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Efficacy of Estimating Pleural Fluid Cholesterol in Diagnosing Tubercular Pleural Effusion

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Original Research Article

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Abstract: Tuberculous pleural effusion (TPE) is diagnosed by biopsy or PCR on clinical suspects and delay in this is still frequent in India. ADA is not readily available mostly in hospitals with limited laboratory facilities. Pleural fluid cholesterol has been used to classify exudates and transudates as it misclassifies fewer cases than any other Light's parameters. To evaluate the utility of cholesterol in lymphocytic exudates in diagnosing TPE in a region of high prevalence of PTB which has never been done before. The study was carried out on 80 patients with PE. Fluid classified as lymphocytic exudates based on light's criteria and lymphoytic proportion >0.75 were differentiated into tubercular and non-tubercular PE based on biopsy or PCR. Fluid ADA and fluid cholesterol were done in both the groups. 49 were positive for TPE. Fluid ADA and fluid cholesterol levels were significantly different in tubercular and non-tubercular PE cases. Fluid cholesterol correlated positively with fluid ADA. Sensitivity, specificity, PPV, NPV with fluid cholesterol value of 50 mg/dL as cut off were 95.9 %, 100 %, 1005, 84.6 % which was better than using fluid cholesterol value of 60 mg/dL as cut off and fluid ADA. Fluid cholesterol estimation could be a feasible option for cheaper diagnosis of TPE and it correlates with fluid ADA. The good accuracy of this method makes it a promising diagnostic tool that could be used for diagnosis of TPE in area where disease has high prevalence. A negative result excludes TPE with a high degree of certainty.
Keywords: fluid cholesterol, pleural effusion, tubercular, exudative, lymphocytic

INTRODUCTION

Tuberculosis (TB) remains one of the major public health concerns in India. With a population of about 1252 million, India is the largest country in the Region. It is ranked first among the high-burden countries and contributed 24% of the estimated global incident TB cases and about 20% of global TB-related deaths in 2013[1].

TB is classified as pulmonary and extrapulmonary. Pleural TB is responsible for 30% to 80% of all pleural effusions (PE) encountered in India. PE in TB usually has lymphocytic and exudative characteristics. Exudates are due to pleural inflammation (pleurisy), with an increased permeability of the pleural surface to proteinaceous fluid and various types of cells. Lymphatic obstruction may also contribute towards the accumulation of pleural fluid[2].

Hypersensitivity to the tubercle bacillus which acts like an antigen in pleural space also plays an important role in determining the occurrence and amount of PE. This immunologic reaction causes the stimulation and differentiation of lymphocytes, which release lymphokines, which in turn activate macrophages for an enhanced bactericidal effect[2]. The diagnosis of tuberculous pleural effusion (TPE) depends on the demonstration of tubercle bacilli in pleural fluid, a pleural biopsy specimen, or the demonstration of granulomas in the pleura[3].

PCR has been used to detect mycobacterial DNA in pleural fluid, with sensitivities ranging upto 80% and specificities of 78% to 100%, depending on the area of the genome that is amplified and the technique used for DNA extraction. Due to the paucity of Mycobacterium tuberculosis in the PE, the performance of a pleural biopsy has historically been

considered the most reliable method to confirm the diagnosis when tuberculous aetiology is suspected. It is also considered for distinguishing between tuberculosis and neoplasia[2].

However, since pleural tissue sampling is more difficult than simple thoracocentesis, pleural fluid markers of TPE have been extensively evaluated as an attractive alternative to pleural biopsy. Adenosine deaminase (ADA) is considered as the most cost effective pleural fluid marker and is routinely employed as a screening tool, in particular, in countries where tuberculosis is endemic[3].

ADA is a predominant T-cell enzyme involved in converting adenosine to inosine and deoxyadenosine to deoxyinosine[4]. An elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of 90% to 100%.[2] There are several isoforms of ADA, but the prominent ones are ADA1 and ADA2. ADA1 is present in all cells, whereas ADA2 is found only in monocytes. ADA2 is the predominant isoform in TPEs, accounting for most of the total ADA activity, in clinical practice; however, the difference in the use of total ADA or the isoform ADA2 is not considered to be significant. Furthermore, the isoenzyme assay is more expensive and not readily available, so fluid ADA with a cutoff value of > 40 U/L is considered standard for distinguishing between tubercular and non-tubercular[2].

