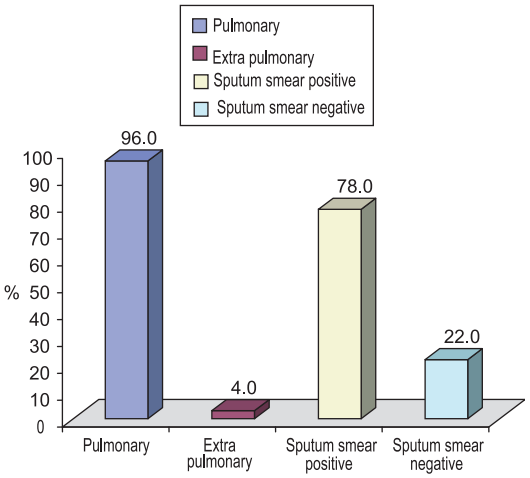

Fig.-1: *Bar diagram of study population according to infection site & sputum examination*

Table-III

Distribution of study population according to the degree of anaemia.

General appearance	Frequency	Hb%	Percent
Mildly anaemic	6	<10gm/dl	6.0
Moderately anaemic	76	6-10gm/dl	76.0
Severely anaemic	18	<6gm/dl	18.0
Total	100		100.0

Table III shows the distribution of study population according to the general appearance. Among the 100 patients the general appearance of maximum cases are moderately anaemic which is 76(76.0%) cases followed by severely anaemic and mildly anaemic which are 18(18.0%) cases and 6(6.0%) cases respectively.

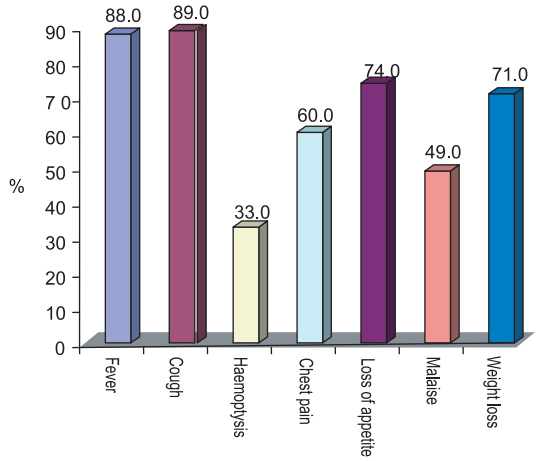

Fig.-2: *Bar diagram of signs & symptoms among the study population*

Table-IV

Distribution of uses of antitubercular drug

Drug	Frequency	Percent
Rifampicin	100	100.0
Streptomycin	35	35.0
INH	98	98.0
Pyrazinamide	92	92.0
Ethambutal	99	99.0

Table IV shows the distribution of uses of antitubercular drug. Rifampicin is used in all 100(100.0%) cases. Streptomycin is used by 35(35.0%) cases. Isoniazide (INH) is taken by 98(98.0%) cases. Pyrazinamide and Ethambutal are taken by 92(92.0%) cases and 99(99.0%) cases respectively.

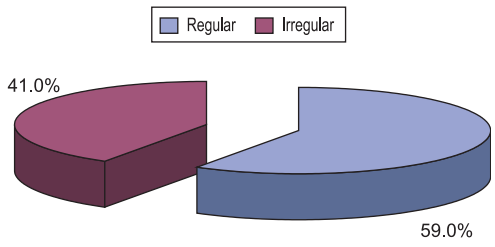

Fig.-3: *Pie chart of regularity of taking antitubercular drugs*

Table-V

Distribution of adverse effects among the study population after taking antitubercular drug.

Adverse effects	Frequency	Percent
Nausea	94	94.0
Vomiting	71	71.0
High colored urine	22	22.0

Table V shows the distribution of adverse effects among the study population. Nausea, vomiting and high colored urine are complained by 94(94.0%) cases, 71(71.0%) cases and 22(22.0%) cases respectively.

Table-VI

Finding of chest X-ray among the study population

Chest X-ray finding	Frequency	Percent
Positive	85	85.0
Negative	15	15.0
Total	100	100.0

Table VI shows the distribution of chest X-ray among the study population. Positive chest X-ray finding like cavity, patchy opacity etc. are found in 85(85.0%) cases and the rest 15(15.0%) cases shows normal findings.

Table VII

Distribution of direct antiglobulin test (DAT)

Direct antiglobulin test	Frequency	Percent
Positive	5	5.0
Negative	95	95.0
Total	100	100.0

Table VII shows the distribution of direct antiglobulin test (DAT). Among 100 cases DAT is positive in only 5(5.0%) cases and the rest 95(95.0%) cases are negative.

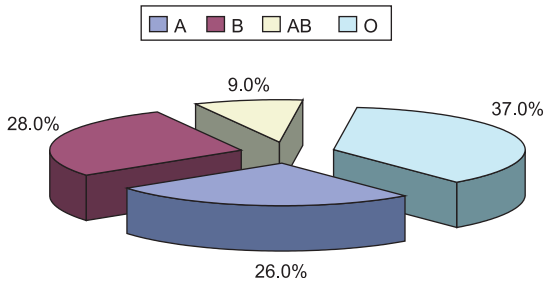

Fig.-4: *Pie chart of study population according to ABO blood grouping*

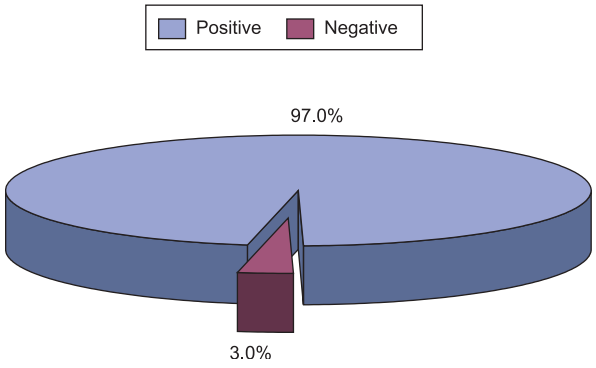

Fig.-5: *Pie chart of Rh typing of blood grouping*

Discussion:

The World Health Organization[9] estimates that 32% of the world population is infected with *Mycobacterium tuberculosis*, the causative agent of Tuberculosis. Approximately one third of the world population is infected and about three millions die each year from this disease[10].

A total number of 100 patients who were diagnosed as cases of tuberculosis at age group between 5-70 years of both sexes and who were admitted in NIDCH under antitubercular chemotherapy were enrolled in this study. In this study among 100 patients maximum are in the age group of 21-30 years which is 35(35.0%) cases followed by 10-20 years of age group, 31-40 years group, 41-50 years group and 51-60 years which are 20(20.0%) cases, 16(16.0%) years, 13(13.0%) cases and 11(11.0%) cases respectively. 5-10 years age group and between 60-70 years group are in only 4(4.0%) cases and 1(1.0%) case. Musellim et al[11] reported a similar result and has shown that patients showed high prevalence of TB in the younger age group like 20-29 years of age. An explanation for this finding remains unclear, but it suggests that endocrine factors might play a role[12].

In this study male is predominant than female which is 62(62.0%) cases and 38(38.0%) cases respectively. The male and female ratio is 1.63: 1. A similar result was reported by Borgdorff et al[13] and mentioned that in most countries, tuberculosis is diagnosed more often in men than in women, in both routine notifications and prevalence surveys. Martinez et al[14] reported similar findings in USA and published that the sex difference was larger in clustered cases than in non-clustered cases. However, little appears known about sex differences in generating infections and secondary cases. In an earlier study in the Netherlands, restricted to clustered cases with epidemiologic confirmation of contact, source cases were significantly more likely than the average tuberculosis patient to be male[15]. Pulmonary tuberculosis is found in 96(96.0%) cases. Extrapulmonary tuberculosis is found in only 04(4.0%) cases. Smear positive and smear negative tuberculosis are found in 78(78.0%) cases and 22(22.0%) cases respectively. Musellim et al[19] reported a similar result and has shown that increased likelihood of tuberculosis presenting with an pulmonary disease manifestation was particularly pronounced. This may be due to decreased local immunity in the lungs in the elderly as a result of associated life-style factors (smoking) or diseases such as emphysema and bronchitis[16].

The distribution of study population according to the general appearance is recorded in this study. Among the 100 patients the general appearance of maximum cases are moderately anaemic which is 76(76.0%) cases followed by severely anaemic and mildly anaemic which are 18(18.0%) cases and 6(6.0%) cases respectively. The distribution of signs & symptoms among the study population recorded[17]. Fever is recorded in 88(88.0%) cases. Cough is present in 89(89.0%) cases. Haemoptysis is found in 33(33.0%) cases. Chest pain is present in 60(60.0%) cases. Loss of appetite is present in 74(74.0%) cases. Malaise is present in 49(49.0%) cases. Weight loss is present in 71(71.0%) cases. Contact with active diseases is observed in 10(10.0%) cases. The distribution of clinical history is recorded. History of taking anti-tubercular drugs is given by 97(97.0%) cases. The history of yellow colored urine is given by 03(03.0%) case. Ismail[18] was done a study among 232 patients and found a reported a similar result and mentioned that patients had typical symptoms of pulmonary tuberculosis like prolonged recurrent fever, cough, anorexia and weight loss.

Rifampicin is used in all 100(100.0%) cases. Streptomycin is used by 35(35.0%) cases. Isoniazide (INH) is taken by 98(98.0%) cases. Pyrazinamide and Ethambutal are taken by 92(92.0%) cases and 99(99.0%) cases respectively. The distribution of regularity of taking anti-tubercular drugs is recorded in this study. Regularly taken anti-

tubercular drugs is in 59(59.0%) cases and the rest 41(41.0%) cases have taken irregular basis. The distribution of adverse effects among the study population is recorded. Nausea, vomiting and high colored urine are complained by 94(94.0%) cases, 71(71.0%) cases and 22(22.0%) cases respectively[19].

The distribution of study population according to blood grouping is recorded in this study. Among the 100 cases the most common blood group is O group which is 37(37.0%) cases followed by B group and A group which are 28(28.0%) cases and 26(26.0%) cases respectively. The AB group is found in only 9(9.0%) cases. Similar result was reported by Dean[24] and mentioned that the blood group O is common and blood group AB is the least common. The distribution of Rh typing of blood grouping is recorded in this study. Among the 100 cases mostly are positive Rh blood group which is 97(97.0%) and the rest 3(3.0%) cases are Rh negative blood group. The finding of chest X-ray among the study population is recorded. Positive chest X-ray finding like cavity, patchy opacity etc are found in 85(85.0%) cases and the rest 15(15.0%) cases shows normal findings. Barnes et al[20] reported a similar result and mentioned that almost 88% patients shows such positivity of chest x-ray. From these findings it may be established that routine CXRs are useful in hospitals serving populations and the probability of detecting AFB on sputum smear is greatly influenced by the roentgen graphic findings[21].

Among 100 cases DAT is positive in only 5(5.0%) cases and the rest 95(95.0%) cases are negative. Lawrence et al[1] reported four mechanisms by which drugs induce a positive DAT. Many drugs can cause a positive DAT however, only some of these will cause hemolytic anaemia[22]. As such the serologic data must be correlated with the patient's clinical history and drug history. The clinical evidence like fever, anaemia, jaundice that would support the diagnosis of drug induced immune hemolytic anaemia includes the association of hemolysis with the initiation of drug therapy and resolution with discontinuation of the drug[25].

Worlledge[24] also reported that Rifampicin dependent antibodies have been found in the Sera of 1 to 50% patients on Rifampicin, depending primarily on the schedule of administration of drug. Also antirifampicin antibodies were reported in 33% of those receiving once weekly dosing compared to 0.8% on daily therapy.

Martinez J. et el[25] also reported Streptomycin induced antibodies which cause positive DAT and immune haemolytic anaemia by immune complex mechanism.

Ahren et el[27] reported that the DAT were strongly positive for IgG and C3d and tests for rifampicin dependent antibodies. This shows that rifampicin may stimulate the production of autoantibodies and/or drug dependent antibodies.

In this study, DAT in positive in 5% cases and 95% cases are DAT negative.

Among DAT positive cases, 03(3%) are blood group O, followed by A & B blood group which are 01(1%) & 01(1%) respectively[2].

Many other toxicities are reported by antitubercular drugs. Isoniazid (INH) may cause neurotoxic & hepatotoxic effects. Haemolysis has occurred in patients with glucose-6-phosphate deficiency. Rifampicin may cause skin rash, thrombocytopania, nephritis & liver dysfunction[28]. If given less often than twice weekly, it may cause a flu-like syndrome & anaemia. Ethambutal may cause dose dependant visual disturbances[29]. Pyrazinamide may cause non-gouty polyarthralgia. Streptomycin may cause ototoxicities, nephrotoxicities, neuromuscular blockade & skin rashes[30].

Conclusion:

In this study drug induced antibody is detected by direct antiglobulin test. The finding of the study permit to identify the patients who developed antibody which may coat on red cell surface.

This study suggests that positive DAT may be helpful in further management of tuberculous patients who may be at risk of developing anaemia which will cause discontinuation of drug.

The overall goal of tuberculosis control to reduce morbidity, mortality and transmission of tuberculosis is no longer a public health problem.

In conclusion, during treatment with anti-tubercular drug prompt identification of anaemia and close monitoring of patient will be done to find out the cause of anaemia by DAT to establish whether the drug is responsible for anaemia or due to any other cause.

It is also important to identify the blood group for transfusion to the patient due to anaemia and for exclusion of cause of anaemia which may be due to drug or other antibodies produced by transfusion.

Recommendations:

Further study is recommendation with following proposals:

- The study will help the physicians & health workers who are working on tuberculous patients to reduce morbidity &use of appropriate dose of drug which will prevent discontinuation of therapy.
- A large scale national epidemiological study should be done.
- DAT should be performed in all patients receiving anti TB drug after 4weeks & subsequently after initiation of treatment.
- Close monitoring and follow up of the patients should be done to avoid drug induced autoimmune haemolytic anaemia.
- S.bilirubin will be done in every patient after 4weeks of initiation of treatment.
- DOTs corner of Primary Health care Centre (Upazilla Health Complex) should be closely monitored for the evidence of drug induced haemolytic anaemia.
- A further study will be done for the detection of specific antitubercular drug which cause haemolytic anaemia frequently.
- Multicentered study will be included.
- Study period will be prolonged.

References:

1. Lawrence LW, Harmening DM, Green R: Haemdytic Anaemias: Extra corpuscular Defects and Acquired Intra corpuscular Defects. In Clinical Hematology and Fundamentals of Hemostasis, 4th edition. Philadelphia, FA Davis Company, 2002, 212
2. Jefferies LC, Elder AF: Transfusion Therapy in Autoimmune Hemolytic Anaemia. In Mintz PD(ed): Transfusion Therapy: Clinical Principles & Practice,Bethesda, American Association of Blood Banks Press,1999, 43
3. Harmening DM, Prihoda LA, Green R: Autoimmune Hemolytic Anaemia. In Harmening DM,(ed): Modern Blood Banking and Transfusion Practices, 4th edition. Philadelphia, FA Davis Company, 1999, 455-456.
4. Arndt PA, Garratty G. The changing spectrum of drug induced immune hemolytic anemia. Semin Hematol. 2005;42:137-144.
5. World Health Organization. Tuberculosis fact sheet. Available from URL: http:// www.who.int/gtb/ publications/factsheet/ index.htm. Accessed on 1 July 2003
6. National Tuberculosis Control Programme (NTCP). National Guidelines and Operational Manual for Tuberculosis Control. Fourth Edition. Directorate General of Health Services. Ministry of Health and Family Welfare. Dhaka, Bangladesh 2004, 17.
7. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of Serious Side Effects from First-Line Antituberculosis Drugs among Patients Treated for Active Tuberculosis. *American Journal of Respiratory and Critical Care Medicine* 2003; Vol 167: 1472-1477
8. Gehrs BC, Friedberg RC. Autoimmune Hemolytic Anemia American Journal of Hematology 2002; 69: 258-271
9. Ahrens N, Genth R, Salama A.. Belated diagnosis in three patients with rifampicin-induced immune haemolytic anaemia. British Journal of Haematology. 2002;117(2): 441–443
10. Krönig B., Fiegel P., Weihrauch Th., Höffler D., Jahnecke J., Arndt-Hanser A. A case of severe repeated immunological reactions to intermittent rifampicin treatment. European J Clin Pharma. 1972; 5(1): 53-57, DOI: 10.1007/ BF00560896
11. Guzzini F, Angiolini F, Cazzaniga L, Gasparini P, Milvio E, Mosconi L. Immune hemolytic anemia and acute kidney failure due to rifampicin. Recenti Prog Med. 1994;85(3):182-5
12. Garratty G. Drug-ind, Fiegel P., Weihrauchuced immune hemolytic anemia. American Society of Hematology, Hematology 2009:73-79

13. Andrés E, Maloisel F. Idiosyncratic drug-induced agranulocytosis or acute neutropenia. Curr Opin Hematol. 2008;15:15-21.

14. Arndt P, Garratty G, Isaak E. Bolger M, Lu Q. Positive direct and indirect antiglobulin tests associated with oxaliplatin can be due to drug antibody and/or drug induced nonimmunologic protein adsorption. Transfusion. 2009;49:711-718

15. Garratty G, Arndt P, Prince HE, Shulman IA. The effect of methyldopa and procainamide on suppressor cell activity in relation to red cell autoantibody production. Br J Haematol. 1993;84:310-315.

16. Broadberry RE, Farren TW, Bevin SV, et al. Tazobactam-induced haemolytic anaemia, possibly caused by non-immunological adsorption of IgG onto patient's red cells. Transfus Med. 2004;14:53-57

17. Sharma SK, Mohan A. Multidrug-resistant tuberculosis. Indian J Med Res 120, 2004, 354-376

18. World Health Organization. WHO report, global tuberculosis control, surveillance, planning, fi nancing. Geneva: The Organization;2007

19. Musellim B., Erturan S., Duman E., Ongen G. Comparison of extra-pulmonary and pulmonary tuberculosis cases: factors influencing the site of reactivation Int J Tuberc Lung Dis 2005;9(11):1220–1223

20. Borgdorff MW, Nagelkerke NJD, Dye C, et al. Gender and tuberculosis: comparison of prevalence surveys with notification data to explore sex differences in case detection. Int J Tuberc Lung Dis 2000;4:123–32.

21. Martinez AN, Rhee JT, Small PM, et al. Sex differences in the epidemiology of tuberculosis in San Francisco. Int J Tuberc Lung Dis 2000; 4:26–31.

22. Asbroek TAHA, Borgdorff MW, Nagelkerke NJD, et al. Estimation of serial interval and incubation period of tubercu- losis using DNA fingerprinting. Int J Tuberc Lung Dis 1999;3: 414–20

23. Ismail Y. *Pulmonary Tuberculosis- A Review of Clinical Features and Diagnosis in 232 Cases*. Medical J Malaysia. 2004;59(1):56-64

24. Dean L. 2005. The ABO blood group. [Online] View at: 15 September; Available at: http:// www.ncbi.nlm.nih.gov/bookshelf/ br.fcgi? book=rbcantigen&part=ch05ABO

25. Barnes P F, Verdegem T D, Vachon L A, Leedom J M, Overturf G D. Chest Roentgenogram in Pulmonary Tuberculosis: New Data on an Old Test. *Chest* 1988;94;316-320

26. Worlledge S. The detection of rifampicin dependant antibodies. Scand J Respir Dis 1973: 84[Suppl] 60-3

27. Martin J, Barbolla L,Frieyro E, et el immune haemolytic anaemia and renal failure induced by Streptommycin.Br J Haematol 1977; 35: 561-571

28. A. Pereira,[1] C.Sanz,[1] F.Cervantes[2] and R.Castillo, Ann Hematol Immune haemalytic anaemia and renal failure associated with rifampicin dependent antibodies with I specificity. 1991; 63: 56-58

29. Aquinas M.Allan WGL,Horsfall PAL,et al. adverse reactions to daily and intermittent rifampicin regimens for pulmonary tuberculosis in Hong Kong. Br Med J 1972;1:765-71

30. Anthony J. Trevor, Bertram G. Katzung : Antimicrobial drugs: Katzung & Trevor's Pharmacology,6th edition,Sunfrancisco 2002, 411-413.

