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Study of cholesterol activity in pleural effusion

Dr. Arunabha Dasgupta*

Associate Professor & i/c HOD
Dept of Medicine
Agartala Government Medical College Agartala Tripura

Email id: drarunabhadasgupta@gmail.com

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ABSTRACT

Background: Pleural effusions are collections of fluid in pleural cavities and are actually manifestations of diverse diseases both infective and non infective and are either cardio-pulmonary in origin or extra-pulmonary. Based on the pathology of formation the fluid can be either transudate or exudate. The most commonly accepted criteria of differentiation is the Light's criteria published by Richard Light in 1972 where pleural fluid protein and lactate dehydrogenase enzyme were estimated along with their respective levels in serum and necessary calculation were done. In1987 Hamm et.al suggested that estimation of pleural fluid cholesterol too reliably can differentiate in between transudate verses exudates as the cholesterol level increases in infective and malignancy like pleural fluid protein and it is low in transudates. The present study was done to estimate pleural fluid cholesterol in infective, malignant and other non infective cases in our setup and to study its accuracy in differentiation.

Aims & objective: To study pleural fluid cholesterol level and to study its accuracy in differentiation of exudates verses transudates and to study correlation of pleural fluid cholesterol with serum cholesterol and pleural fluid protein

Methodology: There were 100 cases of pleural effusions and 75 were due to infective and malignant cases and rest were transudative. Fluids were aspirated and analysed accordingly. Pleural fluid protein and pleural fluid cholesterol both were estimated along with their levels in serum. American society of clinical pathologist laid down value of pleural fluid cholesterol≥54mg/dl & ratio of pleural fluid cholesterol/serum chlosterol≥0.3 as exudates. Further correlation in between cholesterol and protein level in pleural fluid were studied and also serum cholesterol and pleural fluid cholesterol. Results achieved were analysed statistically. A P≤0.05 was considered as statistically significant.

Results & observation: With a cut of value of pleural fluid cholesterol≥ 54mg/dl as exudates all malignant and parapneumonic cases were classified as exudates. Among the 57 tubercular cases 52 cases were classified as exudates and among the 25 transudate cases 24 are successfully dedifferentiated. So the misclassification rate is 6% with accuracy of 94%; the sensitivity achieved is 98.5% & specificity82.7%. When the ratio of pleural fluid cholesterol/serum cholesterol is used as the differentiating marker misclassified rate is 5% & accuracy 95%.The sensitivity is 98.6% & specificity 85.7%. The study of correlation reveals that there is a strong positive correlation in between pleural fluid protein and pleural fluid cholesterol in both exudate and transudate and the result being statistically significant (P≤0.05).

Discussion: In this study pleural fluid cholesterol level and its ratio with serum cholesterol were used as differentiating parameter for classifying exudate and transudate in 100 cases of pleural effusion in our setup and compared with studies of Light et.al and Hamm et.al . We got similar accuracy of differentiation like the pioneer study in separation of serous fluid i.e. Light's criteria. The study of correlation also signifies that in infective causes and malignancy pleural cholesterol increases as pleural fluid protein and decreases in transudate and this is statistically significant.
Conclusion: To overcome few fallacies of Light's criteria there was a search for new parameters in pleural fluid for better differentiation and cholesterol was used in this regard. In our study pleural fluid cholesterol and its ratio with serum cholesterol can reliably be used as differentiating parameter and in our results the misclassification rate was 5% with accuracy of 95%

KEY WORDS: PLEURAL EFFUSION, CHOLESTEROL, EXUDATE, TRANSUDATE, PLEURAL FLUID CHOLESTEROL TO SERUM CHOLESTEROL RATIO

1 BACKGROUND

The pleural cavity is a potential space that is present in between the visceral pleura and the parietal pleura. Its importance is illustrated from its development from the coelom – the primitive body cavity; where it is lined by mesothelial cells. These cells envelops all the subsequently developed organs that have a constant motion as the heart and the lungs. [1]

Normally a thin layer of fluid 10-27 m thick and a total amount of 15ml-25ml is present in each cavity. [2]

The pleural cavity is perceived for its vices as ' Pleural Effusions' than its physiological functions as the cavity is vulnerable to various infections and non infective cardio-pulmonary diseases and shows alternations in the form of collection of fluid inside the cavity. [3]

Once the pleural fluid inside the cavity is localized its cause has to be determined for treatment. Thoracocentesis and pleural fluid analysis are undertaken for evaluation of the cause- the results of which supports the clinical impression.