Theoretically, according to Bayes theorem, the predictive value of ADA depends not only on its sensitivity and specificity, but also on the local prevalence of the disease: in a high prevalence setting the positive predictive value (PPV) of elevated ADA would increase, while in a low prevalence setting the PPV would decline but the negative predictive value (NPV) would remain high, so a low concentration of ADA might rule out TPE[3].

High levels of ADA have also been reported in some non-tubercular conditions involving lungs like pleural fluid lymphocytosis, leukemias, lymphomas and collagen vascular diseases (e.g., rheumatoid pleuritis and systemic lupus erythematosus)[2]. Measurement of pleural interferon-γ, an alternative cytokine derived from lymphocytes, may also be utilized in the diagnosis of TPE, but the sensitivity and specificity of this marker is lower than that of ADA[5].

Although making the diagnosis is more likely with the measurement of ADA, its availability may be problematic in some countries like India and pleural culture, biopsy and PCR is not easily available, especially in some small patient care units[6]. Manual ADA can be done but is laborious and prone to human errors. The use of automated kits is expensive. In developing countries like India with a large rural population, the need for a sensitive and easily available diagnostic test for TPE is even greater.

Therefore, the aim of this study was to evaluate some other parameter in standard pleural fluid analysis for a confident diagnosis of TPE in a region of a high prevalence of tuberculosis, without the determination of ADA.

Pleural fluid cholesterol has been used to classify exudates and transudates as it misclassifies fewer cases than any other Light's parameters. From meta-analysis, Heffner *et al*. have identifed pleural effusion of exudative type with at least one of the following conditions[7].

- Pleural fluid protein >2.9 gm/dL
- Pleural fluid cholesterol >45 mg/dL
- Pleural fluid LDH >2/3rd of upper limit of serum

Therefore the present study was planned to evaluate the utility of cholesterol in lymphocytic exudates in a region of high prevalence of PTB which has never been done before.

MATERIALS AND METHODS

The present study was done in department of Chest and TB in SHKM, GMC, Nalhar, Mewat during a period of 6 months from March 2015 to August 2015. Pleural fluid obtained during thoracocentesis was sent for the following investigations: cytology, microbiology, and biochemistry, which included total protein, lactate dehydrogenase (LDH), cholesterol, glucose, ADA. The same biochemical testing was performed on blood samples along with serum albumin. All biochemical measurements were performed on a clinical chemistry analyser using standard methodology.

ADA was done by enzymatic deamination method and cholesterol with the enzymatic method CHOD PAP (cholesterol oxidase peroxidase)[7]. Depending on the biochemical parameters and cytological reports, lymphocytic exudates were identified.

DIAGNOSTIC CRITERIA

Exudate was considered according to Light's criteria, i.e. pleural fluid LDH >200 IU/L, pleural fluid to serum LDH ratio >0.6 and pleural fluid protein to serum protein ratio >0.5. The presence of any one of the three criteria established that the pleural fluid was exudative while the diagnosis of transudative effusion was made in the absence of all three criteria[2]. Lymphocytic effusion was identified as lymphocyte/ neutrophil ratio in the pleural fluid of >0.75. Patients with lymphocytic exudates were further divided into

two groups: (i) tuberculous exudate, (ii) malignant exudates.

A TPE was diagnosed if all the following clinical criteria were present:

- Fever, cough, pleuritic pain, malaise, anorexia, toxaemia, etc. compatible with a clinical diagnosis of TPE;
- Exudative PE with predominantly lymphocytic pleocytosis and a few mesothelial cells;
- Response to antitubercular therapy.