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ORIGINAL ARTICLE

Adenosine Deaminase Activity in Bronchoalveolar Lavage in Patients with Smear Negative Pulmonary Tuberculosis

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Abstract:

Background: *Tuberculosis (TB) is still one of the major health problems across the world and a leading cause of preventable morbidity and mortality from an infectious agent. TB most commonly involves lungs and its diagnosis is mostly based on acid-fast bacilli (AFB) in sputum smear or a positive sputum culture for AFB. The sputum smear negative patients have been a diagnostic challenge world wide. Adenosine deamianase (ADA) activity has been shown to rise in various body fluids of patients with TB. This study was carried out to determine the diagnostic value of ADA activity in bronchoalveolar lavage (BAL) in patients highly suggestive of pulmonary TB but with negative sputum smear for AFB. We decided to measure the ADA activity in BAL fluid and compare it with Sputum and BAL fluid cultures for AFB.*

Materials and methods: *A cross-sectional study was performed at the National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka from January 2010 to December 2010. Total 100 (one hundred) patients with smear negative suspected pulmonary TB & non-tuberculous pulmonary diseases were included in the study. By Fiber Optic Bronchoscopy (FOB), BAL fluid was obtained from all patients. As per final diagnosis the patients were categorized in two groups, pulmonary TB group and non-tuberculous pulmonary diseases group. Patients with positive sputum culture and /or BAL fluid culture for AFB were considered as pulmonary TB group and the patients with negative results for TB, having lung diseases other than TB were considered as non-tuberculous pulmonary diseases group. ADA levels in BAL fluids were measured in both groups and then compared with each other.*

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Results: *A total number of 100 (M/F: 82/18, mean age 49.51±16.44 years) sputum smear-negative patients were enrolled in the study, of which 43 cases (M/F: 29/14, mean age 38.81±15.66 years) were finally diagnosed as pulmonary TB and 57 cases (M/F: 53/4, mean age 57.58±11.84years) were with non-tuberculous pulmonary diseases. The mean ADA level in BAL fluid was 6.58 ± 1.31 U/L in pulmonary tuberculosis and 4.00 ± 0.93 U/L in non-tuberculous pulmonary diseases. The ADA level was significantly higher in the pulmonary TB than non-Tuberculous pulmonary diseases which is statistically significant (p=0.001). Using ROC curve, the cut off value of ADA in BAL fluid was obtained 5.00 U/L for diagnosis of pulmonary TB. The sensitivity, Specificity, PPV, NPV and Accuracy of ADA level in BAL fluid at Cut off value >5.00 U/L were 90.7%, 87.7%, 84.8%, 92.6% and 89.0% respectively.*

Conclusion: *In conclusion, the results of this study showed that, ADA level in BAL fluid of patients with pulmonary TB is significantly higher than that of other pulmonary diseases though a negative test does not rule out pulmonary TB. It may be useful, faster and cost effective diagnostic test in sputum smear-negative patients highly suggestive for pulmonary TB.*

Key words: *Tuberculosis, Sputum smear, Bronchoalveolar lavage, Adenosine deamianase.*

[Chest & Heart Journal 2011; 35(2) : 95-102]

Introduction:

Mainstay of successful treatment of Tuberculosis largely depends on early diagnosis. The most important diagnostic method is based on finding acid fast bacilli (AFB) in sputum smear or a positive sputum culture for AFB[3]. Sputum microscopic examination can diagnose 50-60% of pulmonary tuberculosis in well equipped laboratory. In developing countries, poor access to microscopic service contributes to even lower rate of AFB detection[4]. A definitive diagnosis is based on the isolation and culture of *Mycobacterium tuberculosis* in sputum samples. A 4-8 weeks period is required to prepare sputum culture results; delay is cumbersome for some patients, it is necessary to look for a simple, rapid and reliable test for diagnosis of pulmonary tuberculosis. This is more important in patients who do not produce sputum. In smear negative patients antituberculous treatment is delayed until culture results are available. This costs the patient a very valuable time in which he or she could have received the initial antituberculous treatment. Sometimes delay is unacceptable in emergency situations. Thus it is necessary to find faster methods with higher sensitivity[5].Fiber optic bronchoscopy (FOB) with endobronchial biopsy and bronchoalveolar lavage (BAL) has proved valuable tools for diagnosis of pulmonary TB[6]. Polymerase chain reaction (PCR) in sputum and bronchoalveolar washings is a rapid and established diagnostic method for pulmonary TB but it is not cost effective and available in few centers[7]. An alternative rapid test for these specimens was the assay of adenosine deaminase (ADA) activity[8].Adenosine deaminase (ADA) is an enzyme catalyzing the hydrolytic & irreversible demamination of adenosine to ionosine & deoxy adenosine to deoxy ionosine[9]. It is known as a cellular immunity marker and its physiologic activity is due to its release from differentiating lymphocytes[10]. ADA is used for the diagnosis of pleural TB and there are studies showing high levels of ADA in sera & bronchoalveolar lavage fluids of TB patients[6]. Very few studies have been employed to assess the ADA activity in BAL and its diagnostic value is still uncertain. So, the aim of the present study is to evaluate ADA activities in BAL fluid among pulmonary tuberculosis patients compared with patients having non tubercular pulmonary disease in order to assess

the diagnostic value of this tests which is elucidated the possible applicability of the ADA activity in BAL fluid in the early detection of patients with AFB smear negative, who are strongly suspected to have pulmonary tuberculosis and thereby initiating treatment without undue delay.

Materials and Methods:

An observational cross-sectional study was carried out in National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh from January 2010 to December 2010 for a period of one (1) year. Samples were collected from both outpatient department (OPD) & inpatient department of NIDCH. Total 100 (one hundred) patients of 14-80 years of age, both males and females of suspected pulmonary tuberculosis with negative sputum smear for AFB and non-tuberculous pulmonary diseases were enrolled in the study. Prior to the commencement of this study, the research protocol were approved by the ethical committee of the NIDCH, Dhaka; informed consents were taken from the respondents prior to interview.

A pretested semi-structured questionnaire and a checklist developed by using selected variables according to the objectives. Laboratory findings were collected from the record. The questionnaire was prepared in English and expressed to the patients in Bengali. Conventional investigations like complete blood counts (CBC), Chest X-ray P/A view, mantoux test (MT), sputum smear for AFB were done for the patients attended in NIDCH. After excluding the positive cases of sputum smear for AFB. 100 patients with negative sputum smear for AFB suspected of having pulmonary TB and other pulmonary diseases were included in the study.

The sputum samples of all patients included in the study were sent to the laboratory for culture for AFB. According to the standard approach FOB was carried out of all (100) patients. Bronchoalveolar lavage (BAL) fluid was obtained and sent to laboratories for smear for AFB, Culture for AFB, ADA level, cytology and malignant cell. Other related investigations were performed to reach the diagnosis of the patients. Those Patients who had positive sputum cultures or BAL fluid cultures for AFB, considered as pulmonary TB group. Those who had other forms of pulmonary diseases and negative for TB, were considered as non-tuberculous pulmonary diseases group. ADA levels in BAL fluids were measured and compared among the groups. The laboratory technicians were not apprised of the tentative diagnosis of each patient; furthermore, the clinicians were unaware of the ADA level when the diagnosis were decided.

All data were compiled and statistical analysis was performed by using SPSS for windows version 15.0. 95% confidence limit was taken. Probability value <0.05 was considered as level of significance. Statistical analysis was made by two-tailed paired t test for differences between ADA levels in BAL fluid of Pulmonary TB and non-TB pulmonary diseases groups. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratios, pre-test probability and post-test probabilities were evaluated in the measurement of ADA level in BAL fluid.

Result:

A total number of 100 patients (82 males, 18 females) in an age group of 14-80 years (mean age 49.51±16.44 years) were enrolled in this study. Out of those, 43 patients (M/F: 29/14; mean age: 38.81±15.66) had pulmonary tuberculosis and 57 (M/F: 53/4; mean age: 57.58±11.84) had non-TB pulmonary diseases. Among the non-TB pulmonary disease cases, 34 patients had bronchial carcinoma, 03 had pneumonia, 07 had ILD, 05 had chronic obstructive pulmonary disease (COPD), 06 had Fibrosis, 03 had Bronchiectasis and 01 had lung Abscess.

Sputum culture for AFB was positive in 16 (37.2%) cases of pulmonary tuberculosis. Mantoux test (MT) was positive in 25 (58.1%) cases of pulmonary tuberculosis and 19 (33.3%) cases of non-tuberculous pulmonary diseases. MT was negative in 18 (41.9%) cases of pulmonary tuberculosis and 38 (66.7%) cases of non-tuberculous pulmonary diseases. Smear for AFB in BAL examination was positive in 21 (48.8%) cases and BAL culture for AFB was positive in 30 (69.8%) cases of pulmonary tuberculosis (Table 1,2)

Table-I

Distribution of different investigations

Investigations	Pulmonary Tuberculosis	Non TB Pulmonary diseases	Total
Sputum culture for AFB			
• Positive	16 (37.2)	0 (.0)	16 (16.0)
• Negative	27 (62.8)	57 (100.0)	84 (84.0)
Mantoux test (MT)			
• Positive	25 (58.1)	19 (33.3)	44 (44.0)
• Negative	18 (41.9)	38 (66.7)	56 (56.0)
FOB impression			
• Normal	3 (7.0)	32 (56.1)	35 (35.0)
• Inflammatory lesion	40 (93.0)	13 (22.8)	53 (53.0)
• Mitotic lesion	0 (.0)	12 (21.1)	12 (12.0)

Figure within parentheses indicates in percentage.

Table-II

Distribution of BAL fluid examination

BAL fluid examination Smear for AFB	Pulmonary Tuberculosis	Non TB Pulmonary diseases	Total
• Positive	21 (48.8)	0 (.0)	21 (21.0)
• Negative	22 (51.2)	57 (100.0)	79 (79.0)
Culture for AFB			
• Positive	30 (69.8)	0 (.0)	30 (30.0)
• Negative	13 (30.2)	57 (100.0)	70 (70.0)

Figure within parentheses indicates in percentage.

The mean ADA level in BAL fluid was 6.58 ± 1.31 U/L in pulmonary tuberculosis and 4.00 ± 0.93 U/L in non-tuberculous pulmonary diseases. The level was significantly higher in the pulmonary tuberculosis than non-tuberculous pulmonary diseases, the difference was statistically significant (p=0.001). Among the non-tuberculous pulmonary diseases, The ADA level was - in bronchial carcinoma 3.89 ± 0.95 U/L, in ILD 4.17 ± 0.64 U/L, in Fibrosis 3.90 ± 0.34 U/L, in COPD 3.52 ± 0.38 U/L, in Pneumonia 5.00 ± 1.11 U/L, in Bronchiectasis 4.50 ± 1.82 U/L, in Lung abscess 4.50 ± 1.82 U/L (Table 3).

In this study, the cut-off value of ADA in BAL fluid for diagnosis pulmonary TB was determined to be 5.0 U/L by using ROC curve analysis. Pulmonary TB was diagnosed when BAL fluid ADA level was >5.0 U/L in a case and when ADA level was <5.0 U/L it was considered to be negative for pulmonary TB, the diagnosis was non-tuberculous pulmonary disease. ADA level in U/L

Table-III

Distribution of ADA level (U/L) in BAL fluid by final diagnosis

Group	Final diagnosis	Frequency (%)	Mean ± SD	Range
Pulmonary TB	Pulmonary tuberculosis	43 (43.0)	6.58 ± 1.31	3.90-9.20
Non-TB Pulmonary diseases	• Bronchial carcinoma	34 (34.0)	3.89 ± 0.95	2.20-5.70
	• ILD	7 (7.0)	4.17 ± 0.64	3.20-5.30
	• Fibrosis	4 (4.0)	3.90 ± 0.34	3.70-4.40
	• COPD	5 (5.0)	3.52 ± 0.38	3.10-4.10
	• Pneumonia	3 (3.0)	5.00 ± 1.11	4.00-6.20
	• Bronchiectasis	3 (3.0)	4.50 ± 1.82	3.40-6.60
	• Lung abscess	1 (1.0)	4.80	4.80
	Total	57 (100.0)	4.00 ± 0.93	2.20-6.60
Total		100(100.0)	5.11±1.69	2.20-9.20

Figure within parentheses indicates in percentage.

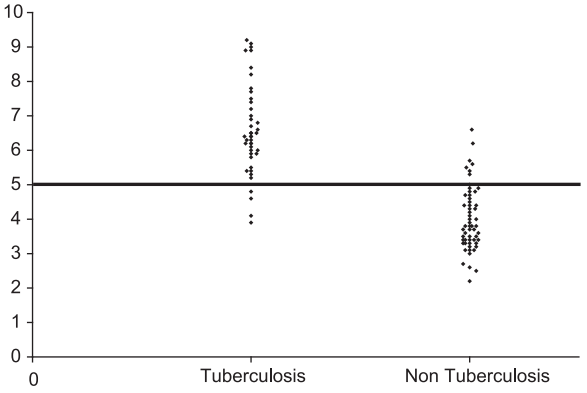

Fig.-1 : *ADA levels in BAL fluid of pulmonary tuberculosis and Non-TB pulmonary diseases patients.*

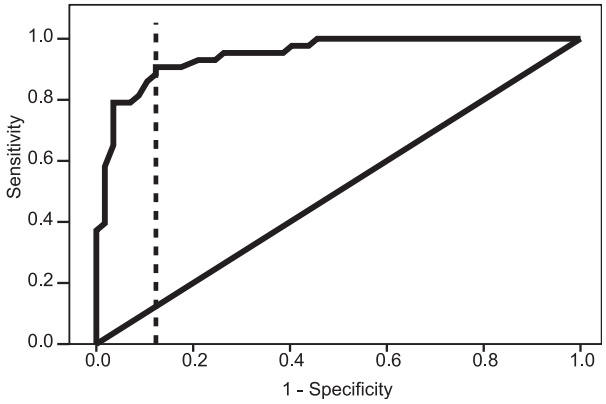

Fig.-2: *ROC curve of ADA level by tuberculosis positive (Area under the ROC curve = 0.948)*

At cut-off value of ADA level in BAL fluid >5.0 U/L for the diagnosis of pulmonary TB, the sensitivity was 90.7%, specificity was 87.7%, positive predictive value (PPV) was 84.8%, negative predictive value (NPV) was 92.6% and accuracy was 89.0%. Moreover the likelihood ratio for positive results (LR+ve) was 7.39% and likelihood ratio for negative results (LR-ve) was 0.11. Both the ratios were in 'fair' level statistically. The pre-test probability was 43%. Using the nomogram the post-test probability for LR+ve was 82% which was quite higher than the pre-test probability, and the post-test probability for LR-ve was 8% which was quite lower than pretest probability. These statistical results indicate that, the investigation ADA level assay in BAL fluid was applicable for the diagnosis of pulmonary TB.

At BAL fluid ADA level >5.00 U/L, other investigations like sputum culture for AFB, BAL fluid culture for AFB, BAL fluid smear for AFB and MT results were compared statistically. The sensitivity, Specificity, PPV, NPV and Accuracy of Sputum culture for AFB were 37.2%, 100.0%, 100.0%, 67.9% and 73.0% respectively; of BAL fluid Culture for AFB were 69.8%, 100.0%, 100.0%, 81.4% and 87.0% respectively; of BAL fluid Smear for AFB were 48.8%, 100.0%, 100.0%, 72.2% and 78.0% respectively; of Mantoux test (MT) were 58.1%, 66.7%, 56.8%, 67.9% and 63.0% respectively. Among ADA positive cases (ADA level in BAL fluid >5.0 U/L), sputum culture for AFB was positive in 33% cases. BAL fluid culture for AFB was positive in 74.4% cases, BAL fluid smear

Table-IV

Validity tests of different investigations

	BAL fluid ADA level >5.00 U/L	Sputum culture for AFB	BAL fluid Culture for AFB	BAL fluid Smear for AFB	Mantoux test (MT)
True positive	39	16	30	21	25
False positive	7	0	0	0	19
False negative	4	27	13	22	18
True negative	50	57	57	57	38
Sensitivity	90.7%	37.2%	69.8%	48.8%	58.1%
Specificity	87.7%	100.0%	100.0%	100.0%	66.7%
PPV	84.8%	100.0%	100.0%	100.0%	56.8%
NPV	92.6%	67.9%	81.4%	72.2%	67.9%
Accuracy	89.0%	73.0%	87.0%	78.0%	63.0%
LR+	7.39	-	-	-	1.74
LR-	0.11	0.63	0.30	0.51	0.63

PPV = positive padictive value value, NPV = negative pradictive value, LR +ve = likelihood ratro for positive results, LR -ve = likelihood ratro for negative results

Table-V

Result of different investigations in pulmonary TB patients

BAL fluid ADA level >5.00 U/L	Sputum culture for AFB Positive	BAL fluid Culture forAFB Positive	BAL fluid Smear for AFB Positive	Mantoux test Positive
ADA positiven=39	13 (33.3)	29 (74.4)	20 (51.3)	22 (56.4)
ADA Negative n=4	3 (75.0)	1 (25.0)	1 (25.0)	3 (75.0)
Total n=43	16 (37.2)	30 (69.8)	21 (48.8)	25 (58.1)

Figure within parentheses indicates in percentage.

for AFB was positive in 51.3% cases, Mantoux test was positive in 56.4% cases. So, in comparison to other investigations ADA level assay in BAL fluid was found to be better for diagnosis of pulmonary TB patients with negative sputum smear for AFB.

Discussion:

Various workers had tried different biochemical tests from time to time which might help in early diagnosis and confirmation of pulmonary tuberculosis[12]. M. tuberculosis PCR in bronchial aspirates is a rapid diagnostic procedure with a high sensitivity (~95%) and specificity (~70%) in sputum smear negative pulmonary TB patients[13]. This test is very costly, not so available in our country and the result would be available at 24-48 hours. Whereas, although the invasive procedure, BAL fluid ADA seems to be a more cost-effective diagnostic procedure than PCR test in our country. Moreover, the results of this test are available in a couple of hours and with high sensitivity, specificity, positive predictive value and negative predictive value. ADA is used for the diagnosis of TB and there are studies showing high levels of ADA in Bronchoalveolar lavage fluids of pulmonary TB patients[6]. The reason of high ADA level is that, ADA is an enzyme found in peripheral blood and tissue lymphocytes and is increased in BAL fluid in diseases with lymphocytic reactions[14]. Inflammation due to TB in lung parenchyma is a type of lymphocytic inflammation and is expected to increase in pulmonary TB patients[15].

In our study, the mean ADA level in BAL fluid was 6.58 ± 1.31 U/L in pulmonary tuberculosis and 4.00 ± 0.93 U/L in non-tuberculous pulmonary diseases. The difference was statistically significant (p=0.001). By using ROC curve analysis the cut-off value of ADA in BAL fluid for diagnosis pulmonary TB was determined to be 5.0 U/L, at which level the sensitivity was 90.7%, specificity was 87.7%, PPV was 84.8%, NPV was 92.6% and accuracy was 89.0%. These statistical results indicate that, the investigation ADA level assay in BAL fluid was applicable for the diagnosis of pulmonary TB.