Traditionally transudates or exudates are classified based on the aetiology & underlying pathology with transudates the treatment is directed towards the cause rather than the fluid collection itself whereas in exudates if infective treat-ment is directed towards the cause as well as to the fluid accumulation and if malignancy is the cause more extensive diagnostic aids are required. [4]

The first diagnostic step in evaluation is to determine if the fluid is a transudate or exudate. The Gold standard which is followed in this respect is the Light's Criteria which was established as early as 1972 and followed still now & it shows a correct classification of 93% of pleural fluids. The criteria rely on the level of total protein in the fluid and its ratio with serum protein level and the level of Lactate dehydrogenase (LDH) both in serum and pleural fluid collection. [5]

But other researchers could only reproduce specificities of 70-86% by using the Light's criteria & 25% of transudates are mistakenly classified as exudates subjecting the patients to undergo extensive investigations. [6]

Efforts are undertaken for search for new biochemical pa-rameters in pleural fluid and with analysis of other factors which can help in differentiation of transudates verses exu-dates. [7]

Apart from analysis of gross characteristics of the fluid the other factors which are important in analysis are estimation of protein, glucose, pH, specific gravity, enzymes like amylase, Lactate dehydrogenase, adenosine deaminase and cells. [8]

It was known that cholesterol exists in pleural fluids and its level increases in infective causes of effusions and malignancy whereas in transudative causes is low. Thus its value can be used reliably as an indicator in discrimination of transudates from exudative effusions. [9]

Various studies using cut-off pleural fluid cholesterol levels ranging from 47mg/dl to 60mg/dl with sensitivity ranging from 73%- 96% and specificity ranging from 81-100% in comparison to Light's criteria sensitivity range of 99% and specificity of 98% has been used where cholesterol was used as a differentiating parameter. [10]

As cited by American Society of Clinical Pathologists depicted in Henry's Clinical diagnosis and management by Clinical methods (1993) the recommended routine tests required for analysis of pleural effusion fluids for diagnosis includes variety of tests and has recommended the use of cholesterol in pleural fluid and its ratio with serum cholesterol for determination in between transudates and exudates as a diagnostic marker with diagnostic efficacy similar to Light's criteria. Even the fluid can be assessed for estima-tion of tumour markers and immunological study like ANA. The cut of value used for pleural fluid cholesterol for exu-date is 54mg/dl and above and the ratio of pleural fluid cholesterol to serum cholesterol ≥0.3[11]

Luis Valdes et.al in their study of pleural effusions compared several parameters of differentiation: Pleural fluid LDH, Pleural fluid LDH/ Serum LDH ratio, Pleural fluid protein, Pleural fluid protein/ serum protein ratio with Pleural fluid Cholesterol and Pleural fluid Cholesterol/ Serum Cholesterol ratio. The differentiation of transudates and exudates was statistically significant for cholesterol ($P<0.001$) and the sensitivity was 92.5% and specificity 87.6% as against for LDH both sensitivity and specificity was 84.6% and for pleural protein both the values were 98%. [12]

In one Indian study by Guleria R et.al pleural fluid choles-terol level and its ratio and also pleural fluid triglyceride were used as markers of differentiation and an efficiency rate of 92% with sensitivity 98% & specificity 84%. They estimated the lipid profile in pleural fluid in tubercular and non tubercular cases and found only cholesterol can be used as a differentiating parameter. [13]

An effort was made in our setup to study the accuracy of pleural fluid cholesterol and its ratio with serum cholesterol in differentiating transudates and exudates and to study the correlation with serum cholesterol and pleural fluid protein.

2 AIMS & OBJECTIVES

1. To study pleural fluid cholesterol level
2. To assess correlation in between serum cholesterol & pleural fluid cholesterol level
3. To establish the correlation in between pleural fluid protein content & pleural fluid cholesterol
4. To assess the importance of pleural fluid cholesterol & & its ratio with serum cholesterol in differentiating exudates and transudates in pleural effusion

3 MATERIALS & METHODS

The study is a cross-sectional observational study done for a period of one and half years

The study was conducted after taking due permission from Institutional Ethics Committee.