Erythrocyte sedimentation rate (ESR) was done in all such patients. Sputum was evaluated for mycobacteria by Ziehl–Neelsen staining and culture on Lowenstein–Jensen medium for 8 weeks whenever an acceptable sample could be obtained. Pleural fluid culture was also done whenever possible. In addition, a pleural biopsy was done wherever indicated and if consent was obtained or PCR was done to obtain a confirmed diagnosis of TPE.

Patients who had granulomas on pleural biopsy or those in whom mycobacteria were isolated from the pleural fluid, pleural biopsy or any other clinical specimen by smear and/or culture were diagnosed to have tuberculous pleural effusion even if they did not fulfil any of the clinical criteria outlined above, or if gene is identified by PCR[2].

A malignant pleural effusion was diagnosed if one of the following criteria were fulfilled:

- Demonstration of malignant cells on cytological examination of the pleural fluid;
- Demonstration of malignant tissue in a pleural biopsy specimen;
- Histologically proven primary malignancy with exclusion of any other cause known to be associated with pleural effusion.

Cases with congestive heart failure, pulmonary infiltrate associated with an inflammatory process, nephrotic syndrome, cirrhosis of the liver were excluded. Patients with history of previous thoracentesis and bleeding diathesis were also excluded.

Statistical Analysis

Data are expressed as mean ± SD. The Student t test was used for the comparison of the continuous variables between TPE and non-tubercular groups. The results of the diagnostic tests were expressed as sensitivity, specificity, predictive values (positive and negative) and accuracy. Receiver operator characteristics (ROC) curve methodology was used to find the optimum cut-point.

RESULTS

80 cases of pleural effusion were enrolled in the study; of these 60 were lymphocytic exudates. Mean age of presentation was 36.77±17.32 yrs, with male to female ratio of 41:18. The mean age in men was 37.48±17.12 yrs, while in females was 35.11±18.17yrs. After pleural biopsy and/or PCR, 49 were identified as tubercular effusion while 11 were identified as non-tubercular and were malignant. Culture was positive in one sputum and five cases of pleural fluid. Only one of the tubercular lymphocytic effusions was sputum smear positive. The basic characteristics of patient are shown in table 1.The value of various parameters analysed in serum and pleural fluid are shown in table 2. The value of ADA in pleural fluid in tubercular group was 66.0±32.2 U/L which was significantly different from that of non-tubercular group (16.2±4.8 U/L). Fluid cholesterol also showed the same pattern, fluid cholesterol in TPE was 81.5±31.3 mg/dL which was significantly different from 28.8±8.8 mg/dL of non-tubercular group. Rest of the parameters were comparable in both the groups.

Table−1: Basic characteristics of lymphocytic exudates patients

Parameter	Values
Age	36.77±17.32 yrs
M:F	41:18
Age Males	37.48±17.12 yrs
Age Females	35.11±18.17 yrs
Tubercular: non-tubercular	49:10
Sputum smear positive	1
Sputum culture positive	1
Fluid culture positive	5
Cough	32%
Fever	30%
Cough + fever	20 %
Breathlessness	80 %

Table−2: Value of various parameters analysed in serum and pleural fluid

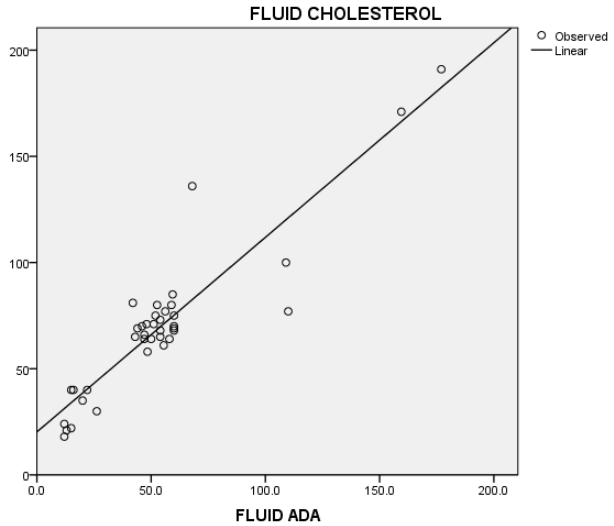
Parameter	TUBERCULAR	NON-TUBERCULAR
SERUM PROTEIN (g/dL)	6.8 ± 1.0	6.2 ± .5
SERUM ALBUMIN (g/dL)	3.4 ± .56	3.4 ±.47
SERUM LDH (U/L)	439 ± 125	548 ± 315
FLUID SUGAR (mg/dL)	70.16 ± 33.31	61.33 ± 32.63
FLUID PROTEIN (g/dL)	4.8 ± 0.93	3.47 ± 0.90
FLUID LDH (U/L)	2.01 ± 1.29	1.83 ± 1.23
FLUID ADA (U/L0	66.00 ± 32.19*	16.2 ± 4.82
FLUID CHOLESTEROL (mg/dL)	81.52 ± 31.26*	28.75 ± 8.76