A few authors have studied ADA activity in BAL fluid for diagnosis of pulmonary tuberculosis. Similar result was found by Halvani and Binesh (2008) and added that, ADA activity in BAL fluid of pulmonary TB patients was higher than that seen in other diseases. Orphanidou et al (1998) examined the ADA activity in BAL fluid of pulmonary TB patients and non-TB pulmonary disease patients and found that ADA level in BAL fluids of pulmonary TB patients was significantly higher than that of non-TB lung disease patients ($p<0.001$), which was consistent with the present study.

Halvani et al. (2008) carried out a study on 63 (sixty three) patients. They obtained mean ADA level in BAL fluid 4.13±2.55 U/L in pulmonary TB group, 2.42±1.06 U/L in nontuberclous pulmonary disease group and 1.93±0.88 U/L in control group. The level was also significantly higher in the pulmonary TB group compared to the other two groups (P=0.001). They obtained the cut-off value of 3.5 U/L with 57% sensitivity and 84% specificity.

In the study by Kayacan et al. (2002), ADA level in BAL fluids of pulmonary TB patients, non-TB pulmonary diseases patients and controls were 3.1±2.0 U/L, 0.4±5.0 U/L and 0.2±0.4 U/L respectively (P< 0.001). They obtained the cut-off value of 1.0 U/L with 100% sensitivity and 83% specificity.

In a study conducted by Kubota et al. (1999), the mean ADA level in BAL fluid of miliary TB patients, sarcoidosis patients, idiopathic interstitial

pneumonia patients and control group was 5.02±3.75 U/L, 1.06±0.99 U/L, 0.21±0.43 U/L and 0.3±0.51 U/L respectively ($p < 0.001$).

Orphanidou et al. (1998) compared ADA activity and lysozyme levels in BAL fluid of smear negative pulmonary TB patients and non-TB pulmonary disease patients. They found no significant difference in lysozyme level of BAL fluids between two groups but the ADA level in BAL fluids of pulmonary TB patients was (6.0 ± 05.8 U/L) significantly higher than that of non-TB lung disease patients ($P<0.001$). They obtained the cut-off value of 2.5 U/L with 71.4% sensitivity and 87.5% specificity for pulmonary TB .

Other studies showed controversial results. Reechaipichitkul et al. (2004) compared ADA levels in BAL fluid of pulmonary TB patients, lung cancer patients and those with other forms of pulmonary diseases and found no significant difference among those three groups ($P=0.56$)

ADA activity assessed in different types of samples, had different diagnostic values in various studies in different countries of the world. Studies conducted in regions with high incidence of TB , measurement of ADA activity found to be a valuable method for diagnosis of TB. In contrast, in low TB incidence regions it is not a precise method for TB diagnosis. This is also true about ADA level in BAL fluid. It seems that this laboratory assessment is remarkably dependent on the method of measurement, materials and location of study. Some differences between our results of this study and other studies can be somehow related to different methods of measurement and different TB prevalence in our country.

European Respiratory Society (ERS) Task Force recommended to instill 240 ml of normal saline in BAL procedure[16]. Kayakan et al. (2002) used 100 ml, Halvani et al. (2008) used 150 ml, Reechaipichitkul et al. (2004) used 150 ml, Orphanidou et al.(1998) used 150 ml normal saline. In this study, we used 100ml (Five aliquots of 20 ml) normal saline solution.

Conclusion:

The findings of this study permit to conclude that, ADA level determination in BAL fluid of pulmonary TB patients is diagnostically important. The ADA level is significantly higher in pulmonary TB patients than that of other pulmonary diseases, and is a rapid and cost effective method with high sensitivity. We therefore believe that, ADA activity in BAL fluid in patients with smear negative pulmonary TB may serve as a rapid and reliable parameter in establishing the diagnosis. A large scale and multi centered study should be done to evaluate the accurate efficacy of ADA level assay in BAL fluid in patients with pulmonary TB.

References:

1. World Health Organization. WHO report 2007; Global tuberculosis control, surveillance, planning, financing. Geneva: The Organization; 2007.
2. Shaikh MS, Dholia KR, Jalbani MA, Shaikh SA. Prevalence of intestinal tuberculosis In cases of acute abdomen. *Pakistan Journal of Surgery* 2007; 23(1): 52-56.
3. Orphanidou D, stratakos G, Rasidakis A, Toumbis M, Samara J, Bakakos P, et al. Adenosine deaminase activity and Lysozyme levels in bronchoalveolar lavage fluid in patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1998; 2(2): 147-152.
4. Dimakou K, Bakasos P, Samaa I. Adenosine deaminase activity in sputum of patients with Pulmonary tuberculosis. *Eur. Resp J* 1997; 10(25): 450.
5. Kayacan O, Karnak D, Delibalta M, Beder S, Karaca L, Tutkak H. Adenosine deaminase activity in bronchoalveolar lavage in Turkish patients with smear negative pulmonary tuberculosis. *Respiratory Medicine* 2002; 96(7): 536- 541.
6. Reechaipichitkul W, Lulitanond V, Patjanasoontorn B, Boonsawat W, Phunmanee A. Diagnostic yield of Adenosine deaminase in bronchoalveolar lavage, *Southeast Asian J Trop Med Public Health* 2004; 35(3): 730-734.
7. Recheldi L, Barnini S, Saltini C. Molecular diagnosis of tuberculosis, *Eur Respir J Suppl* 1995: 20: 689-700.
8. Kurata N. Adenosine deaminase, *Nippon Rinsho* 1995; 53: 1178-1183.
9. Dilmac A., Ucoluk GO, Ugurman F, Gozu A, Akkalyoncu B, et al. The diagnostic Value of

Adenosine deaminase activity in sputum In pulmonary tuberculosis, *Respir Med* 2002; 96: 632-634.

10. Ungerer JPJ, Oosthuizen HM, Bissbort SH, Vermaac WJH. Serum adenosine deaminase: isoenzymes and diagnostic application, *Clin Chem* 1992; 38(7): 1322-1326.
11. Yew WW, Leung CC. Update in Tuberculosis 2006, *Am J Respir Crit Care Med* 2007; 175: 541-546.
12. Rao SK. Kumar HA, Rudresh BM, Srinivas T, Bhat KH. A Comparative study and evaluation of serum adenosine deaminase activity in the diagnosis of pulmonary tuberculosis, *Biomedical Research* 2010; 21(2): 189-194.
13. Wong CF, Yew WW, Chan CY, Au LY, Cheng AF. Rapid diagnosis of smear-negative pulmonary tuberculosis via fiberoptic bronchoscopy: utility of polymerase chain reaction in bronchial aspirates as an adjunct to transbronchial biopsies. *Respir Med* 1998; 92: 815-819.
14. Kubota M, Katagiri M, Imasaki T, Yanase N, Soma K, Tomita T. Adenosine deaminase activity in bronchoalveolar lavage fluid of sarcoidosis patients, *Nihon Kokyuki Gakkai Zasshi* 1999; 37(5): 374-379.
15. Halvani A, Binesh F. Adenosine Deaminase Activity in Bronchoalveolar Lavage Fluid in Patients with Smear-Negative Pulmonary Tuberculosis, *Tanaffos* 2008; 7(2): 45-49.
16. Haslam PL. BAL standardization and measurement of acellular components. *Eur Respir Rev* 1998; 8: 1066-1071.

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ORIGINAL ARTICLE

Fluorescein Diacetate (FDA)" A staining based method for early diagnosis of Category II Treatment Failure Tuberculosis Patient

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Abstract

Objectives: *Rapid diagnosis of true treatment failure tuberculosis patient among suspected cat II treatment failure tuberculosis patients.*

Design: *Prospective type of study among ZN smear positive suspected cat II failure TB patients to find out true treatment failure by FDA staining and conventional culture.*

Result: *Total 100 patients of cat II failure ZN positive were included. 90 patients were FDA positive. Eighty seven (87%) were culture positive. Eighty six (95.5%) were positive by both culture and FDA staining. Sensitivity of FDA staining was 98.5%.*

Conclusion: *FDA staining on fresh sputum can be used for early and accurate diagnosis of true treatment failure. This early information is of great advantage in clinical settings to choose an appropriate drug regimen.*

[Chest & Heart Journal 2011; 35(2) : 103-106]

Introduction:

Tuberculosis is a major health problem in Bangladesh. In 2007 Bangladesh ranked 6th on the list of 22 highest TB burden countries in the world [1]. Estimates suggest that in Bangladesh about 880 new TB cases and 176 TB deaths occur daily [2]. The estimated prevalence of all forms of TB and incidence rates in Bangladesh was 425 and 225 respectively per 1, 00,000 per year and the mortality rate was 51 per 1,00,000 per year [3].

Diagnosis of Pulmonary tuberculosis in developing countries mainly depends on result of ZN stained sputum smear. Treatment regimens are usually prescribed without assessing the drug susceptibility profile of infecting strain [4].

Category I treatment are given to those patients who are newly diagnosed TB patients and have never taken anti-TB drug or have taken anti-TB drug for less than one month. Treatment duration is six months. If any patient remains smear positive after 5th month or later during treatment of category I, is called category I failure. Category II treatments are given to those patients who are previously treated with anti-TB drug for more than one month, relapse cases, category I failure, and patients with history of drug default. Treatment duration is eight (8) months. If any patient remains smear positive after 5th month or later during treatment of category II, that patient is called category II failure. These patients will be treated

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with category IV regimens which are mainly second line drug [5].

Sputum smears microscopy by Ziehl-Neelsen staining (Z-N) for acid fast bacilli (AFB) remains the most important and widely available diagnostic method for tuberculosis in high prevalence countries. Its specificity is very high on diagnosis of new TB cases, but not for the case detection during treatment [6]. ZN stained sputum smear microscopy cannot distinguish between live and dead AFB [7]. Smear may often found positive due to prolonged excretion of dead bacilli. At continuous treatment phase of Cat I a Z-N positive smear is already more indicative of resistance and in 50% cases during Cat II it even indicates Multi Drug Resistant Tuberculosis (MDR-TB). Later, during the treatment and at the end of the treatment, it usually indicates failure. However, one third of sputum specimens of these failure patients did not grow TB bacilli on culture, which may have been due to dead AFB, resulting in false declaration of failure and unnecessary re-treatment. Besides, rapid determination of drug susceptibility remains problematic all over the world and especially in TB endemic countries. A reliable test to distinguish live from dead AFB might thus be very useful as a criterion to change treatment early, i.e. to MDR treatment or prolong intensive phase and as a true indicator of failure. Fluorescein Diacetate (FDA), a salt of the green fluorophore fluorescein, is detectable by fluorescence microscopy only after its cleavage by cell esterase. These are present in viable cell only [8]. .

Materials and Methods:

Patients diagnosed as Category II Treatment Failure by physician referred to the National Tuberculosis Referral Laboratory (NTRL) was enrolled for sputum examination. Those patients who showed AFB positive by Z-N method were included for FDA staining and conventional culture. A total 100 patients of different age and sex were enrolled in this study. Sample collection and laboratory work were done in the NTRL, NIDCH, during the period of January 2010 to December 2010.

Staining procedures:

Ziehl-Neelsen (ZN) staining was performed as per standard procedure [9]. The FDA staining procedure was adapted from the publication by Tsukiyama *et al.*[8]. After drying, unfixed smears were covered for 30 min with the FDA reagent, which was made up weekly by diluting 100µl FDA stock (5% w/v fluorescein diacetate in acetone, stored in aliquots at 20° C for up to 2 years) in 10 ml phosphate buffer solution.. After rinsing with water and de-staining with 1% acid alcohol for 1–2 min, bacilli were killed using 5% watery phenol for 10 min, followed by a final water rinse and air-drying in the dark. One length of the smear was read under a fluorescence microscope at 1000 magnification.

Conventional culture:

Culture were done on Lowenstein-Jensen media (LJ) media as was described by Kantor *et al.*, [10].

Result

Among 100 patients, sputum Z-N staining grading showed maximum positivity, i.e., 3^+ in 48 (48%) cases, followed by 1^+ in 29 (29%) cases, 2^+ in 17 (17%) cases and scanty in 6 (6%) cases. Among these cases 90 (90%) were positive and 10 (10%) cases were negative by FDA staining. Of these 90 cases, FDA staining grading showed, 8 (8.8%) cases were scanty, 30 (33.3%) cases were 1^+, 25 (27.7%) cases were 2^+, and 27 (30%) cases were 3^+.

The results of FDA staining were compared with conventional culture, among 100 Z-N positive TB cases 86 cases were positive and 9 were negative by both the methods. Out of 10 FDA negative, 1 (10%) case was found culture positive and 4 (4.5%) cases of FDA positive were culture negative. Sensitivity of FDA staining was 98.5% and specificity was 70.0%

Table-I

Distribution of the study patients according to FDA grading (n=100).

FDA grading	Number of patients	Percentage
	(n=90)	100
S	8	8.8
1^+	30	33.3
2^+	25	27.7
3^+	27	30.0
Total	90	100

Note:FDA: Fluorescein Diacetate

Table II

Results of FDA staining and Conventional culture in L-J media (n-100).

FDA staining	Number	Conventional culture	
		Positive*n* (%)	Negative*n* (%)
Positive	90	86 (95.5)	4 (4.5)
Negative	10	1 (10)	9 (90)
Total	100	87 (87)	13 (13)

Table III

Sensitivity, Specificity, Positive predictive value , Negative predictive value and accuracy of FDA staining on conventional culture.

	Percentage
Sensitivity	98.5
Specificity	70.0
Positive predictive value	94.5
Negative predictive value	90.0
Accuracy	95.0

Discussion

Early detection of drug resistance could optimize treatment, improve the outcome for patients with drug resistance pulmonary TB and prevent the transmission of MDR-TB. AFB microscopy by Z-N staining is the most important and simple diagnostic method. However, it can not differentiate the live bacilli from the dead. As a result the declaration of treatment failure by Z-N staining some time may give false treatment failure results due to excretion of dead bacilli. So, for final result one should wait for 3-4 weeks for culture result to diagnose true treatment failure case. Whereas, true treatment failures can be diagnosed within one hour by FDA staining. FDA staining is able to differentiate between live and dead bacilli. Harada and Numata [11] reported that FDA staining is a valuable method to detect viable bacilli in sputum on the first day of examination and it is advantageous for doctors and patients as they are informed early about treatment failure rather than waiting for several weeks for culture report. They applied this method for sputum smear in TB cases and found perfect correlation with growth in culture . FDA has been used to determine the viability leprosy and other mycobacteria [12]..

The results of FDA staining were compared with conventional culture, among 100 Z-N positive TB cases, 86 cases were positive and 9 were negative by both the methods. Out of 10 FDA negative, 1 (10%) case was found culture positive and 4 (4.5%) cases of FDA positive were culture negative. Sensitivity of FDA staining was 98.5%. Similar results were observed in a study conducted by Hamid *et al*.[6] which showed 99% sensitivity and 80% specificity. They recommend FDA staining on fresh smear positive sputum can be used for early and accurate diagnosis of true treatment failure.

The required time for the FDA staining in the present study was 1 hour. On the other hand conventional culture takes 28 days. The simple and inexpensive technique of FDA staining could rapidly assess infectiousness of the patients under treatment and provided potential guidance for infection control measures. FDA staining may allow early field screening for MDR-TB and impending treatment failure.

Conclusion:

Z-N staining can not diagnosed treatment failure accurately, because it can not differentiate live and dead bacilli. FDA staining is able to differentiate the live and the dead bacilli early. So FDA staining on fresh sputum can be used for early and accurate diagnosis of true treatment failure. This early information is of great advantage in clinical settings to choose an appropriate drug regimen.

Refference:

1 National tuberculosis control in Bangladesh, annual report 2009:pp.2-5.available on ,www.ntpban.org.

2 Operational Manual for the Management of Multi drug resistance TB (MDR), National Tuberculosis Control programme, 1st edition 2009, pp-11,21

3 TB epidemiological profile. Revised on December 2010, [on line], Available at :http://www.who.int/tb/data

4 De Cock KM & Wilkinson D. (1995) Tuberculosis control in resource poor countries: alternative approaches in the era of HIV. *Lancet*; 346:675-677

5 National Guideline and Operational Manual for Tuberculosis Control, 4th Edition.Tuberculosis Control Programe, 2009 pp-19.

6 Hamid Salim A, Aung KJ, Hossain MA, Van Deun A. (2006) Early and rapid microscopy-based diagnosis of true treatment failure and MDR-TB. *Int J Tuberc Lung Dis;* 10(11); 1248–1254.

7 International Union against Tuberculosis and Lung Disease (IUATLD), 2005]. Tuberculosis bacteriology, priorities and indications in high prevalence countries: position of the technical staff of the Tuberculosis Division of the International Union Against Tuberculosis and Lung Disease. *Int J Tuberc Lung Dis*; 9: 355–361.

8 Tsukiyama F, Katoh M, Matsuo Y. (1984) Modification of the fluorescent staining method for mycobacterial cells. *Hiroshima J Med Sci*; 33: 293–295.

9 Standard operative procedure. World Health Organization.2007, Geneva, Switzerland.

10 Kantor IND, kim SJ, Frieden T, Laszlo A, Luelmo F, Norval P, Rieder H, Valenzuela P, Weyer K.(1998) Laboratory service in tuberculosis control, Geneva, World Health Organization. (WHO/TB/98.258).pp.16-31 (part-II) & 9-59 (part-III).

11. Harada S, Numata N. (1992) Application of FDA/EB staining for the detection of viable or non-viable mycobacteria in clinical specimens. *Kekkaku*; 67: 113–117.

12 Kvach JT, Veras JR. (1982) A fluorescent staining procedure for determining the viability of mycobacterial cells. *Int J Leprosy*; 50: 183–192.