To calculate the sample size Kish-Leslie formula (1965)[14] was used & sample size of 100 cases of pleural effusions were included.

Participation consent in written has been collected from all cases after explaining the purpose and pattern of the study.

Inclusion criteria

All patients were above 18 yrs of age

All cases of pleural effusions of any aetiology undergoing first thoracocentesis for diagnosis were included in the study after taking proper consent of participation

Exclusion criteria

1. Patients already had thoracocenteis earlier for diagnosis
2. Patients having pyothorax and haemothorax
3. Patients already on antitubercular drugs
4. Patients taking lipid lowering drugs
5. Patients refused to give consent
6. Patients where diagnosis was not achieved
7. Patients of pleural effusion due to renal failure & pulmonary embolism

4 METHODOLOGY

A detailed history was taken from every subjects with enquires on past history, family history and occupational exposures. Detailed physical & general examinations were done and relevant findings noted.

All patients were subjected to all necessary biochemi-cal investigations along with estimation of serum protein & serum cholesterol level.

Total serum protein concentration was estimated by Biuret method [15]

The serum cholesterol was estimated by Enzymatic-CHOD-PAP method [16]

All patients had a chest x-ray done and USG-pleural cavities were done in cases of loculated effusions and also for diagnostic drainage when required.

With total asepsis thoracocentesis was done in all cases with collection of first drawn pleural fluid in sterile vials and the fluid was subjected to the following analysis-

1. Gross appearance
2. Colour
3. Coagulum
4. Sugar
5. Protein
6. Cholesterol
7. Pleural fluid Adenosine deaminase (ADA)
8. Pleural fluid Lactate dehydrogenase level (LDH)
9. Cytology for-

- Total leukocyte count
- Differential leukocyte count
- Red blood cell
- Malignant cell

10. Bacteriology which included gram stain of the fluid & Ziel Neelsen stain of the fluid
11. Biopsy of lung mass for diagnosis of malignancy when suspected by chest x-ray & subsequent CECT-Chest.

The calculation of pleural fluid protein was done by Biuret method and pleural fluid LDH by enzymatic method. Estimation of pleural fluid cholesterol was done by enzymatic Chod-pap method.

For estimation of exudates verses transudates the follow-ing criteria was used-

1. Pleural fluid protein content more than 3gms/dl as exudates
2. Light's criteria

According to this criteria an exudates is defined if it fulfils one or more of the following:

1. Pleural fluid protein / total serum protein > 0.5
2. Pleural LDH / Serum LDH > 0.6
3. Pleural fluid LDH > 2/3 of upper limit of Serum LDH

The results obtained are analysed statistically by using SPSS software version 16 and study of correlation evaluated by Pearson co-relation test [17] & P 0.05 is considered as significant.

5 OBSERVATION & RESULTS

There are 100 cases of pleural effusions in this study and they are divided into following groups as per their diagnosis derived:

- Group A

It consists of cases of pleural effusion due to transudative causes. There were 25 cases of transudative effusions and the cases included cases of nephrotic syndrome, portal hypertension and congestive cardiac failure

- Group B

It consists of 57 cases of exudative pleural effusion due to tuberculosis diagnosed by ADA > 40 U/L

- Group C

It consists of 10 cases of pleural effusion due to malignancy of lungs demonstrated by CECT Thorax and subsequent biopsy of the mass.

• Group D

It consists of 8 cases of pleural effusion due to pneumonia There were 100 cases of pleural effusions of which 70 were male and 30 were females and the age ranged from 24 to 82 years with a mean age of 40.583 years.

Table 1: The distributions of cases of pleural effusion according to aetiology are:

Cause of pleural effusions	*frequency*	*percentage*
1. Congestive cardiac failure	4	4%
2. Hepatic cirrhosis with portal hypertension	12	12%
3. Nephrotic syndrome	9	9%
TOTAL TRANSUDATE	25	25%
1. Tuberculosis	57	57%
2. Neoplastic	10	10%
3. Para pneumonic	8	8%
TOTAL EXUDATES	75	75%

Table 2: The mean ages of incidence of the above causes of pleural effusion are:

Sl No.	Diseases	Mean age in years
1	Congestive cardiac failure	34.6
2	Portal hypertension	40.4
3	Nephrotic syndrome	43.4
4	Tuberculosis	31.1
5	Para pneumonic	33.0
6	Neoplastic	61.0