* Significant w.r.t non-tubercular group (p ≤ .05), LDH: lactate dehydrogenase, ADA: adenosine deaminase

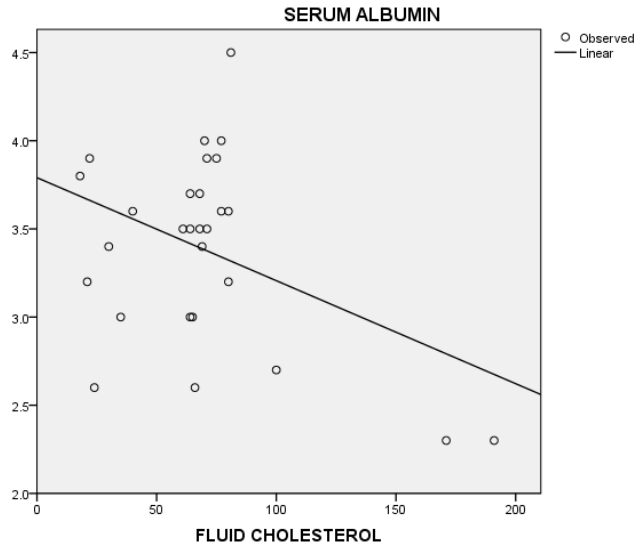
Fluid cholesterol was found to correlate positively with fluid ADA (r2= .837, p<.001) (pearson coefficient= .915), while serum albumin correlated negatively with fluid cholesterol (r2= .171, p<.05) (pearson coefficient= -.413) and fluid ADA (r2=.180, p<.05) (pearson coefficient= -.424) as evident in figure 1, 2 and 3. Of the 49 TPE cases fluid ADA results were positive in 46 patients taking ADA value in pleural fluid of 40 U/L as cut off, 44 were positive taking 45 U/L as cut off.

There is also disparity in the opinion of using fluid cholesterol value of 50 mg/dL or 60 mg/dL for differentiating between exudates and transudate. Considering this fact and finding efficacy of using fluid ADA level with cut off of 40 U/L in effusion cases and fluid cholesterol levels, taking 50 mg/dL and 60 mg/dL, sensitivity, specificity, PPV, NPV for the three was calculated. 48 were positive for TPE taking fluid cholesterol value of 40 mg/dL as cut off while 2 were false positive, 47 were positive taking fluid cholesterol value of 50 mg/dL as cut off, while only 38 were positive taking 60 mg/dL as cut off while 11 were false negative. Table 3 shows the performance characteristics of fluid cholesterol and fluid ADA, using two different cut offs for both in the diagnosis of TPE. Sensitivity, specificity, PPV, NPV with fluid ADA value of 40 U/L as cut off are 93.9 %, 100 %, 1005, 76.8 % respectively which is better than using 45 U/L as cut off. Sensitivity, specificity, PPV, NPV with fluid cholesterol value of 50 mg/dL as cut off are 95.9 %, 100 %, 1005, 84.6 % which is better than using fluid cholesterol value of 60 mg/dL as cut off and fluid ADA.