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REVIEW ARTICLE

Asthma in Pregnancy – An Overview

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Abstract:

Backgrond:*Asthma is an important chronic disorder of the airways with significant morbidity and mortality. Around 300 million people currently have asthma.It is estimated that there may be an additional 100 million people with asthma by 2025. According to First National Asthma Prevalence Study(NAPS) 1999 , in Bangladesh about 7 million people (5.2% of the population) are suffering from current asthma (at least three episodes of asthma attack in last 12 months).Asthma accounts for about 1 in every 250 deaths worldwide,although modern management ,which obviously includes patient education,can prevent 80% of such deaths. Asthma has been reported to affect 3.7 to 8.4% of pregnant woman in the USA making it potentially the most common serious medical problem to complicate pregnancy.Maternal asthma increases the risk of perinatal mortality , preeclamsia ,preterm birth, and low birth weight infants,while better controlled asthma is associated with decreased risks.*

Discussion: *Asthma in pregnancy is often under-recognized and sub-optimally treated. The course of asthma during pregnancy is variable – it improves , remains stable, or worsens in similar proportions of women. Asthma during pregnancy follows the rule of one-third ,that is one-third asthmatics become worse, one-third remains same and one-third improves. The exact mechanism behind this is not known .It is common to experience some breathlessness near the end of the pregnancy,this is related to the size of the fetus and the pressure it puts on the diaphragm.The risk of an asthma exacerbation is high immediately postpartum .Acute asthma attacks can result in dangerously low fetal oxygenation , poor asthma control is associated with pre-eclampsia as well as greater rates of caesarean section ,pre-term delivery ,intra-uterine growth retardation and low-birth weight .Women with well-controlled asthma during pregnancy ,however, have outcomes as good as those in their non-asthmatic counterparts .Triggers should be controlled meticulously during pregnancy.They can influence the probability of giving birth to a wheezy baby. Active and passive smoking should also be avoided at this time .It increases the chances of wheezing in the new born .Woman with severe or uncontrolled asthma are at higher risk for pregnancy complication and adverse fetal outcome than woman with well-controlled asthma Recent evidence based guidelines have concluded that it is safer for pregnant women with asthma to be treated pharmacologically than to continue to have asthma symptoms exacerbation.*

Conclusion: *All asthma medicines have been shown to be absolutely safe for both the the mother and the baby. Inhaled route is always preferred.Acute attack of asthma is very rare in labor, perhaps due to endogenous steroid production.Asthma medications may enter the breast milk but the concentration is extremely small and do not have any adverse effects on the baby.*

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Asthma is a chronic inflammatory disorder causing hyper-responsiveness of the

airways to certain stimuli resulting in recurrent variable airflow limitation , at least partly reversible ,presenting as wheezing , breathlessness ,chest tightness ,and coughing[1] .Asthma is a serious health problem worldwide

- Its prevalence has increased in the past two decades
- Approximately 8% of pregnant women reported current asthma
- It is the most common chronic condition in pregnancy

Changes in Respiratory Functions during Pregnancy

Normal pregnancy is associated with –

- 20% increase in oxygen consumption
- 15% increase in the maternal metabolic rate
- Achieved via a 40-50% increase in resting minute ventilation ,resulting mainly from a rise in tidal volume rather than respiratory rate
- **This hyperventilation causes –**

  - Pao_2 to increase

  -$Paco_2$ to fall ,with a compensatory fall in serum bicarbonate to 18-22 mmol/l

  - A mild respiratory alkalosis is therefore normal in pregnancy (arterial pH 7.44)

- 75% of women experience a subjective feeling of breathlessness during pregnancy
- In late pregnancy the diaphragmatic elevation caused by the enlarging uterus leads to a decrease in FRC.
- No change in PEFR or FEV_1 in pregnancy
- Decrease FRC may exacerbate hypoxaemia because of premature airway closure when acute asthma complicates pregnancy.[2]

Physiological factors affecting asthma in pregnancy

- Increase in free cortisol levels may protect against inflammatory triggers
- Increase in progesterone may improve airway responsiveness
- Increase in prostaglandin F2á may promote airway constriction
- Placental 11â hydrxysteroid dehydrogenase type 2 decreased activity is associated with an increase in placental cortisol concentration and low birth weight
- Placental gene expression of inflammatory cytokines may promote low birth weight
- Modification of cell mediated immunity may influence maternal response to infection and inflammation.[3]

Effects of Pregnancy on Asthma

- The course of asthma in pregnancy in an individual woman is largely unpredictable
- Women with mild disease are unlikely to experience problems , whereas those with severe asthma are at greater risk of deterioration ,particularly late in pregnancy
- Asthma may improve during pregnancy due to progesterone mediated brochodilation and increased serum cortisol levels
- Asthma may deteriorate during pregnancy due to increased stress and increased gasro-oesophageal reflux
- Many asthmatic patients experience worsening of symptoms during pregnancy because they stop or reduce medication due to fears about its safety.
- In most women asthma has no effect upon the outcome of pregnancy
- Severe ,poorly controlled asthma may have an adverse effect on fetal outcome as a result of chronic or intermittent maternal hypoxaemia
- Increase risk of premature labour
- Low birth weight
- Higher rates of pregnancy induced

  -hypertension

  -Pre-eclamsia and

  -Caesarean section

- Increased incidence of tachypnoea of the newborn

- Neonatal hypoglycemia
- Neonatal siezures
- Admission to the neonatal intensive care units in the babies of asthmatic women[4]

Special considerations in pregnant women with asthma

- Ensure optimal asthma control throughout pregnancy
- Manage asthma exacerbations aggressively
- Avoid delay in diagnosis and treatment
- Assess medication needs and response to therapy frequently
- Ensure adequate patient education and acquisition of self management skills
- Treat rhinitis ,gastric reflux and other comorbidities adequately
- Encourage smoking cessation
- Assess pulmonary function with spirometry at least monthly
- Offer a multidisciplinary team approach[3]
- Be aware of the risk of pre-elampsia and intrauterine growth retardation [5]

Main differential diagnosis in pregnant women with dyspnoea

- Asthma
- Physiological dyspnoea of pregnancy
- Pulmonary embolism
- Pulmonary oedema
- Peripartum cardiomyopathy
- Amniotic fluid embolism

Management of Asthma

All patients should be educated regarding the relationship between asthma and pregnancy

Should be taught about

-self-treatment
-Inhaler techniques
-Adherence to medication

Control of potential environmental triggers [6]

Appropriate management of common coexisting conditions such as

-Rhinitis
-Sinusitis
-Gastro-esophageal refux

Women who smoke must be informed of the potential adverse effects of smoking on the fetus

Should be strongly encouraged to quit

Assessment of Asthma control in pregnant women

Variable	Well-controlled Asthma	Asthma not well controlled	Very poorly controlled Asthma
Frequency of symptoms	<2days/wk	>2days/wk	Throughout the day
Frequency of night time awakening	<2 times/mo	1-3 times/wk	>4 times/wk
Interference with normal activity	None	Some	Extreme
Use of short-acting â-agonist for symptom control	<2 days/wk	>2 days/wk	Several times/day
FEV_1 or PEFR(% of the predicted or personal best value)	>80	60-80	<60
Exacerbations requiring use of systemic corticosteroid(no.)	0-1 in past 12 month		>2 in past 12 months

FDA Pregnancy Risk Category

Category	Interpretation
A	Controlled studies show no risk, Adequate, well-controlled studies in pregnant women have failed to demonstrate a risk to the fetus in any trimester of pregnancy
B	No evidence of risk in humans. Adequate well-controlled studies in pregnant women have not shown increased risk of fetal abnormalities despite adverse findings in animals,or in the absence of adequate human studies ,animal studies show no fetal risk .The chance of fetal harm is remote,but remains a possibilities .
C	Risk cannot be ruled out.Adequate well-controlled human studies are lacking and animal studies have shown a risk to the fetus or are lacking as well there is a chance of fetal harm if the drug is administered during pregnancy but the potential benefits may outweigh the potential risk
D	Positive evidence of risk .Studies in humans, or investigational or post marketing data, have demonstrated fetal risk.Nevertheless, potential benefits from the use of the drug may outweigh the potential risk.
X	Contraindicated in pregnancy . Studies in animals or humans or investigational or post marketing reports have demonstrated positive evidence of fetal abnormalities or risk that clearly outweighs any possible benefits to the patients .

FDA Pregnancy risk category, TERIS rating of asthma medication[5]

Medication	Risk Category	TERIS rating(magnitude of teratogenic Risk/Quality, Quantity of Data)
Salbutamol	C	Undetermined/Limited
Levo-salbutamol	C	NA
Sameterol	C	Undetermined/Very Limited
Beclomethasone	C	Unlikely/Limited to Fair
Budesonide	B	Unlikely/Limited to Fair
Fluticasone	C	NA
Mometasone	C	Undetermined/Limited
Triamcinolone	C	Undetermined/Limited
Fluticasone/salmeterol	C	NA
Oral steroid	C	
Cromolyn	B	Unlikely/Fair to Good
Nedocromil	B	Undetermined/Very Limited
Montelukast	D	Undetermined/Limit
Theophylline	C	None/Fair to Good

Steps of Asthma Therapy during Pregnancy

Step	Preferred Controller Medication	Alternative Controller Medication
1	None	
2	LDICS	LTRA,theophylline,or cromolyn
3	MDICS	LDICS plus LABA,LTRAor theophylline
4	MDICS plus LABA	MDICS plus either LTRA or theophylline
5	HDICS plus LABA	
6	HDICS plus LABA plus oral prednisolone	

Patient Education for Self-Treatment of Asthma during pg

Subject	Recommendation
General information	Provide basic information about asthma and relationship between asthma and pregnancy
Use of inhaler device	Demonstrate proper technique for specific device and ask patient to perform the technique ;demonstrate use of spacer device for matered dose inhaler
Adherence to treatment	Discuss self-reported adherence to treatment with controller medication and if needed ,address barrier to optimal adherence
Self-treatment action plan	Provide schedule for maintenance medication and dose of rescue therapy for increased symptoms,explain when & how to increase controller medication & when & how to use prednisolone ,explain how to recognize a severe exacerbation &when &how to seek urgent or emergency care

Environmental Control Measures to Reduce Exposure to Allergens

Allergen	Instructions	Level of Evidence
Animal dander	Remove pet from house; if removal not acceptable, keep pet out of bedroom	Consensus judgement
Dust mites	Encase pillow &other mattress with impermeable covers,wash sheets & blankets weekly in hot water	Data from several RCTs
Cockroaches	Do not leave food or garbage exposed; use poison baits or traps rather than chemical agents , which can aggravate asthma	Few RCTs

Management of acute severe asthma

Acute severe attacks of asthma are dangerous and should be vigorously managed in hospital

Treatment is no defferent from the emergency management of acute severe asthma outside pregnancy .

- Oxygen
- Nebulised $\hat{a}_2$ agonists
- Nebulilised ipratropium
- Oral or i.v steroids and in severe cases
- Intravenous aminophylline or
- Intravenous â2 agonists should be used as indicated

Sadly,pregnant women receive appropriate treatment with corticosteroids less commonly than non-pregnant women.[10]

Management of asthma during labour and delivery

- Acute attacks of asthma during labour and delivery are extremely rare
- Women should be reassured accordingly
- Women may continue their regular inhalers throughout labour
- Those on oral steroids at the onset of labour or delivery should receive parenteral steroids
- Prostaglandin E2 used to induce labour ,to ripen the cervix,or for early termination of pregnancy is a bronchodilator and is safe
- Prostaglandin F2á ,indicated for severe post partum haemorrhage ,should be used with caution as it may cause bronchospasm
- Asthmatic women may safely use all forms of pain relief in labour including epidural analgesia
- Opiates should be avoided
- Ergometrine has been reported to cause bronchospasm[11] .

Management of asthma in breast feeding mother

Women with asthma should be encouraged to breast feed

The risk of atopic disease developing in the child of an asthmatic mother is about one in 10,or one in three if both parents are atopic. This risk may be reduced by breast feeding

All inhaled preparations ,oral steroids and methylxanthines are safe when breast feeding[12]

Conclusions and Recommendations

- Management of asthma in pregnancy does not differ significantly from management outside pregnancy
- The priority should be effective control of the disease process ,with the aim being total freedom from symptoms both day and night
- The medications used to treat asthma are safe in pregnancy ,but concerns on the part of pregnant women and their carers may result in the reduction ,cessation or withholding of important treatments
- Great attention must therefore be given to explanation and reassurance about the safety of the drugs used to treat asthma in pregnancy and lactation
- Although uncontrlled asthma may increase the risk of adverse perinatal outcomes ,women with well-controlled asthma in pregnancy generally have good pregnancy outcomes
- Exacerbation should be prevented by optimal asthma management ,and if they occur they should be treated aggressively
- Asthma care and obstetric care should be carefully integrated
- The patient should be educated regarding the potential risks of uncontrolled asthma for herself and her pregnancy
- The small risk of harm to the fetus comes from poorly controlled severe disease rather than from the drugs used to prevent or treat asthma
- We should choose inhaled budesonide over other ICS because more safety data are available on the use of this drug during the gestational period
- The patient should also be instructed in the use of an optimal inhaler technique
- The patient should be given a personalised self-management action plan for asthma
- We would recommend follow-up every 1 to 2 weeks initially to ensure that asthma control is achieved and then ,once the patient's condition is stable, at least monthly throughout the pregnancy[13]

References

1. Davidson'principal and practices of medicines-chapter,19,21st edition,p-662
2. Asthma and pregnabcy report-NIH publication ,No.-93-3279
3. Kallen B. Rydhstroem H, Aberg A ,et al ,Asthma during Pregnancy –a population based study. Eur J Epidermolol 2000 ; 16 (2) ,167-71
4. Kwon HL, Belanger K, Bracken MB et al.asthma prevalence among pregment and childbearing aged women in the United States; estimates from national health surveys. *Ann Epidermiol* 2003;13(5);317-24
5. Schartz M, Zeiger RS, Hoffman CP,et al.Perinatal outcomes in the in the pregnancies of asthmatic women : a prospective controlled analysis. *Am J Respir Crit Care Med* 1995;151 (4) :1170-4
6. Bracken MB,Triche EW, Belanger K et al. Asthma symptoms,severity,and drug therapy: a prospective study of effects on 2205 pregnancies.Obstet Gynaecol 2003;102(4):739-52
7. Kircher S,Schatz M,Long L. Variables affecting asthma course during pregnancy .*Ann Allergy Asthma Immunol* 2002;89(5):463-6
8. National Guidelines Asthma &COPD- 4th edition,p- 53
9. National Guidelines Asthma & COPD -4th edition, p-54
10. Wendel PJ,et al. Asthma treatment in pregnancy; a randomized controlled study,Am J Obstet Gynecol 1996,175 (1) ,150-4
11. Tata LJ, Lewis SA, McKeever TM, et al. Effect of maternal asthma, exacerbations and asthma medication use on congenital malformations in offspring: a UK population-based study. Thorax 2008; 63:981.
12. Dombrowski MP, Schatz M, ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin: clinical management guidelines for obstetrician-gynecologists number 90, February 2008: asthma in pregnancy. Obstet Gynecol 2008; 111:457.
13. Bakhireva LN, Jones KL, Schatz M, et al. Asthma medication use in pregnancy and fetal growth. J Allergy Clin Immunol 2005; 116:503.

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REVIEW ARTICLE

Female Genital Tuberculosis: An Evaluation and Diagnostic Dilemmas

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Abstract:

Female genital tuberculosis (GTB) is an important cause of infertility in developing countries. The actual prevalence is not exactly known mainly due to its subtle presentation and lack of reliable confirmatory investigations. A rising trend has been reported in last few years due to TB/HIV co-infection and emergence of drug resistant cases. Bangladesh being a 'concentrated epidemic' zone (in relation to HIV infection) is at risk. GTB is a paucibacillary form of TB and almost always secondary to a focus else where in the body commonly lungs. Though an early diagnosis is important to prevent adverse sequel, it remains a challenge for the clinicians. Future fertility is poor even after treatment. This review considers the background information, magnitude of problem, diagnostic difficulties in evaluation and newer developments like PCR, IGRAs in the field of detection of GTB. Finally, the involvement of a respiratory physician to enlighten the diagnostic accuracy and to treat drug-resistant forms has been stressed to optimize the outcome.

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Introduction :

Tuberculosis (TB) remains an important infection in women globally. It predominately presents with pulmonary disease although extra pulmonary TB is not uncommon[1]. In 1993, the World Health Organization (WHO) declared TB a global public health emergency. According to WHO, in 2010, there were 8.8 million incident cases of TB, 1.1 million deaths among HIV negative people and additional 0.35 million deaths from HIV associated TB. It is the second leading cause of death from an infectious disease worldwide after HIV[2]. The probability of developing TB is much higher among people infected with human immune-deficiency virus (HIV) [2]. The morbidity associated with this condition has major health implications[3]. The disease has a worldwide distribution and the incidence is high in the developing countries[4].

Female genital tuberculosis (GTB) is a form of extrapulmonary TB and is almost always secondary to TB infection elsewhere (usually pulmonary) in the body[5]. It is a known cause of infertility [6,7] and has a tremendous impact on reproductive health[7]. This disease is responsible for 5% of all female

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pelvic infections [8,9,10] and occurs in 10% cases of pulmonary TB[8,10]. The first reported case of gynaecological TB was described by Morgagni in 1744[11].

The prevalence of female GTB is largely underestimated as the disease often remains silent or may present with non-specific symptomatology[3]. However a rising incidence, partly as a result of HIV pandemic[10,12,13] and emergence of resistant strains has been reported[8,13]. Majority of patients are in the reproductive age group[5,8,9,14,] but older women are also known to harbour it[15].

This silent invader of genital tract tends to create diagnostic dilemmas[7,13] & remains a challenge for the clinicians. As there is no gold standard test[7], a high index of clinical suspicion aided by a number of investigations may help to reach to a definite diagnosis. A combined approach involving a respiratory physician and gynaecologist may help to achieve the optimum goal in diagnosis, treatment and follow-up particularly in dealing with the multi-drug resistant TB (MDR-TB) cases. An early diagnosis is important otherwise many women in their sexual prime are rendered infertile due to GTB[9]. The chances of pregnancy have so far been poor (5%) even after the completion of treatment[8].

Incidence :

The exact incidence of female GTB is not known partly because more often the disease remains silent and in addition there are lack of reliable confirmatory investigations[10]. In developed countries, such as USA, Australia and Western European countries, the incidence is les than 1 percent[16,17] but the incidence in some African countries is as high as 15-19 percent [18,19]. The global prevalence of genital tuberculosis is estimated to be 8-10 million cases[20] with a rising trend in some developing countries[12]. Increase in the trend of this disease may be partly due to an overall rise in tuberculosis cases[8,10]. The other contributory factors may be HIV infection and emergence of drug resistant forms[8,13]. It is important to mention that, the incidence of TB has decreased significantly worldwide during the last 30 years[22]. However, the pandemic of HIV has altered its incidence, morbidity and mortality in less developed countries[12].

Situation in Bangladesh (BD) :

According to WHO, Bangladesh ranks sixth among countries with high TB burden[23,24]. The estimated prevalence and incidence rates of all forms of TB including HIV were respectively 411 and 225 per 100,000 population and mortality rate excluding HIV was 43 per 100,000 in 2010[2]. But according to a nationwide Tuberculosis Disease – cum – Infection Prevalence Survey 2007-09 – a steep decline in the number of TB cases among urban population has been registered[24]. The prevalence of female GTB is not known but there is a risk of resurgence of TB as the HIV burden has moved from low prevalence to 'concentrated epidemic' following the publication of the HIV sero survey results of more than 5 percent among injecting drug users[25]. Data from national drug resistance surveys indicate low levels of MDR-TB. Isolated survey have indicated that MDR-TB rates among newly diagnosed cases between 3% and 15.4%[23] . The proportion of HIV positive individuals among TB cases has been estimated to be 0.1% and HIV prevalence is 7% among injecting drug users[23].

Impact of female GTB on reproductive health :

Female GTB is one of the major causes for severe tubal disease leading to infertility[3]. There is a wealth of literature on GTB and infertility[7], being first shown by Malkani et al.[26]. When TB affects genital organs of young females, it produces devastating effects by causing irreversible damage to the fallopian tube resulting in infertility which is difficult to cure both by medical and surgical methods [27,28]. Minimal damage may lead to ectopic pregnancy and extensive damage may lead to complete tubal occlusion[15]. Peritubal adhesions and tubo-ovarian mass have been found in 47.2% of cases[29], Various grades of intra-uterine adhesion (Asherman's syndrome) or nonreceptive ednometrium have been reported in association with GTB[30].

However, the average worldwide incidence of female genital TB in infertile population has been reported as 5-10%[31,32]. Despite the advances in chemotherapy, with the WHO's recommended DOTS strategy[33], pregnancy and live birth after diagnosis of GTB has been reported to be low and when it did occur was more likely to be an ectopic pregnancy or resulted in a spontaneous abortion [34,35]. Overall, the conception rate among women

with GTB varies from 10% to 20% throughout the world[7]. Frydman et al suggest that IVF is the only effective treatment for tuberculous infertiltiy[36].