The biochemical parameters of Pleural fluid in all the cases of different aetiology are as follows:

Table 3: The distributions of pleural fluid protein among the tubercular cases are:

Protein in gm/dl	frequency
Less than 3 gm/dl	16
i) ≤ 1.5	6
ii) 1.51-2.99	10
Above 3gms/dl	41
i) 3.0-4.49	36
ii) ≥4.5	5

Table 4: The ratio of pleural fluid protein to serum protein in tubercular effusions shows the following distribution:

THE RATIO	Number of cases (n=57)
≤ 0.5	5
0.51 & more	52

Table 5: The distribution of pleural fluid cholesterol in tubercular cases (n=57) are:

Cholesterol level in mg/dl in pleural fluid	Number of cases
Less than 54	5
i) ≤ 30	0
ii) 31-53	5
More than 54	52
i) 54-89	23
ii) ≥90	29

The mean pleural fluid cholesterol in tubercular cases (n=57) is 84.4 mg/dl.

By Light's criteria of differentiating exudates from transudates in tubercular effusions 52 cases are termed as exudates with the ratio of pleural fluid protein/ serum protein > 0.5 and it has found that in 52 cases of tubercular effusions the value of pleural fluid cholesterol level is ≥ 54mg/dl.
There were 10 cases of malignant pleural effusions and all of them had mass lesions and confirmations of malignancy were done by biopsy of the mass.

The mean pleural fluid cholesterol level amongst the ten malignant cases is 92.6mg/dl. By Light's criteria all ten cases are exudative in nature & all of them had pleural fluid cholesterol content above 54mg/dl.

Table 6: The distributions of pleural fluid protein amongst the malignant cases are:

Protein in gm/dl	frequency
Less than 3gms/dl	0
Above 3gms/dl	10
3.0-4.49	3
≥4.50	7

Table 7: The ratios of pleural fluid protein to serum protein in cases of malignant effusions (n=10) are:

The ratio	frequency
≤ 0.5	0
0.51 & more	10

Table 8: The distributions of pleural fluid cholesterol level amongst the malignant cases (n=10) are:

Cholesterol in mg/dl	frequency
Less than 54 mg/dl	0
Above 54mg/dl	10
54-89	3
≥90	7

The mean pleural fluid cholesterol level amongst the ten malignant cases is 92.6mg/dl. By Light's criteria all ten cases are exudative in nature & all of them had pleural fluid cholesterol content above 54mg/dl.

Table 9: In cases of parapneumonic pleural effusions the distribution of pleural fluid protein content are as follows:

Protein in gm/dl	Frequency
Less than 3gm/dl	0
Above 3gm/dl	8
3.0-4.49	6
≥4.50	2

Table 10: The ratio of pleural fluid protein/ serum protein in all cases of parapneumonic effusions (n=8) are:

The ratio	frequency
≤0.5	0
0.51& more	8

Table 11: The distributions of pleural fluid cholesterol amongst the eight para-pneumonic cases are:

Cholesterol in mg/dl	frequency
Less than 54mg/dl	0
Above 54mg/dl	8
54 -89	3
≥90	5

The mean level of pleural fluid cholesterol amongst the para-pneumonic cases is 86.8mg/dl. By Light's criteria all the eight cases are having pleural effusion exudative in nature and all eight cases of para-pneumonic effusion have pleural fluid cholesterol level above 54mg/dl.

There are 25 cases of transudative pleural effusions with four cases of congestive cardiac failure, twelve cases of hepatic cirrhosis and nine cases of nephrotic syndrome.

Table 12: The distributions of pleural fluid protein in transudative cases are:

Protein in gm/dl	frequency
Less than 3gm/dl	22
≤1.5	6
1.51-2.9	16
≥3gm/dl	3

Table 13: The ratio of pleural fluid protein/ serum protein in transudative cases is:

The ratio	Frequency
≤ 0.5	23
0.51 & more	2

Table 14: The distributions of pleural fluid cholesterol among the 25 transudative cases of pleural effusions are:

Cholesterol in mg/dl	frequency
Less than 54 mg/dl	24
≤ 30	10
30-53	14
≥54mg/dl	1

The mean pleural fluid cholesterol level among the 25 transudative pleural effusions is 32.3mg/dl. By Light's criteria 23 of them fulfils the criteria for transudative cause and 24 of them had pleural fluid cholesterol level below 54mg/dl.