Considering the highest sensitivity achieved and the lowest false negative rate observed, cholesterol value of 50 mg/dL, should be taken as cut off for diagnosis of TPE. This was later confirmed by the results of the ROC curve. Figure 4 shows the ROC curve taking different values as cut offs and Table 4 shows its area under the curve. The ROC curve with cut off of fluid cholesterol of 50 mg/dL has the maximum area under curve of 0.980, which also suggests cut off of fluid cholesterol of 50mg/dL is the best. The cost of each test was Indian Rupees (INR) 15 for the fluid cholesterol and INR 150-200 for fluid ADA.



Fig−1: Correlation between fluid cholesterol and fluid ADA (r2= .837, p<.001)

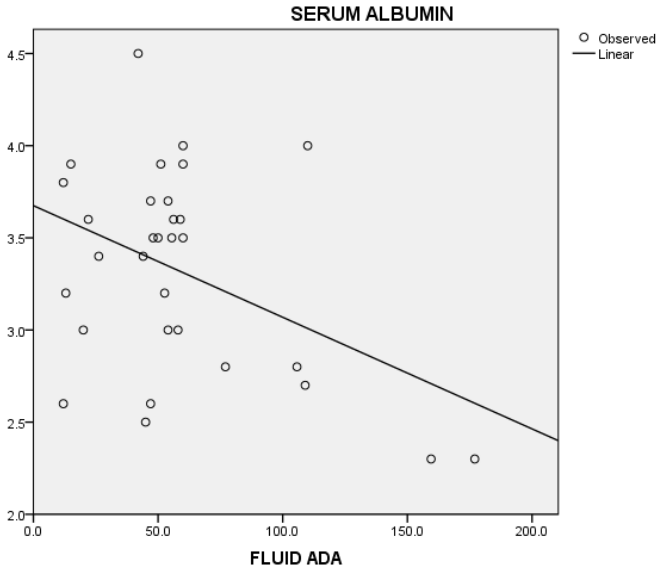


Fig−2: Correlation between serum albumin and fluid cholesterol (r2= .171, p<.05)

Table−3: Performance characteristics of fluid cholesterol and fluid ADA, using two different cut offs

	FLUID ADA(40 U/L)	FLUID ADA(45 U/L)	FLUID CHOLESTEROL (50 mg/dL)	FLUID CHOLESTEROL (60 mg/dL)
SENSITIVITY	93.9 %	89.8 %	95.9 %	77.6 %
SPECIFICITY	100 %	100 %	100 %	100 %
PPV	100 %	100 %	100 %	100 %
NPV	78.6 %	68.8 %	84.6 %	50 %
ACCURACY	.95	.92	.97	.82

ADA: adenosine deaminase



Fig−3: Correlation between serum albumin and fluid ADA (r2=.180, p<.05)

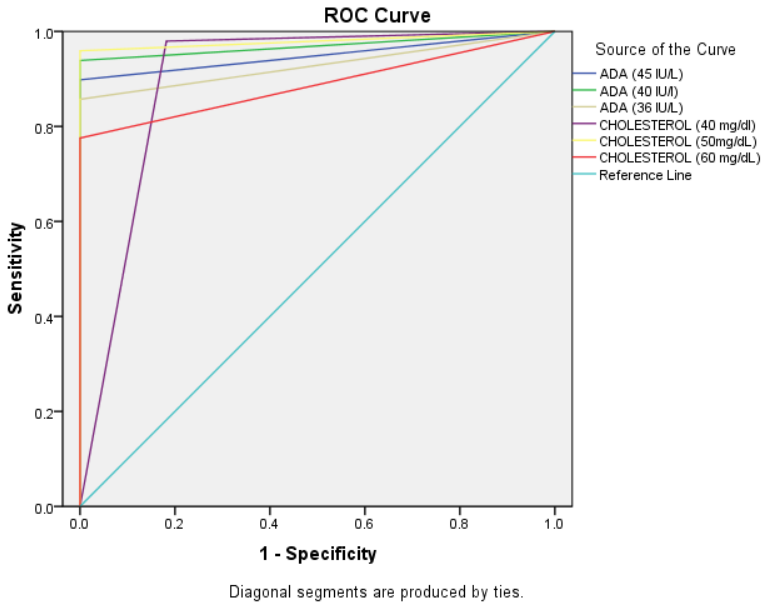


Fig-4: ROC curve with different values as cut offs for fluid ADA and fluid cholesterol in diagnosis of TPE