Pathogenesis :

GTB is almost always secondary to a TB elsewhere in the body most commonly from lungs[8,9,37,38,39]. and sometimes from kidneys, gastrointestinal tract, bones and joints. The mode of spread is usually haematogenous or lymphatic and occasionally via direct contiguity with an intra-abdominal or peritoneal focus[37]. Primary TB infection of the female genital tract is extremely rare but it may occur when the male partner has active genitourinary TB, during orogenital sex or direct inoculation at sexual intercourse and ascending spread from the vagina, cervix and the vulva[22].Following secondary or primary infection, the fallopian tubes are believed to be the initial and most frequently affected genital organ followed by endometrium[8,9,17,40,41]. Frequency of involvement include fallopian tubes (90-100%), endometrium (50-60%), ovaries (20-30%), cervix (5-15%) and vulva & vagina – 1%. The infective agent is *Mycobacterium tuberculosis*, occasionally *M. bovis* may cause human disease[9].

The primary focus is often quiescent or healed by the time the genital lesion becomes active and that a long latent period of may years may intervene between the primary affliction and the appearance of GTB[9,43,44]. The incidence of active TB in infected individuals in only 10% [45,46]. Depending on the virulence of the organism and immune response generated by the host, the disease remains either active or becomes asymptomatic with latent infection persisting for many years[47]. Latent infected individuals contain dormant yet viable bacilli which may reactivate when the host response becomes low. During reactivation, the bacilli induce immune modulation and there is release of harmful cytokines like IL2, TNF alpha and INFgamma[48].

Pathology :

The essential pathology in TB is the production of a characteristic lesion, the tubercle. This is an avascular granuloma, composed of a central zone containing giant cells, with or without caseation and a peripheral zone of lymphocytes & fibroblasts[10].

Clinical profile :

Female GTB is a disease of varied symptomatology. The disease is often discovered incidentally in many patients and remains undiscovered in a large number of symptom less patients[35]. About 10-11% of patients are asymptomatic. A history of poor general health persisting over months or years associated with weight loss, undue fatigue, low grade fever or vague abdominal discomfort is often elicited. In 30-50% of cases there is history of diagnosis/ treatment for extragenital TB[17,49] and in about 20% there is history of TB in immediate family[17]. The four major presenting complaints are infertility, menstrual disturbances, pelvic pain and swelling[22]. Infertility is the most common presentation with an incidence ranging from 40-75%[6,34]. Menstrual disturbances may be in the form of amenorrhoea (usually secondary), menorrhagia, metrorrhagia, oligomenorrhoea, and v. rarely postmenopausal bleeding[43,44]. In about 4%, it is the cause of puberty menorrhagia[9]. There may be offensive discharge & post coital bleeding as in involvement of cervix[38], and painful shallow ulcer in the vulva/vagina[9,50]. **Physical examination** findings may be normal in upto 50% of cases[28,41,49] when abnormal findings are present, adnexal mass or signs of ascities are common. Adnexal mass vary in size and consistency and results from conglomeration of pelvic organs matted together by adhesions[39].

Diagnostic Dilemmas in clinical evaluation :

GTB can mimic ovarian cancer and diagnosis is only made after laparotomy[39]; Pelvic inflammatory disease, often recurrent and failing to respond to standard treatment , is often due to GTB[9]; If a young girl with puberty menorrhagia fails to respond to standard hormonal therapy, she should be investigated for GTB[9]; A pelvic inflammatory mass in a virgin girl, may be due to GTB[9] ; Patients with post-menopausal bleeding should be screened for GTB after excluding mailgnancy[9]; Cervical TB may mimic cancer[9,38,51]. Patients with infertility and menstrual disturbance should raise the suspicion of GTB[9]. Sometimes the patients may present with pyrexia of undetermined origin[52] .

Investigations :

Due to the asymptomatic nature / varied clinical presentation, clinical diagnosis of genital TB is difficult[53,54]. Moreover, the criteria for a definitive

diagnosis of GTB by demonstration of Mycobacterium tuberculosis are hardly ever met in people with paucibacillary GTB[55], because microscopy for acid-fast bacilli (AFB) requires the presence of at least 10,000 bacilli /ml of specimen and culture requires at least 100 bacilli /ml[56]. Molecular diagnosis by PCR (Polymerase chain reaction) has become a useful adjunct in recent years; because PCR detects less than 10 organisms/ml [57]. Peritoneal fluid DNA PCR helps in detecting cases missed by PCR on endometrial aspirate specimen[7]. PCR is a rapid, sensitive & specific molecular method for detecting mycobacteria in both pulmonary & extra pulmonary samples[8]. But PCR cannot distinguish between live and dead bacteria and hence may not reflect active disease [8] and can give false positive results.

Routine laboratory tests are of little value[3]. Traditionally the diagnosis depends on demonstration of causative organism *Mycobacterium tuberculosis* by acid fast staining and/or growth of the organism on Lowenstein-Jensen (LJ) medium[8] but the tests are of limited value as a substantial number of GTB are bacteriologically mute[58]. Diagnositc accuracy may be improved by using fluorescence microscopy (being more sensitive)[59,60,61] in stead of Ziehl-Neelsen staining and using BACTEC radiometric culture which offer results within 2 weeks[8,62] & being more sensitive. If the smear is positive, PCR or gene probe tests should be done as other mycobacteria are also acid-fast.

An absolute diagnosis cannot be made from characteristic features in Hysterosalpingography (HSG) or laparsocopy[3]. HSG in contraindicated in suspected GTB as it can flare up the disease[9] but laparoscopy is now being increasing used for early detection of GTB because it offers dual advantage of pelvic organ visualization & sample collection from inaccessible sites[63].

Histopathological examination of the endometrial tissue is an important test to detect GTB but has limitation of low detection rate[3]. MT (Mantoux test) may be positive in GTB[3] but has limitations, classification is based on risk factors and controversies on use of the test on BCG vaccinated persons[39]; can be positive with non-tuberculous mycobacteria (NTM) or when the patient has had past infection[3]. Interferon – gamma release assays (IGRAs) are exciting new developments which can distinguish latent tuberculosis infection[3]. X ray chest may show features of healed (common) or active tuberculosis [3] .Ultrasonic guided fine needle aspiration (FNAC) from pelvic mass may be helpful in some cases[9]. Semen culture is desirable in GTB, as this is transmitted sexually and test for HIV infection should not be neglected[9].

A high index of clinical suspicion, evaluation of clinical findings and risk factors may direct to the best possible investigation. Identification of the causative organism is of limited value in paucibacillary GTB;there are many other tests which strongly suggest but cannot confirm it. Sometimes a therapeutic test may be needed[8].

Conclusion:

In keeping with the global increase in TB, prevalence of GTB is likely to rise in near future. Bangladesh being a 'concentrated epidemic' (in relation to HIV infection) is also at risk. GTB is an important cause of implantation failure, recurrent pregnancy loss, ectopic pregnancy and infertility. An early diagnosis is important before the tubes are damaged beyond recovery. Future fertility after GTB is poor, even after anti-tubercular therapy (ATT). But GTB presents a dilemma to clinicians. High index of suspicion for GTB aided by intensive investigations may be needed to detect early cases of GTB. PCR seems to be a rapid and more sensitive test than other conventional methods IGRAs are new developments and have excellent specificity to distinguish latent TB from prior vaccination. Involvement of a respiratory physician may improve diagnostic accuracy and offer best available treatment particularly in MDR-TB cases.

References :

1. Rasolofo RV, Menard D, Auregan G, Gicquel B, Chanteau S : Extrapulmonary and pulmonary tuberculosis in Antananarivo (Madagascar): high clustering rate in female patients. *J Clin Microbial* 2002, 40:3964-3969.
2. World Health Organization(WHO) report2011/ Global Tuberculosis Control www.who.int/tb
3. Thangappah R.B.P, Paramasivan C.N, Narayanan S. *Indian J Med Res* 2011; 134 : 40-46

4. Raviglione MC, O'Brien RJ. Tuberculosis, In : Fauci AS, Braunwal E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL *et al* (eds). Harrison's Principles of Internal Medicine, 19th ed. New York : Mc-Graw-Hill; 2008; 1006-36

5. Gatong DK, Gitau G, Kay V, Nigwenya S, Latong C, Hassan A. Female genital tuberculosis. *The Obstetrician and Gynaecologist.* 2005; 7 : 75-79

6. Jahroni BN, Parsanezhad MF, Ghane-Shirazi R. Female genital tuberculosis and infertility. *Int J Gynaecol Obstet.* 2001; 75 (3) : 269-274.

7. Kulshestha V, Kriplani A, Agarwal N, Singh UB, Rana T. Genital tuberculosis among infertile women and fertility outcome after anti-tubercular therapy. *Int J, Gynaecol & Obstet.* 2011; 113 : 229-234.

8. Kumar P, Malhotra N (eds). Genital tuberculosis. In : Jeffcoate's Principles of Gynaecology 7th ed. New Delhi, JAYPEE, 2008, 361-364.

9. Padubidri VG, Daftary SN (eds). Tuberculosis of the genital tract. In : Hawkins & Bourne Shaw's Textbook of Gynaecology 14th ed. India ELSEVIER, 2008, 136-144.

10. Gon Chowdhury R, Paine SK, Bhatacharjee B & Chatterjee S. Infestation of the endometrium by Mycobacterium Tuberculosis Bacilli-cause of reproductive failure. *Al Ameen J Med Sci* 2010; 3 (4) : 322-331.

11. Morgagni JB: De Sedibus et Causis meorhorum. *E pistola* 2003; 1744; 38:34.

12. Harries R, Maher D, Graham S, TB/HIV : A Clinical manual. 2nd ed. World Health Organization (WHO/HTM/TB/2004. 329)

13. Asha B, Hansali N, Manila K, Priti S, Dhawal B. Genital tuberculosis in Infertile Women : Assessment of Endometrial TB PCR results with Laparoscopic and Hysteroscopic features *JOGI,* 2011; May June : 301-306.

14. Qureshi RN, Sammad S, Hamd R, Lakha SF, Female genital tuberculosis revisited. *J Pak Med. Assoc.* 2001; 51 : 16-18.

15. Malik S. Genital tuberculosis and implantation in assisted reproduction. *Rev. in Gynaecological Practice.* 2003; 3 : 160-164.

16. Punnonen R, Kiilholma P, Meurman L. Female genital tuberculosis and consequent fertility. *Int J Fertil* 1983; 28 : 253-8.

17. Schaefer G. Female genital tuberculosis. *Clin Obstet Gynecol* 1976; 19 : 223-239.

18. Abebe M, Lakew M, Didane D, Lakew Z, Kiros K, Harboe M. Female genital tuberculosis in Ethiopia. *Int J Gynaecol Obstet* 2004; 84 : 241-6.

19. Oosthuizen AP, Wessels PH, Hefer JN. Tuberculosis of the female genital tract in patients attending an infertility clinic. *S Afr Med J* 1990; 77:562-4.

20. Figueroa-Damian R, Martinez-Velazoo I, Villagrana-Zesati *et al*. Tuberculosis of the female reproductive tract : effect on function. *Int J Fertil Menopausal Stud*. 1996; 41 (4) : 430-6.

21. Steinbrook Robert. Tuberculosis and HIV in India. *New Eng. J Med*. 2007; 356 (12) 1198-1199.

22. Sin SY, Tang LCH. Female genital tuberculosis : An update. *Hong Kong Practitioner* 1995; 17 (1) : 19-24.

23. http//www.sear.who.int/en/section 1081/section1090. htm

24. http//southasia.oneworld.net/todaysheadlines/tb-prevalence-down-in-Bangladesh

25. Sungkhobal D. (Forward) In : National Guidelines on TB/HIV Program collaboration, 1st ed. Dhaka, 2009.

26. Malkani PK, Royni CK, Pelvic tuberculosis. *Obstet Gynaecol*. 1959; 14 : 600-11.

27. Ben Youssef LB, Chelli H, Belhadj A. Current anatomo-clinical aspects of genital tuberculosis in women. Apropos of 49 cases. J *Gynecol Obstet Biol Reprod (Paris)* 1985; 14 : 59-65.

28. Varma TR. Genital tuberculosis and subsequent fertility. *Int J Gynecol Obstet* 1991; 35 : 1-11.

29. de Vynck WE, Kruger TF, *et al*; Genital tub. Association with Female Infertility in West Cape. *S Afr Med J* 1990; 77 (12) : 630-1.

30. Sharma JB. Roy KK, Kumar S, Malhotra N, Chawla P. Progress in Obstetrics & Gynaecology; edited by John S. Scang LT. Frank AC; 2008.

31. Deshmukh KK, Lopez JA. Naidu TAK, Gaurkhede MD. MV Place of laparoscopy in pelvic tuberculosis in infertile women; *Arch Gynecol* 1985; 237 : 197-200

32. Chhabra S. Genital tuberculosis – a baffling disease : J Obstet Gynaecol India 1990; 40 : 569-73.

33. Haas DW. Mycobacterial disease. In : Mandell GL, Bennet JE, Dolin R (eds). Principles and Practice of Infectious Diseases. Philadelphia. Churchill Livingstone, 2000; 2576-2607.

34. Tripathy SN, Tripathy SN. Infertility and pregnancy outcome in female genital tuberculosis. *Int J Gynaecol Obstet* 2001; 76 (2): 159-63,

35. Bapna N. Swarankar M, Kotia N. Genital tuberculosis and its consequences on subsequent fertility. *J Obstet Gynaecol India* 2005; 55 (6) : 534-537.

36. Frydman R., Eibschitz I, Belaisch-Allart JC, Hazout a, Hamou JE. In vitro fertilization in tuberculous infertily. *J in Vitro Fert Embryo transf.* 1985; 2 (4) : 184-9..

37. Umoh A.V., Gabriel M.A. genital tuberculosis with secondary infertility – a case report of successful treatment and subsequent live birth in Uyo, Nigeria. *J Med Med Sci.* 2011; 2 (5) : 839-842.

38. Singhal S.R. Chowdhury P. Nanda S. Genital tuberculosis with predominant involvement of cervix : A case report. *Clin Rev Opinions*, 2011, 3 (5) : 55-56.

39. Varma I, Glob libr women's med, (ISSN : 1756-2228) 2008 DOI 10. 3843 GLOWM 10034, updated Oct 2008.

40. Nogales – Ortiz F. IldefonsoT., Nogales FF : The Pathology of genital tuberculosis, *Obstet Gynaecol* 1979; 53 : 422-428.

41. Saracoglu OF, et al : Pelvic tuberculosis. *Int J Gynaecol Obstet;* 1992; 37 : 115

42. Burnie JC : The age of onset of genital tuberculosis in women. *J Obstet Gynaecol.* 1956; 63 :96

43. Simon HB. Weinstein AJ, Pasternak MS et al. : Genitourinary tuberculosis : clinical features in a general hospital population. *Am J Med* 1977; 63 : 410

44. Daly JW., Monif GRG : Mycobacteria. In : Monif GRG (ed) : Infectious Diseases in Obstetrics & Gynaecology, 2nd ed. Philadelphia. Harper Row, 1982 : 301

45. Kumar S. Female genital tuberculosis. In : Sharma SK. Mohan A. (eds) tuberculosis, 1st edn. Delhi : Jaypee; 2001; 311:324

46. Sharma JB. Female genital tuberculosis revisited. *Obstet Gynaecol Today* 2007; XII : 61-63

47. Stead WW, Kerby GR, et al. The clinical spectrum of primary tuberculosis in adults : confusion with reinfection in the pathogenesis of chronic tuberculosis. *Ann Intern Med* 1968; 68 : 731-45.

48. Raghupathy R. Makhseed M. Azizieh. Th1 and Th2 cytokine profiles in successful pregnancy and unexplained recurrent abortions In : Gupta Sk. (ed). *Reprod Immunol*. 1994; 14 : 149-57.

49. Falk V, Ludviksson K, Agren G : Genital tuberculosis in women : Analysis of 187 newly diagnosed cases from47 Swedish hospitals during the ten-year period 1968 to 1977. *Am J. Obstet Gynaecol* 1980; 138 : 933.

50. Akhter R, Mohiuddin MG, Afroz H. Atypical presentation of vulval tuberculosis, *Bangladesh Med. J.* 2009; 38 (2) : 65-67

51. Agarwal S. Madon M, Leekha N, Raghunondan C. A rare case of cervical tuberculosis simulating carcinoma of cervix. A case report. Cases J. 2009; 2 : 161.

52. Bakari AG, Onyemelukwe GC, Akolawole MA, Muazu SB, Randawa A, Genital Tuberculosis presenting as pyrexia of undetermined origin (letter) . *African Health Sciences.* 2007; 7 (1):37

53. Goldin AG, Baker WT. Tuberculosis of the female genital tract. *J Ky Med Assoc* 1985; 83 : 75-6.

54. Schaefer G. Tuberculosis of the female genital tract. *Clin Obstet Gynaecol* 1970; 13 : 965-98.

55. Katoch VM. Newer diagnostic techniques for tuberculosis. *Indian J Med Res* 2004; 120 (4) : 418-28

56. Bates JH. Diagnosis of tuberculosis, *Chest*. 1979; 76 (6 suppl) : 757-68.

57. Baum SE, Dooley DP, Wright J, Kost ER, Storey DF, Diagnosis of culture negative female genital tract tuberculosis with peritoneal involvement by polymerase chain reaction. *J Reprod Med*. 2001; 46 (10) : 929-32

58. Bhanu NV, Singh UB, Chakraborty M, Suresh N, Arora J, Rana T, et al. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading of infertility.*J Med Microbiol* 2005; 54 : 927-31

59. Steingart K, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis : A systemic reviews. *Lancet Infect Dis*. 2006; 6 (9) : 570-81.

60. Islam MR. Qayyum/MA, Rahim M. Huq S.R., Jessy MKH, Mahmud AM. Role of Fluorescence Microscopy for diagnosis of Pulmonary Tuberculosis. *Chest & Heart Journal*. 2010, 34 (2) : 76-82.

61. Schoeman JH, Westaway MS, Neethling A. The relationship between socio-economic factors and pulmonary tuberculosis. *International J Epidemiology* 1991; 20 (2) : 435-440

62. Ketch VM. Newer diagnostic techniques for TB. *Indian J Med Res* 2004; 120 : 418-28.

63. Merchant R. Endoscopy in the diagnosis of genital tuberculosis. *J Reprod Med* 1989; 34 (7) : 468-74.

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REVIEW ARTICLE

Incorporating Omalizumab into Asthma Management: A Review

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Abstract

Effective asthma management is hindered by several factors, such as (1) variability in clinical diagnosis and treatment patterns, (2) low adherence to therapy caused by improper use of medications, (3) poor technique in using therapeutic devices, (4) medication side effects, (5) inadequate responses to therapy, and (6) patients' acceptance of a poor quality of life. These barriers increase hospitalizations and emergency department visits, reduce productivity, and cause persistent morbidity and mortality. New therapies target mechanisms that cause airway disease, reduce utilization of health care resources, and improve patient adherence. Omalizumab, an immunoglobulin E (IgE) blocker, reduces the clinical burden of asthma and the associated use of health care resources. This report advises health care professionals on how to incorporate IgE blocker therapy into current treatment guidelines.