Table 15: Study of pleural fluid cholesterol in correlation with serum cholesterol of all the patients including both transudates and exudates shows the following distribution:

Causes of pleural effusions	Mean serum cholesterol level	Mean pleural fluid cholesterol level	Study of co-relation by Pearson co relation test	P Value
Tubercular effusions(n= 57)	199.33	84.4	r = 0.7656	P < 0.00001*
Neoplastic effusions(n= 10)	306.2	92.6	r=-0.5903	P = 0.72585
Parapneumonic effusions(n= 8)	160.5	86.8	r=-0.5056	P = 0.201
Transudative effusions(n= 25)	232.2	32.3	r= 0.3594	P =0.7764

(* statistically significant)

Table 16: Study of Pleural fluid Protein in correlation with Pleural fluid Cholesterol of all the patients including both transudates and exudates shows the following distribution:

Causes of pleural effusions	Mean Pleural fluid Protein level	Mean pleural fluid cholesterol level	Study of co-relation by Pearson co relation test	P Value
Tubercular effusions (n= 57)	3.26	84.4	r = 0.7322	P < 0.00001*
Neoplastic Effusions (n= 10)	4.77	92.6	r = 0.8659	P = 0.0012*
Parapneumonic effusions (n= 8)	3.62	86.8	r = 0.5642	P = 0.145
Transudative effusions (n= 25)	1.98	32.3	r = 0.6857	P =0.000155*

(* statistically significant)

Discussion:

The present study was carried out to study the pleural fluid cholesterol level in cases of pleural effusions of diverse aetiologies and to study the correlation in between serum cholesterol and pleural fluid cholesterol level and also to study the correlation of pleural fluid cholesterol and pleural fluid protein.

There were 100 cases of pleural effusions of diverse aetiologies for the study.

Among the cases there were 70 male and 30 female patients. The age of incidence have shown that the most of the cases are in between 30-40yrs and neoplastic cases are above 60 yrs.

Analysis of side of involvement showed that right sided involvement was (65%) more common than left sided involvement (20%) and in 15% of cases we had bilateral involvement.

Among the tubercular cases 8 cases had radiologically apparent tubercular parenchymal infiltrates and among them 6 cases had positive AFB in sputum examination.

Estimation of serum protein showed that 85% of cases had normal serum protein level. All 9 cases of nephrotic syndrome and 6 cases of tubercular effusions had decreased serum protein level.

The mean serum cholesterol level was normal for all exudates 190 mg/dl where as for transudates it was 220mg/dl. This is because all cases of nephrotic syndrome had hypercholesterolemia.

Apart from nephrotic cases the neoplastic cases too had higher serum cholesterol values.

The differentiation between exudates and transudates by estimation of pleural fluid protein is an old and important parameter and it was shown by Carr & Power[18] in 1956 that a value of pleural fluid protein 3gm/dl and above in pleural fluid were exudative effusions and Storey[19] et.al recommended its use in routine analysis.

The mean pleural fluid protein level in tubercular cases was 3.26 gm/dl; in malignant cases was 4.77gm/dl and para-pneumonic cases were 3.62gm/dl as against transudative cases where the mean value was 1.98gm/dl.

By application of the ratio of pleural fluid protein to serum protein for classification of exudates and transudates(where exudates≥0.5)in this study we found this parameter is having sensitivity of 88.89%; specificity of 93.33%; positive predictive value of 97.5%; negative predictive value of 73.6% with an efficiency rate of 93% and misclassification rate of 7%.

Table 17: When compared with Light.et.al study the present study showed the following:

Study	Misclassification rate	Sensitivity	Specificity	Efficiency
Light et.al	5%	99%	98%	95%
Present study	7%	88.89%	93.33%	93%

The study of pleural fluid cholesterol showed that in exudative effusions the mean cholesterol value is 89.64 mg/dl and transudative effusions is 32.3 mg/dl. American Society of Pathologist (1993) laid down a value of pleural cholesterol 54mg/dl and above as exudates.[11]

This parameter of pleural fluid cholesterol level 54mg/dl and above as a differentiating value among transudates and exudates is having a sensitivity of 98.5%; specificity of 82.7%; positive predictive value of 93.3%; negative predictive value of 96.0% ; efficiency rate as 94% and misclassification rate of 6% in this present study.