Table-4: Area under the ROC curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ADA (45 IU/L)	.949	.027	<.001	.892	1.000
ADA (40 IU/l)	.969	.021	<.001	.000	1.000
CHOLESTEROL (50mg/dL)	.980	.017	<.001	.000	1.000
CHOLESTEROL (60 mg/dL)	.888	.041	<.001	.807	.969

DISCUSSION

Exudative lymphocytic pleural effusions commonly encountered in clinical practice often constitute difficult diagnostic problems. The two most common causes are malignancy and tuberculous effusions. For tuberculosis, the limitations of diagnostic tests include few positive staining and culture from pleural fluid, as well as time consumption for identification[2].

The present study was conducted in Department of Chest and TB in SHKM, GMC, Mewat, with the objective of finding the efficacy of estimating fluid cholesterol in diagnosing TPE. The college is situated in a region with high prevalence of TB. Mewat is a region of high prevalence of TB is proven by the fact that annual report of 2014 of TB in India reported 1120 cases were smear positive of the 6959 number of suspects examined in Mewat region of Haryana[1].

In present study, productive cough and fever were present in 32 % and 30 % of patients with tuberculous effusions and both symptoms in combination were present in 20% of patients with pleural TB. Breathlessness was the most common presenting complains and was present in 80% of the patients.

ADA is an enzyme catalyzing the conversion of the adenosine and deoxyadenosine to the inosine and deoxyinosine in the purine degradation pathway. Its quantity increases in the immature and non-differentiated T-lymphocytes following mitogenic and antigenic stimulation. Prominent rise of ADA observed in TPE is due to presence of gradually increasing CD4 blastogenesis after the mycobacterial antigenic stimulus[8]. Our study also revealed the same results in TPE. The present study showed mean TPE ADA level of 66.00±32.19 U/L and of 16.2±4.82 U/L in non-tubercular PE, which is similar to results shown by Subhakar *et al.* [9] and Bamaniya *et al.* [10]. The sensitivity and specificity in our study of diagnosing TPE using fluid ADA is 94% and 100% respectively which is similar to (100%) studies by P. K. Sinha *et al.* [11] and Baldev Raj *et al.* [12].

The study reveals that in diagnosis of TPE, estimation of fluid cholesterol is really useful and is

even better than fluid ADA with a high sensitivity and specificity (of 95.9% and 100 % respectively) in an area of high prevalence of TB. The best cut off of fluid cholesterol that should be used in diagnosis of TPE is 50 mg/dL. Numerous studies have reported the utility of fluid cholesterol estimation for differentiating between exudative and transudative effusions but none of studies to our knowledge has reported its utility for diagnosing TPE. The results can be attributed to the fact that study was carried out in an area of high prevalence of TB.

Pleural cholesterol is thought to be derived from degenerating cells and vascular leakage from increased permeability [7]. Though inflammation may be the major reason for vascular permeability in TB but significant reduction is observed in serum albumin along with increased levels of gamma globulin [13] and this hypoalbuminemia can also contribute to increased permeability. This can explain the cause of significant inverse relationship between serum albumin and fluid cholesterol. Though the exact cause of the rise in cholesterol levels in pleural exudates is unknown, two possible explanations have been put forward.

According to the first, the cholesterol is synthesized by pleural cells themselves for their own needs (extrahepatic synthesis of cholesterol is now known to be much greater than was once thought, depends on the metabolic needs of cells, and is in dynamic equilibrium with cholesterol supply by low density lipoprotein (LDL) and cholesterol removal by high density lipoprotein (HDL)), and the concentration of cholesterol in pleural cavity is increased by the degeneration of leukocytes and macrophages, which contain large quantities of cholesterol[7].

TB infections have unique virulence factors compared to most pathogens. They infect host cell and persist inside phagosomes where there are limited nutrients[14]. M. tuberculosis's (causative organism of TB) unique ability to utilize cholesterol, which is a common component of human cell membranes, helps in its persistence inside leukocytes and macrophages[14]. Furthermore, because the cholesterol catabolism pathway requires a large number of oxygenases, TB infects the lungs where oxygen concentrations are highest[15].