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Introduction:

Asthma remains a daunting challenge for health care professionals. Management of this chronic condition is complicated by the dynamic nature of the disease, the presence of comorbid conditions, widespread variation in the implementation of treatment guidelines, the improper use of medications, the incorrect use of asthma devices, adverse drug events, poor patient compliance, and acceptance by patients of a substandard quality of life. In response to these challenges, treatment guidelines have been published and new therapies have been developed to improve clinical outcomes and to reduce the use of health care resources. Despite these advances, however, the prevalence, morbidity, and mortality associated with asthma remain high.[1] Successful management of asthmatic patients requires therapies that treat the underlying causes of the disease, reduce hospitalization and patients' use of the emergency department, maximize patient adherence to therapy, and improve quality of life. Without these, optimal asthma management will remain elusive.[2]

Pathophysiology of Asthma: Current Knowledge

Asthma is a disease that causes debilitating daily symptoms and unexpected acute exacerbations of symptoms. Symptoms lead patients to limited activity, absences from work or school, hospitalizations and visits to the emergency

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department, and a reduced quality of life. As a result, patients and their family members have both personal and economic hardship. From the perspective of public and societal health, approximately 17 million people in the United States have been estimated to have asthma, one third of them children. Asthma in the United States costs an estimated $12.7 billion annually, with most of this cost attributable to direct medical expenditures and medication. [3]

IgE plays a central role in the pathophysiology of asthma. The two essential phases in this pathophysiology are sensitization to allergen and clinical expression of symptoms on re-exposure to the sensitizing allergen. During sensitization, inhaled antigen (i.e., aeroallergen) is taken up by antigen-presenting dendritic cells lining the airways. The allergen is then processed and presented to antigen-specific T cells. In some persons, these T cells respond by producing cytokines that stimulate the development of IgE-producing B cells. The Fc portion of circulating IgE then binds to high-affinity receptors (FcåRIs) present on the surfaces of mast cells and basophils.[4,5,6] On re-exposure, the sensitizing allergen cross-links IgE molecules present on mast-cell and basophil surfaces. This initiates degranulation and the release of inflammatory mediators, including histamine, prostaglandins, leukotrienes, chemokines, and cytokines. These mediators precipitate an immediate acute-phase reaction, resulting in acute bronchospasm, expressed clinically as an episode of acute asthma. Continued expression of mediators enlists an inflammatory response designated the late-phase reaction, which causes persistent symptoms, airway hyper-responsiveness, and bronchospasm.

IgE may also facilitate sensitization to allergens. Dendritic cells express FcåRIs and have been found to bind IgE, which is thought to focus antigen at the cell surface. In addition IgE binds to low affinity FCe receptors on B cells, which it alters differentiation and regulation of further IgE synthesis.[3,4]

Unmet Needs: Barriers to Improved Outcomes

Patients with the greatest unmet needs are those with moderate-to-severe asthma that is sub-optimally controlled. These patients remain symptomatic despite the use of multiple medications. The hallmark feature of these patients is frequent, severe exacerbations that often require costly emergency treatment, hospitalization, or both.[7,8] This observation supports the theory that current therapies are underused, are used inappropriately, or are not consistently effective.[9] The Epidemiology and Natural History of Asthma Outcomes and Treatment Regimen (TENOR) Study was initiated to describe the natural history of asthma patients, to elucidate the relationship between IgE and disease, and to examine the relationship between severity of asthma, its treatment, and clinical outcomes.[8,9]

Emerging Asthma Therapies

Omalizumab is a recombinant humanized IgG1 monoclonal anti-IgE antibody that binds to the IgE molecule at the same epitome on the Fact region that binds to FcåRI.[4,5] This design means that Omalizumab is not anaphylactogenic, since it cannot interact with IgE that is already bound to cell surfaces and thus cannot induce degranulation of mast cells or basophils.[4,5] Instead, Omalizumab binds to circulating IgE, regardless of allergen specificity, forming small, biologically inert IgE–anti-IgE complexes without activating the complement cascade.[3,4,5] An 89 to 99% reduction in free serum IgE (i.e., IgE not bound to Omalizumab) occurs soon after the administration of Omalizumab, and low levels persist throughout treatment with appropriate doses. Proof-of-concept studies have shown that Omalizumab reduces both early- and late-phase asthmatic responses after allergen inhalation challenge, has a marked effect on late-phase as compared with early-phase skin responses, decreases eosinophil numbers in sputum and submucosal bronchial specimens, and also down-regulates FcåRI on basophils, mast cells, and dendritic cells. A reduction in the expression of FcåRI on basophils and mast cells decreases the binding of circulating IgE, thus preventing the release of inflammatory mediators. A reduction in the expression of FcåRI on dendritic cells may decrease allergen processing and presentation.

Evidence of Clinical Benefit

There are four core randomized, double-blind clinical trials that have compared Omalizumab, administered subcutaneously, with placebo. In

these trials, patients had had asthma for at least one year and required treatment with inhaled corticosteroids. All patients had at least one positive skin test to a perennial aeroallergen (specifically, dust mites, cockroaches, or dog or cat dander), as well as an elevated total serum IgE level. During the course of each trial, inhaled corticosteroids were initially maintained at a stable dose, followed by a phase of dose reduction to the lowest dose required for asthma control. [6,7,8]

These trials all demonstrated a clinical benefit from Omalizumab, although the specific findings varied. Three of the trials evaluated patients with moderate-to-severe persistent asthma (requiring doses of inhaled Beclomethasone, or its equivalent, ranging from 168 to 1200 ìg per day). Two of these three trials included adolescents and adults, and one was a study of children 6 to 12 years of age. In these three trials, treatment with Omalizumab as compared with placebo was associated with significantly fewer exacerbations of asthma per patient, and a significantly lower percentage of patients had an exacerbation. In addition, the dose of inhaled corticosteroids required to control symptoms was significantly less among patients treated with Omalizumab than among those who received placebo.[6]

The fourth trial evaluated patients with more severe asthma who required high-dose inhaled corticosteroids for symptom control (Fluticasone, e"1000 ìg per day). In this trial, no significant effect on the frequency of exacerbations was seen, although the dose of inhaled corticosteroids required to control symptoms was significantly lower among patients treated with Omalizumab.[6]

A fifth clinical trial involved patients who required at least 1000 ìg per day of inhaled Beclomethasone plus a long-acting bronchodilator for symptom control. The study demonstrated a decrease in the rate of exacerbations of asthma only after adjustment for an imbalance in the number of exacerbations in the year before enrollment. Among several secondary outcomes in these trials, quality-of-life measures stand out as being notably improved. [6,7,8,9]

Considerations for IgE blocker Therapy

- Patient at least 12 years of age
- Evidence of reversible disease (such as 12% or greater improvement in FEV1 with at least a 200-ml increase or 20% or greater improvement in PEF)
- IgE level e" 30 IU/ml
- Evidence of specific allergic sensitivity (i.e., positive skin test or blood test for IgE)
- Inadequately controlled Asthma despite medium dose of inhaled corticosteroids for at least three months in combination with a trial of long-acting inhaled beta2 agonists or a Leukotriene modifier
- Systemic corticosteroids or high-dose inhaled corticosteroids required to maintain adequate control
- As directly observable therapy in patients who are not adherent to prescribed therapy

Clinical Use

The role of Omalizumab in the management of asthma has not yet been precisely defined. Patients with persistent asthma (defined as asthma with symptoms that occur more than two days a week or nocturnal symptoms that occur more than twice a month[9,10] have several treatment options in addition to the use of inhaled â-adrenergic agonists. These include environmental control (i.e., the elimination or minimization of exposure to aeroallergens), pharmacologic control (i.e., the use of inhaled corticosteroids, Leukotriene modifiers, or both), and possibly, immunologic control (i.e., immunotherapy for relevant antigens). In addition, evaluation for coexisting conditions such as allergic rhinitis, sinusitis, and gastroesophageal reflux disease may prove beneficial.

Patients who are particularly likely to benefit from the use of Omalizumab include those with evidence of sensitization to perennial aeroallergens who require high doses of inhaled corticosteroids that have a potential for adverse side effects, those with frequent exacerbations of asthma associated with unstable disease, and possibly, those with severe symptoms related in part to poor adherence to daily medication. Analyses of pooled data from published clinical trials have indicated that patients who had a response to Omalizumab had a ratio of observed to expected forced expiratory volume in one second (FEV1) of less than 65%, were taking doses of inhaled corticosteroids equivalent to more than 800 ìg of Beclomethasone dipropionate per

day, and had had at least one visit to the emergency department in the past year. Patients requiring daily oral corticosteroids to control their asthma may be less likely to have a response to Omalizumab.

A total serum IgE level should be measured in all patients who are being considered for treatment with Omalizumab, because the dose of Omalizumab is determined on the basis of the IgE level and body weight. The recommended dose is 0.016 mg per kilogram of body weight per international unit of IgE every four weeks, administered subcutaneously at either two-week or four-week intervals (Table 1). This dose is based on the estimated amount of the drug that is required to reduce circulating free IgE levels to less than 10 IU per milliliter.

Monitoring of total serum IgE levels during the course of therapy with Omalizumab is not indicated, because these levels will be elevated as a result of the presence of circulating IgE–anti-IgE complexes. No other laboratory tests seem to be necessary, since there have been no clinically significant laboratory abnormalities noted during treatment. [7,9,10]

* Adapted from the Xolair package insert. The recommended dose is 0.016 mg per kilogram of body weight per international unit of IgE every four weeks, administered subcutaneously at either four-week or two-week intervals for adults and adolescents (persons 12 years of age and older) with allergic asthma. Dashes indicate that no dose should be prescribed.[6]

Preparation for Use

Omalizumab is supplied as a lyophilized, sterile powder in single-use, 5-ml vials designed to deliver either 150 or 75 mg on reconstitution with sterile water (not normal saline) for injection. The powder requires 15 to 20 minutes or more to dissolve. There are fewer injection-site reactions when the solution is not injected until it is completely clear. The solution is viscous and must be carefully drawn up into the syringe before it is administered. The injection itself may take 5 to 10 seconds to administer. Once prepared, the drug must be used within four hours if at room temperature or eight hours if refrigerated. Because of these requirements for preparation and the high cost of the drug, some practitioners require patients to schedule appointments for injection, and many do not prepare the injection until the patient arrives. This results in visits that take 60 minutes or more, since 30 minutes of observation is recommended after the injection. In general, current asthma symptoms are not a contraindication to the administration of Omalizumab.

Total serum IgE levels will generally increase during treatment, because of the presence of circulating IgE–anti-IgE complexes. An investigative method for measuring free serum IgE levels has recently been reported and may provide an opportunity for monitoring optimal Omalizumab dosing. In addition, recent in vitro studies of the effect of Omalizumab on the accuracy and reproducibility of assays of total and allergen-specific IgE antibodies suggest that the use of a specified Commercial assay may help optimize

Table-I

Dosing Schedule for omalizumab, According to the Baseline Serum IgE Level and Body weight,

Baseline Serum IgE Level IU/ml	Body weight 30-60 kg	61-70 kg	71-80 kg	81-90 kg	91-150 kg
	dose in milligrams				
30-100	150	150	150	150	300
101-200	300	300	300	300	225
201-300	300	225	225	225	300
301-400	225	225	300	300	-
401-500	300	300	375	375	-
501-600	300	375	-	-	-
601-700	375	-	-	-	-

dosing and maximize Omalizumab therapy. There is, at present, no reported clinical experience with such approaches. [9,10,11]

Cost

Omalizumab is considerably more expensive than conventional asthma therapy. The cost of treatment may range from $4,000 to $20,000 per year, depending on the dose, with an average of approximately $12,000 per year. This compares with approximate costs per year of $1,280 for Montelukast, $2,160 for the combination of Fluticasone dipropionate and Salmeterol, and $680 for extended release Theophylline. [12]

Response to treatment

Response to treatment can take several weeks to become apparent.[11] Among patients in a clinical trial who had had a response to Omalizumab by 16 weeks, 87% had done so by 12 weeks. These data suggest that patients should be treated for at least 12 weeks before efficacy is assessed. Given that serum IgE levels and the numbers of FcåRIs increase after therapy is discontinued[12], it seems that treatment needs to be continued for efficacy to persist, but no studies have been reported on the duration of effects after discontinuation. If treatment is interrupted before nine months have elapsed since the last injection, treatment should be resumed at the dose initially prescribed. [11,12] Dosing may need to be adjusted in the event of substantial changes in body weight (Table 1).

Adverse Effects

Potential safety concerns identified by the Food and Drug Administration (FDA) in reviewing trial data on Omalizumab included risks of the development of cancer and anaphylaxis. Cancer developed in more patients exposed to Omalizumab than in those who received placebo (20 of 4127 [0.5%] and 5 of 2236 [0.2%], respectively). They were predominantly epithelial or solid-organ cancers; one case of haematologic or lymphatic cancer was noted. Since the majority of patients treated with Omalizumab have been observed for only a year, the effect of longer exposure or of use in patients who are at increased risk for cancer is not known. Therefore, Omalizumab probably should not be used in patients with a history of cancer or a strong family history of cancer until this risk relationship is better understood. [11,12,13]

Omalizumab is intended to prevent any risk of anaphylaxis, since the agent cannot interact with IgE that is already bound to cell surfaces. However, in clinical trials, three patients (<0.01%) had anaphylaxis. Two of the reactions were temporally associated with Omalizumab administration; the reactions were not immediate but occurred within two hours after the first injection.

Other adverse events seen more often with Omalizumab than with placebo in clinical trials have included rash, diarrhea, nausea, vomiting, epistaxis, menorrhagia, haematoma, and injection site reactions. The most common adverse events were viral infections, upper respiratory tract infections, sinusitis, and headaches, but these were not more common with Omalizumab than with placebo and therefore are unlikely to be side effects of the drug. An analysis of the safety data among children after the use of Omalizumab for 1 year, which included a 28-week core study and a 24-week open-label extension, showed upper respiratory tract infections and headache to be more frequent in the Omalizumab group than in the placebo group. Eleven patients (4.9%) in the Omalizumab group had urticaria; only one case was severe enough that the patient discontinued participation in the study.

Theoretically, the administration of Omalizumab could induce antibodies to the murine components of the drug. However, no immune complex–mediated pathologic conditions that might develop as a result of the formation of such antibodies have been observed. Also, the potential for Omalizumab to interfere with the role of IgE in the clearance of parasitic infections is a possible concern. Although no clinical problems related to such an effect have been seen, this might be a potential concern in specific populations. Further information is needed on the safety profile of Omalizumab after long-term use. [10,11,13]

Areas of Uncertainty

The clinical trials of Omalizumab enrolled patients with precisely defined characteristics of asthma, including sensitivity to specific perennial aeroallergens (i.e., dust mites, cockroaches, and dog or cat dander). The role of Omalizumab in patients with asthma who have allergies to other aeroallergens, such as molds or pollens, or who

have negative allergy skin tests, has not been defined. It is also not clear to what extent Omalizumab might be effective in patients with total serum IgE levels outside the trial ranges (30 to 700 IU per milliliter for patients 12 to 75 years of age).

In clinical practice, there is considerable variability of response to Omalizumab therapy. The reasons for this variability have not been established; studies are needed to determine whether specific characteristics of individual patients may help to predict response. [11,13]

The clinical trials performed to date have evaluated Omalizumab only as adjunctive therapy with inhaled corticosteroids as compared with placebo. They have not evaluated the relative benefit of this agent in comparison with other available therapies, such as Leukotriene modifiers or Theophylline. Also needed are comparisons with asthma therapies that are available for patients for whom low-dose inhaled corticosteroids do not control the asthma and who need step-up management (i.e., an increased dose of the corticosteroid or the addition of another medication). Furthermore, the clinician should give consideration to the actual clinical relevance of the moderate corticosteroid-sparing effects observed in the trials, even if these reductions were significant, as well as to the substantial improvements noted in placebo groups. Given that the cost of Omalizumab is substantially greater than that of conventional asthma therapy, the potential cost effectiveness of this form of treatment will be important to assess. [11,13,14]

The efficacy and safety of Omalizumab have not been established for durations of treatment that exceed one year, and it is not known how long clinical effects may persist after therapy is discontinued. Since asthma is a chronic disease, long-term studies, especially in children, are needed to evaluate the effect of serum IgE suppression throughout development; adverse effects may become apparent only with follow-up into adulthood. We know of only one study to date that has been performed exclusively in the pediatric age group. Efficacy and safety studies are also needed for geriatric and nonwhite patients.

Summary:

Asthma continues to impose a significant clinical and economic burden on society. Patients with sub-optimally controlled, moderate-to-severe asthma pose a considerable challenge to physicians and are among the most frequent users of health care resources. Current treatment strategies recommend the use of multiple medications to control symptoms and to preserve surrogate markers of clinical efficacy, such as FEV1. The complexity of these regimens contributes to patient non-adherence.

Successful asthma management is also hindered because of the cost of asthma care and limited access to health care resources. Novel treatments, such as IgE blockers, have demonstrated a direct impact on disease indicators by minimizing exacerbations, reducing hospitalization and emergency department visits, and improving quality of life in patients with moderate-to-severe, sub-optimally controlled asthma. Proposed treatment guidelines encourage the use of IgE blockers in these patients. The use of such therapies has proved beneficial in reducing the clinical and economic burden of asthma and therefore has important implications for patients, health care providers, and third-party payers.

As additional clinical experience is gained, the ultimate role of IgE blockers will be further defined. Issues such as cost, long-term acceptability, and safety remain to be addressed.

References:

1. Gold DR, Wright R. Population disparities in asthma. Ann Rev Public Health 2005; 26: 89-113
2. Rosenwasser LJ. Incorporating Omalizumab into Asthma Treatment Guidelines: Consensus Panel Recommendations. Pharmacy and Therapeutics 2003; 28(6): 400-10
3. Castro M, Schechtman KB, Halstead J, Bloomberg G. Risk factors for asthma morbidity and mortality in a large metropolitan city. J Asthma 2001; 38: 625-35
4. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class switch recombination. Nat Rev Immunol 2003; 3: 721-32

5. Infuhr D, Crameri R, Lamers R, Achatz G. Molecular and cellular targets of anti-IgE antibodies. Allergy 2005; 60: 977-85

6. Strunk RC, Bloomberg GR. Omalizumab for Asthma. N Engl J Med 2006; 354: 2689-95

7. Owen CE. Anti-immunoglobulin E therapy for asthma. Pulm Pharmacol Ther 2002; 15: 417-24

8. Schulman ES. Development of a monoclonal anti-immunoglobulin E antibody (Omalizumab) for the treatment of allergic respiratory disorders. Am J Respir Crit Care Med 2001;164: S6-S11

9. Soler M, Matz J, Townley R, et al. The anti-IgE antibody Omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. Eur Respir J 2001;18: 254-61. [Erratum, Eur Respir J 2001;18:739-40]

10. Holgate ST, Chuchalin AG, Hebert J, et al. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (Omalizumab) in severe allergic asthma. Clin Exp Allergy 2004;34:632-8

11. Barnes PJ, Woolcock AJ. Difficult asthma. Eur Respir J 1998;12: 1209-18

12. Hoskins G, McCowan C, Neville RG, et al. Risk factors and costs associated with an asthma attack. Thorax 2000; 55: 19–24

13. TENOR Investigators. Data on file: The epidemiology and natural history of asthma: Outcomes and treatment regimens. South San Francisco, CA: Genentech, Inc.

14. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody for the treatment of severe allergic asthma. J Allergy Clin Immunol 2001; 108: 184–90

15. Weiss KB, Sullivan SD. The health economics of asthma and rhinitis. I. Assessing the economic impact. J Allergy Clin Immunol 2001; 107: 3-8

16. Xolair (Omalizumab). San Francisco; Genetech, 2003 (package insert)

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REVIEW ARTICLE

Massive Hemoptysis - An Overview

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Abstract:

Massive hemoptysis has been variably defined as 100 to more than 1000 ml of blood expectorated from lungs over 24 to 48 hours. Most hemoptyses are not massive but true hemoptysis is a medical or surgical emergency.