Table 18: The result of this present study of estimation of Pleural fluid cholesterol level when compared with other studies shows the following:

Study	Misclassification rate	Sensitivity	Specificity	Efficiency
Light et.al	5%	99%	98%	95%
Heffner et.al[9]	5.5%	92%	81.4%	94.1%
Present study	6%	98.5%	82.7%	94%

The American Society of Pathologists advocated the use of the ratio of Pleural fluid Cholesterol level to Serum Cholesterol level also as a differentiating parameter of classifying exudates & transudates in cases of pleural effusions and they recommended the cut of value for exudates ≥ 0.3[11].

Table 19: Based on this criterion we had the following distribution:

Cause of pleural effusions	The ratio	Frequency of cases
Tubercular cases(n=57)	<0.3	4
	≥0.3	53
Neoplastic cases (n=10)	<0.3	0
	≥0.3	10
Para-pneumonic cases(n=8)	<0.3	0
	≥0.3	8
Transudative Cases (n=25)	<0.3	24
	≥0.3	1

This parameter in this study is having the following characteristics: sensitivity 98.6%; specificity 85.7%; positive predictive value 94.6%; negative predictive value 96.0%; misclassification rate 5% and efficiency 95%.

Table 20: When compared with other studies it shows the following:

Study	Misclassification rate	Sensitivity	Specificity	Efficiency
Light et.al	5%	99%	98%	95%
Heffner et.al	5.5%	92%	81.4%	94.1%
Present study	5%	98.6%	85.7%	95%

The study of correlation showed in spite of high serum cholesterol values in transudates the value of pleural fluid cholesterol is low, the mean value being 32.3mg/dl and there exists a weak positive correlation in between the two parameters. But in cases of tubercular pleural effusions there is a positive correlation in between pleural fluid cholesterol and serum cholesterol and the relation is statistically significant ($P < 0.00001$). Similar results are also observed by Gil SV et.al study[20]. This finding supports the notion that increased permeability of pleural capillaries during tubercular inflammation account for pouring in of cholesterol from serum into pleural cavity and thus leads to the rise of pleural fluid cholesterol in tubercular cases.

The correlation in between pleural fluid protein with pleural fluid cholesterol reveals a positive correlation. In exudates the value of pleural fluid protein is high (above3gm/dl) and so is value of pleural fluid cholesterol (above 54mg/dl) and the findings are statistically significant (P<0.05). And also in cases of transudates both the values are on lower side and this also reveals a positive correlation and the finding too is statistically significant. It means a rise of pleural fluid protein in exudates are associated with elevated levels of pleural fluid cholesterol & in transudates low pleural fluid protein is associated with low pleural fluid cholesterol.

This study shows that the ratio of pleural fluid cholesterol to serum cholesterol efficiently classified transudates and exudates with misclassification rate of 5% and can be regarded as a superior parameter of differentiation.

Conclusion:

Pleural fluid protein and its ratio with serum protein have long been used as two parameters to differentiate between exudates and transudates. The introduction of Light's criteria as early as 1972 formulated the use of Pleural fluid Lactate Dehydrogenase combining with Pleural fluid Protein for differentiation of transudates and exudates. Still now it is accepted as the Gold Standard in the field of work for 'separation of Transudates & Exudates' and Richard Light concluded that 'a single test or a set of chemical test is rarely 100% effective in separating two populations but increasing number of tests results in more reliable separation.'

Cholesterol is known to exist in pleural fluid and this study was conceptualized to study the activity of cholesterol in pleural fluids in infective, neoplastic and other non infective causes. And it was found that Cholesterol value in pleural fluid increases in infective and neoplastic cases like pleural fluid protein and there exist a positive correlation between them and likewise in transudative effusions both the values are less and there too exist a positive correlation and the findings are statistically significant(P≤0.05).

The value of pleural fluid cholesterol and its ratio with serum cholesterol can effectively be used for differentiation of exudates verses transudates with a misclassification rate of 5% and efficiency rate of 95%.

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AUTHOR BIOGRAPHY

Dr. Arunabha Dasgupta
Associate Professor & i/c HOD
Dept of Medicine
Agartala Government Medical College Agartala
Tripura