For cholesterol transport to be important during intracellular growth, bacteria resides predominantly in a cholesterol-rich region of the macrophage, and cholesterol containing membranes may be directly adjacent to the pathogen so that mycobacteria may have access to adequate local stores of cholesterol. This compound may be even more abundant in tuberculosis lesions in vivo, where M. tuberculosis is often found within cholesterol-laden foamy macrophages or in extracellular spaces in which cholesterol is deposited as insoluble crystals. The degradation of cholesterol by M. tuberculosis provides carbon for energy and has effects on the structure and abundance of bacterial components that are critical for virulence. The preferential use of cholesterol suggests that the flux of cholesterol in the bacterium may be increased when the bacteria use this compound[14].

Within the cholesterol catabolism pathway, there are many metabolites that may be toxic. Cholestenone, the first product of the cholesterol pathway, has shown to be toxic [17] and accumulation of toxic cholestenone kills the cell [17], and causes release of this mobilised cholesterol from membranes into the pleural space. This can also explain the direct correlation between fluid ADA and fluid cholesterol as both released from cells (lymphocytes and macrophages) which are increasing in pleural fluid in response to inflammation.

Cholesterol constitutes 30% of lipid content of plasma membrane and affects its fluidity. Secretory process of phagocytic cells like macrophages requires cholesterol (such as cell motility, exocytosis and endocytosis)[18]. In addition, sterol metabolism by invaded bacterium in macrophages and have important effects on the host by reducing the local concentration of membrane cholesterol [14]. Their phagocytic activity was found to be deranged in cholesterol deficiency in plasma membrane [18], and helps the bacteria to flourish in conducive environment.

Around 250 genes potentially involved in lipid metabolism have been discovered in M. tuberculosis. These observations indicate utilization of cholesterol by M. tuberculosis and hence higher level in TPE [18].

The second possible explanation is that pleural cholesterol derives from plasma; some 70% of plasma cholesterol is bound to LDL, HDL and the rest to very low density lipoproteins (VLDL), and the increased permeability of pleural capillaries in pleural exudate patients would allow plasma cholesterol to enter the pleural cavity [7].

Misclassification between tubercular and non-tubercular PE can lead to inappropriate patient management or potentially unnecessary and invasive diagnostic investigations that increase morbidity and health care costs [19]. Pleural cholesterol levels were associated with a significantly lower misclassification rate. We conclude that pleural cholesterol level shows substantial promise as a test for distinguishing tubercular and non-tubercular pleural lymphocytic exudates.

Limitation of the study can be small sample size, so the results should be validated on large sample size.

CONCLUSION

Taking into account our results, we believe that the fluid cholesterol estimation could be a feasible option for cheaper diagnosis of TPE. The delay of tubercular effusion diagnosis by biopsy or PCR is still frequent in India. ADA is not readily available mostly in hospitals with limited laboratory facilities. Thus, we should find a low cost method that could be used anywhere and whose results could be readily available for a fast diagnosis of this infection. The good accuracy of this method makes it a promising diagnostic tool that could be used for diagnosis of TPE in area where disease has high prevalence. However, it does not suggest that standard pleural fluid analysis be systematically replaced by the use of fluid cholesterol estimation, but a positive result is surely an indicator for starting empirical anti tubercular therapy, and a negative result excludes tubercular effusion with a high degree of certainty.

There is reasonable amount of evidence to support the use of fluid cholesterol in the work-up of patients suspected of having PE but now it should be used for TPE also. Remote hospitals and less affluent health systems should realize the benefit of fluid cholesterol estimation in TPE and incorporate them in their pleural fluid handling routine.

It is a kind of “new kid on the block” that has just appeared in the race against TPE, if further validation studies worldwide are supportive in the near future, cholesterol-based assays may prove useful as a non-invasive confirmatory test to complement current screening procedures and as a rapid clinical test to guide the comprehensive management of patients with TPE.

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