The etiologies of massive hemoptysis are tuberculosis (85.5%), bronchiectasis, carcinoma (2.3%), infections, CHF, Pericarditis and many others. Patients die from asphyxiation or exsanguinations. In pulmonary tuberculosis hemorrhage is due to aneurysmal dilatations of pulmonary arteries leading to a rupture of a vessel in the wall of a tuberculous cavity. Friction of fungal ball against the hypervescularized walls of the cavity leads to hemorrhage in intracavitary fungal lesion. In bronchiectasis, there is proliferation and enlargement of bronchial arteries and precapillary bronchopulmonary anastomosis which become eroded that leads to bleeding.

To localize the site of bleeding, some investigations are necessary like radiography, bronchoscopy, C.T scan of chest, Bronchial arteriography, Pulmonary angiography.

Aim of management of massive hemoptysis is to prevent asphyxiation, to localize the site of bleeding, and to arrest the hemorrhage. It includes medical, some invasive procedures as well as surgical approach. Medical treatment implies rest in bed with Trendelenburg position with affected site down, wide i.v. line, arterial blood gas monitoring, sedatives, 0_2, antibiotic, blood transfusion, reversal of anticoagulation, corticosteroids in immunologic cases, and anti-TB in tuberculosis. Endobronchial control measures with ice cold saline lavage, balloon tamponade, vasoconstrictive agents, selective coagulative treatment with laser, topical thrombin, arterial embolisation, i.v. angiotensin, radiotherapy and intracavitary treatment. If all the measures fail, then surgical intervention is to be considered.

Endobronchial control measures and arterial embolization after medical therapy have radically changed the management of patients with massive hemoptysis. Surgical candidates should be assessed accurately, thus allowing an elective, less morbid operation.

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Introduction:

Hemoptysis is defined as the spitting of blood derived from the lungs or bronchial tubes as a result of pulmonary or bronchial hemorrhage.[1] Hemoptysis is classified as nonmassive or massive based on the volume of blood loss; however, there are no uniform definitions for these categories. Massive hemoptysis has been variably defined as

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100 to more than 1000 ml of blood expectorated from lung over 24 to 48 hours.[2] Expectorated blood is often swallowed and can not be measured. Many patients with hemoptysis have compromised lung function and even a small quantity of blood in bronchial tree can lead to acute airway obstruction and asphyxiation.

The coughing up of blood prompts most people to seek medical attention. Rapid bleeding leaves no opportunity for meaningful intervention.

Etiology:

The etiologies of massive hemoptysis are *Bronchitis, Bronchiectasis, Aspergilloma, Tumor, Tuberculosis, Lung abscess, Emboli, Coagulopathy, Autoimmune disorders, AVM, Alveolar hemorrhage, Mitral stenosis, Pneumonia.* (BATTLE CAMP).[3]

Male: female ratio is 76.6:23.4. For most etiologies, massive hemoptysis involves disruption of high pressure bronchial circulation or pulmonary circulation. The bronchial circulation plays an important role in hemoptysis because of its intimate association with the tracheobronchial tree.

In many cases, bronchial arterial tree becomes hyperplasic and tortuous. Because the arterioles associated with airways are under systemic pressure, they have a propensity to bleed profusely when airways are diseased.[4]

Vascular Anatomy

The pulmonary circulation carries deoxygenated blood from the right ventricle across the pulmonary capillary bed and returns oxygenated blood via the pulmonary veins. This is a low pressure circuit with normal pressures of 15-20/5-10 mmHg.[5]

?*The bronchial circulation* is a nutritional source for the structural elements of the lung. Bronchial arteries branch from the aorta and are at systemic pressure. They can bleed profusely when airways are diseased.

Mechanism of hemorrhage:

Abnormalities of lung vasculature is observed in pulmonary tuberculosis is due to *aneurysmal dilatations of pulmonary arteries called Rasmussen aneurysms* that leads to a rupture of a vessel in the wall of a tuberculous cavity.[6]

An *intracavitary fungal ball* is one of the most important causes of systemic hypervacularization. Friction of fungal ball against the hypervescularized walls of the cavity, toxins or fibrinolytic enzymes elaborated by fungus and antigen-antibody reactions in the cavity wall leads to hemorrhage.[10]

In *bronchiectasis,* there is proliferation and enlargement of bronchial arteries and precapillary bronchopulmonary anastomosis which become eroded that leads to bleeing.[7]

Patients with *chronic necrotizing pneumonitis* can bleed massively; alcoholism is often a predisposing factor.

In *lung abscess*, bacterial infection destroys the lung tissue by the process of suppuration and necrosis. When necrosis involves vascular granulation tissue, the capillaries bleeds into the cavity of the abscess.[5]

Lung cancer usually causes massive bleeding by direct invasion of central pulmonary arteries.[8] There is also proliferation of bronchial arteries in primary pulmonary neoplasm.

Diagnosis:

History and physical examination

Although the history and physical examination findings rarely are pathognomonic of hemoptysis, they can confirm hemoptysis and provide valuable clues to the cause. The initial objectives are to differentiate hemoptysis from epistaxis and hematemesis and then to establish the severity.[9]

The appearance of specks of blood a few days after the acute onset of a productive cough, congestion, sore throat, and low-grade fever suggests bronchitis. Malignancy is a primary concern in patients who present with hemoptysis, especially those with risk factors such as age over 50 years, male sex, and a smoking history of more than 40 pack-years.15 Weight loss, fever, and night sweats suggest infection, such as pulmonary tuberculosis or lung abscess.[9]

Patients with hemoptysis caused by pulmonary embolism are frequently dyspneic. In these patients, hemoptysis is generally a late phenomenon; the hemoptysis represents pulmonary infarction, which usually means there is a large blood clot. Hemoptysis also is a late presentation of both pulmonary arterial hypertension and mitral stenosis. In the latter, dyspnea usually precedes the hemoptysis.

A history of rheumatic fever and progressive exertional dyspnea in a patient with sudden hemoptysis or the pink, frothy sputum associated with pulmonary edema points to mitral stenosis. Concomitant hematuria suggests a vasculitis or immunologically mediated disease, such as Wagener's granulomatosis, SLE, or Goodpasture syndrome. A cyclical pattern associated with menses can occur with a rare condition, thoracic endometriosis.

The physical examination findings occasionally suggest the diagnosis, but more reliably indicate the level of the patient's distress, since blood in the alveoli often leads to hypoxemia and respiratory embarrassment. With approximately 400 mL of blood in the alveoli, significant hypoxemia, asphyxiation, and death can occur. Rarely, shock due to exsanguinations causes death.[9]

Nasal ulcerations suggest Wagener's granulomatosis, and oral mucosal and cutaneous telangiectases suggest hereditary hemorrhagic telangiectasia and arteriovenous malformation. An opening snap and a diastolic murmur may suggest mitral stenosis, whereas an accentuated second heart sound in the pulmonic valve area suggests pulmonary hypertension.

Findings on chest auscultation are variable. Localized wheeze caused by bronchial obstruction can occur with an endobronchial lesion, such as lung cancer. In patients with diffuse alveolar hemorrhage or massive hemoptysis, there may be rhonchi and crackles or fairly normal auscultatory findings. Clubbing can occur with lung cancer, bronchiectasis, or lung abscess.[9]

Radiology

To localize the site of bleeding, some investigations are necessary like *radiography, bronchoscopy, C.T scan of chest, Bronchial arteriography, Pulmonary angiography*. Some clinicians claim that in massive hemoptysis only the rigid bronchoscope can provide adequate cleaning of blood and maintain a satisfactory airway. It should be carried out by an endoscopist skilled in the use of both types of bronchoscope[10].

Laboratory

Certain laboratory studies assist in the diagnosis and management of hemoptysis. These include measurement of hemoglobin and hematocrit to assess blood loss and platelet count, international normalized ratio, activated partial thromboplastin time, and creatinine level to assess coagulation status. These tests should be done in patients with persistent mild or minimal, moderate, or massive hemoptysis. However, if bronchitis is the working diagnosis, the laboratory tests may be delayed.

Management:

Aim of management of massive hemoptysis is to *prevent asphyxiation, to localize the site of bleeding, to arrest the hemorrhage,* to determine the cause of hemoptysis and to treat the patient definitely.[11] A patient with massive hemoptysis requires treatment in the ICU

Treatment of patients with massive hemoptysis includes *medical, some invasive procedures as well as surgical approach*. Medical treatment implies rest in bed with Trendelenburg position with affected site down, wide I.V. line, arterial blood gas monitoring, sedatives, 0_2, antibiotic, Blood transfusion, reversal of anticoagulation, corticosteroids in immunologic cases, and anti-TB. in selected cases, Endobronchial control measures with ice cold saline lavage, balloon tamponade, vasoconstrictive agents, selective coagulative treatment with laser, topical thrombin, arterial embolisation, i.v. angiotensin, radiotherapy and intracavitary treatment. If all the measures fail, then surgical intervention is to be considered.

Endobronchial control measures: - The introduction and spread of endobronchial control measures revolutionized the management of massive hemoptysis. The systemic lavage of the bleeding lung with large volumes of ice-cold saline solution can induce slowing and ultimate cessation of bleeding by hypothermic vasospasm of the bronchial arterial branches that supply it.[3] Under sedation and use of topical anesthesia rigid bronchoscope is rapidly inserted with 100% 0_2 pumped thought it. All blood and clots are suctioned from the trachea and major bronchi, the bleeding side is identified and non-bleeding main bronchus is snugly cannulated, ventilation is begun. After the patient is clinically stable the bleeding bronchus is cannulated and 50 ml. aliquots of iced- saline solution are injected into the endobronchial tree for 15 seconds and are then rapidly suctioned out and the process is repeated if necessary. After

cessation of bleeding, bronchocope is withdrawn.

Balloon Tamponade: - Massive hemoptysis can be controlled by placement of Fogarty- type embolectomy catheters and subsequent balloon inflation in the bleeding bronchus using bronchoscope.

Selective coagulative treatment: - Topical thrombin and fibrinogen- thrombin solutions have been used with reported success in the treatment of patients with massive hemoptysis. The fibrin precursors sprayed into the bronchus selectively to the site of bleeding forms a fibrin clot which plugged the bronchus and hemoptysis ceased promptly.[2]

Arterial Embolization: - Bronchial artery embolization is a definitive treatment in patient with massive hemoptysis when a bleeding site is identified by arteriograply. It is an attractive alternative to surgery in patient with bilateral diseases, multiple bleeding sites or in patients with borderline pulmonary reserve.[2]

Pulmonary resection has been shown to be the most effective method for the control and prevention of recurrent bleeding in most patients.[8,9] The criteria for selecting surgical cases include to localize the site of bleeding, adequate pulmonary function, no medical contraindication, resectable carcinomas without distant metastasis, no mitral diseases[7]. In elective cases we select patients for pulmonary resection based on the forced expiratory volume in 1 second (FEV_1) Patient who has a minimum FEV_1 of 2 or 1.7 L considered fit for pneumonectory or lobectomy respectively. Bilateral parenchymal diseases, unresectable carcinomas or the inability to localize the bleeding site also prohibit surgical resection. [12]

With the introduction of ice-cold saline lavage and arterial embolization it is possible to control majority of cases of massive hemoptysis. Urgent surgery (i.e., within 24 to 48 hours after initial control) is required only in cases of fungal ball, lung abscess, failure of any control method, presence of cavity, obstruction of the main or lobar bronchus with a clot that can not be suctioned during a rigid bronchoscope.[13]

Surgical procedures required are classified into 4 groups-pulmonary resections (pneumonectomy, lobectomy, wedge resections, segmentectomy), collapse therapy (thoracoplasty), cavernostomies, & intrathoracic vascular ligatures. Bronchoscopy either flexible or rigid should be performed at the end of the surgical procedure.[14]

Message:

1. Most hemoptysis is not massive, but true massive hemoptysis is a medical emergency. Patients die from asphyxiation or exsanguination.
2. The top 3 causes of massive hemoptysis are TB, bronchiectasis, and carcinoma.
3. Remember the 3 principles of management: 1) maintain airway patency and oxygenation, 2) localize the source of bleeding, 3) control hemorrhage

Conclusion:

Endobronchial control measures and arterial embolization after medical therapy have radically changed the management of patients with massive hemoptysis.[15] With the control of hemorrhage, the clinician is able to identify non-surgical patients and assess surgical candidates accurately, thus allowing an elective, less morbid operation.

References:

1. Stedman TL. Stedman's Medical dictionary. 27th ed. Philadelphia: Lippincott Williams & Wilkins, 2000
2. Thompson AB, Teacher H, Rennard SI: Pathogenesis, Evaluation and Therapy for Massive Hemoptysis. Cline Chest Med 1992;13:69.
3. *medicine.ucsf.edu/education/resed/Chiefs_cover_sheets/hemoptysis1.pdf* de Gracia J, de la Rosa D, Catalán E, et al. Use of endoscopic fibrinogen-thrombin in the treatment of severe hemoptysis. Respir Med 2003; 97:790
4. Chun HJ, Byun JY, Yoo SS, Choi BG. Added benefit of thoracic aortography after Tran arterial embolization in patients with hemoptysis. AJR Am J Roentgenol 2003; 180:1577.
5. Remy-Jardin M, Bouaziz N, Dumont P, et al. Bronchial and nonbronchial systemic arteries at multi-detector row CT angiography:

comparison with conventional angiography. Radiology 2004; 233:741.

6. Menchini L, Remy-Jardin M, Faivre JB, et al. Cryptogenic haemoptysis in smokers: angiography and results of embolisation in 35 patients. Eur Respir J 2009; 34:1031.

7. Jougon J., Ballester M., Delcambre F., Mac Bride T., Valat P., Gomez F., and Laurent F., Velly J.F. Massive hemoptysis: what place for medical and surgical treatment. Eur J Cardiothoracic Surg 2002; 22:345-351

8. WU M, Zhang L-W, Zhu H, Qian Z-X. Surgical treatment for thoracic hydatidosis: review of 1230 cases. Chinese Medical J 2005;118:1665-1667.

9. JACOB L. BIDWELL, M.D. and ROBERT W. PACHNER, M.D., University of Wisconsin Medical School, Milwaukee, Wisconsin *Am Fam Physician.* 2005 Oct 1; 72(7):1253-1260

10. Toker A, Tanju S, Bayrak Y, et al. Life threatening hemoptysis in a child; the only symptom. Ann Thorac Surg 2004;77:336-8

12. Humphrey LL, Teutsch S, Johnson M, U.S. Preventive Services Task Force. Lung cancer screening with sputum cytologic examination, chest radiography, and computed tomography. *Ann Intern Med*. 2004; 140:740–53.

13. JACOB L. BIDWELL, M.D. and ROBERT W. PACHNER, M.D., University of Wisconsin Medical School, Milwaukee, Wisconsin. *Am Fam Physician.* 2005 Oct 1; 72(7):1253-1260

14. Bharti S, Bharti B: Hydatid disease of the lung – unusual cause of hemoptysis. *Indian Pediatr* 2002, 39:1062-1063.

15. Ibrahim WH. Massive haemoptysis: the definition should be revised. Eur Respir J 2008; 32:1131.

Chest & Heart Journal
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CASE REPORT

Subclavian Artery Pseudoaneurysm in a Nonagenarian Man after Closed Fracture of the Clavicle- A Case Report

Md. Saif Ullah Khan[1], Md. Naimul Hoque[2], Md. Mahbubur Rahman[3]

Abstract:

A ninety-year-old man was admitted with a subclavian artery pseudoaneurysm (PSA) after a closed fracture of the middle third of the right clavicle. This manifested itself a few days after the injury as a slowly growing, pulsatile and tumour-like mass in the right supraclavicular fossa. The distal pulses were normal. This is an uncommon but potentially dangerous complication as it jeopardizes both the extremity and the life of the patient.

[Chest & Heart Journal 2011; 35(2) : 132-134]

Introduction:

Injuries to the brachial plexus and subclavian artery are serious complications of shoulder girdle trauma. Due to the close anatomical relationship between the brachial plexus and the subclavian artery in the thoracic outlet, both structures are often simultaneously involved in shoulder girdle injuries. Isolated lesions of the subclavian artery or the brachial plexus can also occur, especially with clavicular fractures. When a false subclavian aneurysm leads to a gradually increasing compression of the brachial plexus, the neurological signs and symptoms develop insidiously after the traumatic. Vascular complications in closed clavicular fractures are very uncommon[2,3,4]. In a series published only two out of 93 patients with an injury of the subclavian vessels had a clavicular fracture[5]. Duplex scan can confirm diagnosis of pseudoaneurysm. Criteria for diagnosis of pseudoaneurysm sac are; extravascular arterial sac with flow, communication between sac and artery and to-and-fro signal in the neck of PSA . In addition the proximal native artery of origin may have a lower resistance spectral waveform when compared with the distal artery.

Case Report:

A 90-year-old man was seen in a district hospital after a fall on the right shoulder following RTA. The radiographs showed a displaced fracture of the

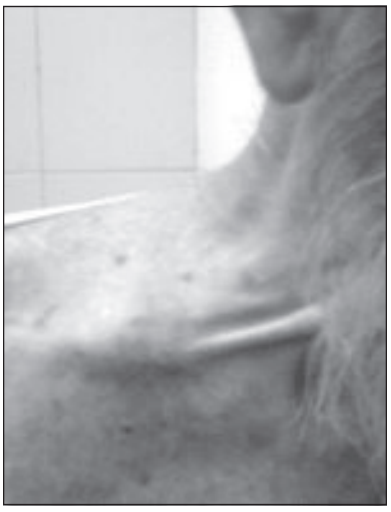

Fig.-1: Tumour-like mass in the right supraclavicular fossa

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middle third of the right clavicle. The patient was treated conservatively with a figure-of-eight-bandage. A few days after the fall, the patient noticed a mass in the right supraclavicular fossa which was progressively growing. Two months after the fracture, a pulsatile tumour-like mass was palpated. The distal pulses and the neurological examination were normal. Color Duplex scan showed a pseudoaneurysm of the right subclavian artery. The patient was operated upon under general anaesthesia. A right supraclavicular incision was used. After dissecting the pseudoaneurysmal sac subclavian artery was clamped partially by a curved vascular clamp to repair the artery.

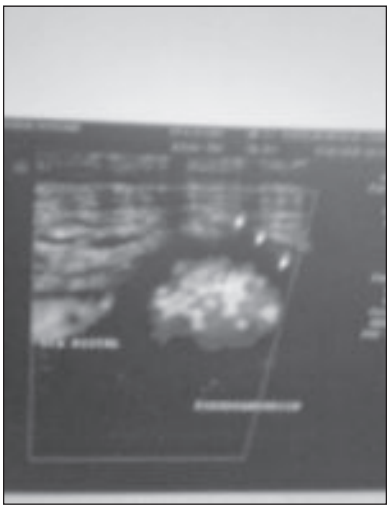

Fig.-2: *Color Duplex Scan image showing extravascular arterial sac having color flow*

Discussion:

The anatomical space between the clavicle and the first rib, where the neurovascular structures exit from the thorax towards the upper extremity, can be narrowed by exuberant callus formation, pronounced fracture displacement or pseudarthrosis of the clavicle. A subclavian artery pseudoaneurysm can clinically present as a palpable, pulsatile and sometimes visible mass in the supraclavicular fossa. This mass can compress the subclavian vein, making the venous return difficult. Distal pulses can be normal, as in other vascular lesions[3]. The brachial plexus, in close relationship with the subclavian vessels, can also be affected; in fact, the neurological symptoms can be noticed first[6,7,8]. In other cases there may be distal ischaemic arterial symptoms in the extremity due to embolic episodes coming from the pseudoaneurysm[8,9]. Among the possible complications the worst is rupture of the pseudoaneurysm, as it threatens the life of the patient. In the series this happened in 10% of all cases[10]. Arterial ischemia and even cerebral ischemia (due, probably, to retrograde embolisation) are other possibilities. Subclavian artery pseudoaneurysm can jeopardise both the extremity and the life of the patient. Treatment is usually surgical. The pseudoaneurysm is either removed, bridging the arterial defect along its length with an end-to-end anastomosis or with a graft, or opened longitudinally and closed with an angioplasty or a simple suture[8,10]. The latter option was the one used in this case. Recently, open surgical procedures have been partly replaced by percutaneous transluminal placement of endovascular devices. Uncovered endovascular flexible self-expanding stent placement with transstent coil embolization of the pseudoaneurysm cavity is a promising new technique to treat posttraumatic pseudoaneurysm vascular disease by minimally invasive methods, while preserving the patency of the vessel and side branches[11.]

References:

1. B. Hansky, E. Murray, K. Minami, R. Kiirfer. Delayed brachial plexus paralysis due to subclavian pseuoaneurysm after clavicular fracture. (Eur J Cardio-thorac Surg 1993; 7:497-4981.
2. Berga C, Prat S, Ninot S, et al. Complicaciones arteriales de los traumatismos cerrados de la cintura escapular. *Angiologia* 1992 ; 44 : 139-143.
3. Chervu A, Quinones-Baldrich WJ. Vascular complications in orthopedic surgery. *Clin Orthop* 1988 ; 235 : 275-288.
4. Craig EV. Fractures of the clavicle. Rockwood CA,Matsen FA III editors. *The Shoulder*. WB Saunders,Philadelphia, 1998, 428-482.

5. Graham JM, Feliciano DV, Mattox KL, et al. Management of subclavian vascular injuries. *J Trauma* 1980 ; 20 : 537-544.

6. Aycock TM, Isom W, Crenshaw CA, et al. Monoplegia and false aneurysm – Case report. *South Med J* 1971 ; 64 : 1165-1171. de l´épaule. *J Chir (Paris*) 1978 ; 115 : 151-157.

7. Hansky B, Murray E, Minami K, Körfer R. Delayed brachial plexus paralysis due to subclavian pseudoaneurysm after clavicular fracture. *Eur J Cardiothorac Surg* 1993 ; 7 : 497-498.

8. Dolibois JM, Matrka PJ. False aneurysm of the brachial artery complicating closed fracture of the humerus. A case report. *Clin Orthop* 1975 ; 113 : 150-153.

9. Johnson B, Thursby P. Subclavian artery injury caused by a screw in a clavicular compression plate. *Cardiovasc Surg* 1996 ; 4 : 414-415.

10. Pairolero PC, Walls JT, Payne WS, et al. Subclavian-axillary artery aneurysms. *Surgery* 1981 ; 90 : 757-763.

11. Assali, A. R., Sdringola, S., Moustapha, et al. Endovascular repair of traumatic pseudoaneurysm by uncovered self-expandable stenting with or without transstent coiling of the aneurysm cavity. Catheterization and Cardiovascular Interventions, 53: 253–258. doi: 10.1002/ccd.1160

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CASE REPORT

A Man with Chronic Cough – Evaluated As Kartagenar's Syndrome

MK Uddin, S Ahmed,Qayyum MA, MM Attar, Z Alan

Abstract:

Bronchiectasis in young patient always requires a through evaluation to find out the aetiology, Patient from tuberculosis prevalent countries many a times failed to achieve due attention regarding the aetiology of bronchiectasis. The numbers of rare diseases which cause bronchiectasis outnumber our prediction on account of vast population (around 150 million). So, it is not infrequent to see the severe bronchiectasis along with complications which probably may be delayed if not able to avoid as most of congenital causes are orphan diseases.

Key Words: cilia • primary ciliary dyskinesia • bronchiectasis • ciliary beat pattern • Kartagener's syndrome.

[Chest & Heart Journal 2011; 35(2) : 135-139]

Case report:

Sohel Ahmed (Figure-1), aged 20 years, tailor, non-diabetic, normotensive, has been suffering from persistent cough since childhood and shortness of breath for last five years, Cough is more marked at morning. Cough is associated with mucoid sputum but there is history of infection 2-3 times per year as evidenced by copious amount of yellowish sputum along with fever. He developed gradual progressive shortness of breath over last 5 years. Now dyspnoea occurs on minimal exertion. On enquiry he stated that there is frequent runny nose along with headache. There is no history of childhood pneumonia, measles, and whooping cough. There are no features suggestive of hemoptysis, arthritis, deafness, chronic diarrhea, visual impairment.

Examination showed stunted growth, clubbing, cyanosis, pitting oedema and raised JVP. Chest is barrel shaped, auscultation reveals shower of coarse crackles altered with coughing. Apex beat is found at right 5^{th} intercostal space medial to mid clavicular line. Pulmonary component of 2^{nd} heart sound (P2) is loud. There is no hepatosplenomegaly and features of ascites. Clinical impression is severe bronchiectasis, cor pulmonale, dextocardia and respiratory failure due to? primary ciliary dyskinesia (PCD). X-ray of the patient confirmed dextocardia along with multiple ring shadows (figure-2). HRCT scan of chest revealed extensive bronchiectasis all over lung more marked in lower lobes (figure -3).

USG of abdomen revealed that the patient has situs inversus. Semen analysis showed that 100% sperm are immotile. Sweat test are within normal limit but saccharine test become positive. X-ray PNS are in favour of chronic sinusitis with absence of frontal sinuses while CT scan of para nasal sinuses

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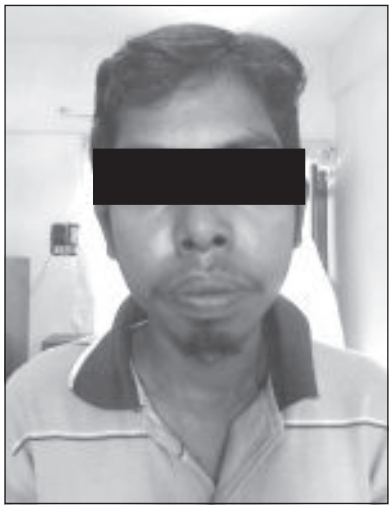

Fig.-1: *Patient's picture*

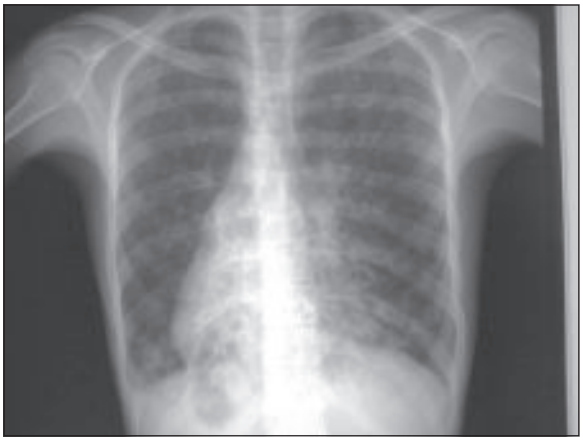

Fig.-2: *Chest x-ray P/A view - bilateral bronchiectasis with dextocardia.*

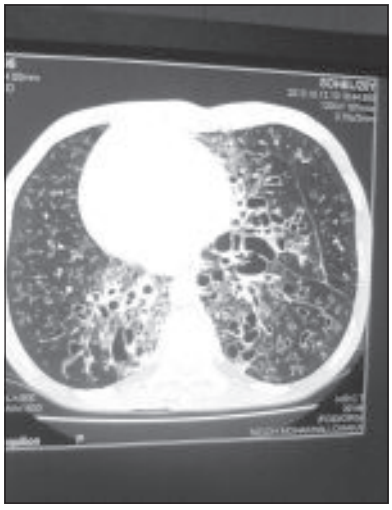

Fig.-3: *CT scan of chest - extensive bronchiectasis in all lobes.*

established the agenesis of frontal sinuses and sphenoid sinuses along with chronic maxillary sinusitis (Figure-3 & 4). ECG, echo, ABG revealed that the patient compensated type –II respiratory failure with cor pulmonale.

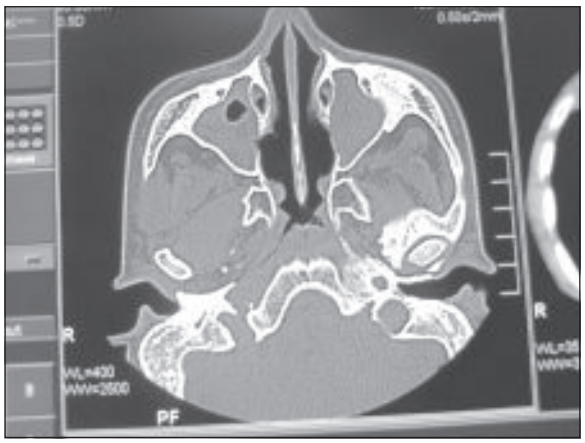

Fig.-4: *CT scan of paranasal sinuses - agenesis of frontal sinuses with chnonic sinusitis maxillary.*

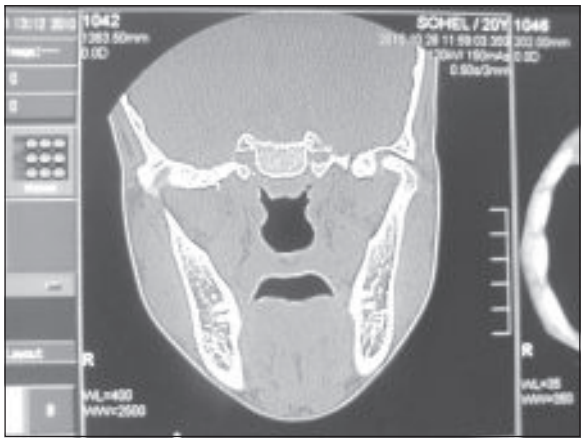

Fig.-5: *CT scan of paranasal sinuses - agenesis of sphenoid sinuses.*

So the history, clinical examination and investigations confirmed the case as a Primary ciliary dyskinesia. The individual phenotypically is a case of Kartagenar's syndrome as there is conclusive evidence of sinusitis, bronchiectasis and situs inversus, although we didn't able to carry out the genetic testing and electron microscopic examination to see the ciliary movement.

Discussion:

Primary ciliary dyskinesia (PCD) is a genetic disease, autosomal recessive genetic disorder,

associated with defective ciliary structure and function and characterized by chronic oto-sino-pulmonary disease [1, 2]. Situs inversus occurs randomly in approximately 50% of subjects with PCD [3, 4]. The prevalence is estimated at approximately 12,000 to 17,000, as extrapolated from radiographic studies in Norway and Japan involving situs inversus in association with bronchiectasis, though precise figures for the United States are lacking [5, 6]. Diagnosis relies on a combination of clinical evaluation and electron microscopic analysis of ciliary ultra structure and may be difficult to establish in some subjects [7–9]. Diagnostic delay leading to inadequate therapy may result in poorer outcomes for patients with PCD and that is happened to this particular case. [10]. Kartagener's syndrome is a variety of primary ciliary dyskinesia where the clinical triad of Situs inversus, Bronchiectasis, Pan-sinusitis exists together. Primary ciliary dyskinesia, also known as immotile ciliary syndrome is a rare, ciliopathic, autosomal recessive genetic disorder that causes a defect in the action of the cilia lining the respiratory tract (lower and upper, sinuses, Eustachian tube, and middle ear) and fallopian tube. The classic symptom combination associated with PCD was first described by A. K. Zivert in 1904.Kartagener published his first report on the subject in 1933. [11-15] Situs inversus incidence: 1: 8000-24000 live birth. Situs inversus occurs randomly in half the patients with primary ciliary dyskinesia; therefore, for every patient with Kartagener's syndrome, another patient has primary ciliary dyskinesia but not situs inversus.PCD is a genetically heterogeneous disorder affecting motile cilia[17] which are made up of approximately 250 proteins.[18] Around 90%[19] of individuals with PCD have ultra structural defects affecting protein(s) in the outer and/or inner dynein arms which give cilia their motility, with roughly 38%[19] of these defects caused by mutations on two genes, DNAI1 and DNAH5, both of which code for proteins found in the ciliary outer dynein arm.

Ciliary dysfunction (partial or complete absence of dyenin arm) causes failure of normal embryonic rotation. Patients with PCD bronchiectasis develop much slower than CF due less mucus hyper secretion & airway plugging. Bronchiectasis occurs due to recurrent lower respiratory tract infection, which is therefore acquired; because of delay in the removal of infected material from the bronchi.[16] Classical patients develop severe bronchiectasis in 3rd & 4th decade. The other clinical features are infertility, Cough & recurrent otitis media, Recurrent fever, hemoptysis, digital clubbing & cyanosis, deafness (conductive hearing loss).

Diagnosis depends on phenotypical clinical presentation along with Microscopic examination of nasal scrapings for ciliary movement, ciliary beat frequency and examination of ciliary ultra structure. The diagnosis should be considered in any patient with a history of recurrent upper and lower respiratory tract infection since childhood and can be regarded as established if I) the features of Kartagener's syndrome are present II) an adult male gives a consistent respiratory history and is found to have immotile live sperm or III) a woman or child gives a consistent respiratory history and has a sibling with kartagener's syndrome or has consistent ultra structural defects on a nasal or bronchial epithelial brush biopsy. [20]Nasal NO has been reported to be low in PCD [13–15, 17, 21]. In summary, PCD may be diagnosed using a combination of a careful clinical history together with an examination of ciliary structural analysis and measures of nasal NO. Ciliary ultra structure and functional studies may appear normal in some instances, despite a strong phenotype otherwise. Conversely, PCD may be excluded if the phenotype is very weak (absence of lifelong oto-sino-pulmonary disease) with normal nasal NO levels. Electron microscopy (EM) of ciliated epithelium is widely used to diagnose primary ciliary dyskinesia (PCD). Ciliary beat frequency (CBF) has been used to screen samples to determine whether EM is indicated. Beat pattern analysis has been advocated as an additional diagnostic test [24, 25]

Wendy A. Standard ET el showed in their study, upon 371 suspected primary ciliary dyskinesia patients, the use of ciliary beat frequency alone to screen which biopsies should have EM will result in a significant number of missed diagnoses. Ciliary beat pattern analysis is a more sensitive and specific test for PCD with higher positive predictive values and negative predictive values [25].

Treatment of kartagener's syndrome is supportive and to delay the complication along with

improvement in life expectancy by chest physiotherapy & judicious use of antibiotics. Gene therapy may be an option of treatment in near future.

So in Kartagenar's syndrome as involvement & severity are quite variable.

Persons with PCD live an active life. The rate of decline of lung function is much slower than that in cystic fibrosis. But delayed diagnosis and improper treatment results poorer outcome. So we must give due priority for evaluation of bronchiectasis to exclude PCD.

References:

1. Afzelius BA. A human syndrome caused by immotile cilia. Science 1976; 193:317–319.
2. Mossberg B, Afzelius BA, Eliasson R, Camner P. On the pathogenesis of obstructive lung disease: a study on the immotile cilia syndrome. Scand J Respir Dis 1978; 59:55–65.
3. Afzelius BA. Inheritance of randomness. Med Hypotheses 1996; 47:23–26.
4. Noone PG, Bali D, Carson JL, Sannuti A, Gipson CL, Ostrowski LE, Bromberg PA, Boucher RC, Knowles MR. Discordant organ laterality in monozygotic twins with primary ciliary dyskinesia. Am J Med Genet 1999; 82:155–160.
5. Torgersen J. Situs inversus, asymmetry, and twinning. Is J Hum Genet 1950; 2:361–370.
6. Katsuhara K, Kawamoto S, Wakabayashi T, Belsky JL. Situs inversus totalis and Kartagener's syndrome in a Japanese population. Chest 1972; 61:56–61.
7. de Iongh RU, Rutland J. Ciliary defects in healthy subjects, bronchiectasis, and primary ciliary dyskinesia. Am J Respir Crit Care Med 1995; 151:1559–1567.
8. Bush A, Cole P, Hariri M, Mackay I, Phillips G, O'Callaghan C, Wilson R, Warner JO. Primary ciliary dyskinesia: diagnosis and standards of care. Eur Respir *J* 1998; 12:982–988.
9. Escudier E, Couprie M, Duriez B, Roudot-Thoraval F, Millepied MC, Pruliere-Escabasse V, Labatte L, Coste A. Computer-assisted analysis helps detect inner dynein arm abnormalities. Am J Respir Crit Care Med 2002; 166:1257–1262.
10. Coren ME, Meeks M, Morrison I, Buchdahl RM, Bush A. Primary ciliary dyskinesia: age at diagnosis and symptom history. Acta Paediatr 2002; 91:667–669.
11. Siewert A. Uber einen fall von bronchiektasien bei einen patienten mit situs inversus viscerum. Klin Wochenschr 1904; 41:139.
12. Kartagener M. Zur pathogenese der bronchiektasien bei situs viscerum inversus.Beitr Klin Tuberk 1933; 83:489.
13. Pederson H, Rebbe H. Absence of arms in the axoneme of immobile human spermatozoa. Biol Report 1975:12:541.
14. Pederson H, Mygind N. Absence of axonemal arms in nasal mucosa cilia in Kartagener's syndrome. Nature 1976; 262:494.
15. Afzelius BA.A human syndrome caused by immotile cilia. Science 1976; 193:317.
16. Eliasson R, Mossberg B, Cammer P, Afzelius BA. The immotile cilia syndrome: a congenital ciliary abnormality as an etiologic factor in chronic airway infections and male sterility. N Engl J Med 1977; 297:1.
17. Chodhari, R; Mitchison, Hm; Meeks, M (March 2004). "Cilia, primary ciliary dyskinesia and molecular genetics". Paediatric respiratory reviews 5 (1): 69–76. doi:10.1016/j.prrv.2003.09.005. PMID 15222957.
18. Gene Reviews. "Primary Ciliary Dyskinesia". http://www.genetests.org/servlet/access?db= geneclinics& site= gt & id= 8888890&key =ujnJJL1T9E6XN&gry= &fcn= y&fw= 8zBM&filename = /profiles/pcd/index.html. Retrieved 2007-11-16 s.
19. Zariwala, Ma; Knowles, Mr; Omran, H (2007). "Genetic defects in ciliary structure and function". Annual review of physiology 69: 423–50. doi:10.1146/annurev. physiol.69. 040705.141301. PMID 17059358.

20. Afzelius BA. Immotile Cilia syndrome past, present and prospects for the future. Thorax 1998:53:894.

21. Lundberg JO, Weitzberg E, Nordvall SL, Kuylenstierna R, Lundberg JM, Alving K. Primarily nasal origin of exhaled nitric oxide and absence in Kartagener's syndrome. Eur Respir J 1994; 7:1501–1504.

22. Wodehouse T, Kharitonov SA, Mackay IS, Barnes PJ, Wilson R, Cole PJ. Nasal nitric oxide measurements for the screening of primary ciliary dyskinesia. Eur Respir J 2003; 21:43–47.

23. Zariwala M, Noone PG, Sannuti A, Minnix S, Zhou Z, Leigh MW, Hazucha M, Carson JL, Knowles MR. Germline mutations in an intermediate chain dynein cause primary ciliary dyskinesia. Am J Respir Cell Mol Biol 2001; 25:577–583.

24. Narang I, Ersu R, Wilson NM, Bush A. Nitric oxide in chronic airway inflammation in children: diagnostic use and pathophysiologic significance. Thorax 2002; 57:586–589.

25. Wendy A. Stannard Et el. Diagnostic Testing of Patients Suspected *of Primary Ciliary Dyskinesia. Am J Respir Crit Care Med 181. pp.* 307-314, (2010)