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RESEARCH ARTICLE

DIAGNOSTIC ACCURACY OF INTERFERON-GAMMA IN DIFFERENTIAL DIAGNOSIS BETWEEN TUBERCULAR PLEURAL EFFUSION AND MALIGNANT PLEURAL EFFUSION

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ABSTRACT

In India, most Common cause of pleural effusion is tuberculosis followed by malignancy. Aim of present study was to evaluate IFN-gamma in tubercular and malignant pleural pleural effusion by ELISA method to know its diagnostic accuracy in differentiating both conditions. This observational and case-control study was conducted between june2015 to august 2016. 31 patients of Tubercular pleural effusion and 31 of Malignant pleural effusion were taken in the study. Concenteration of IFN-gamma in pleural fluid was measured by ELISA-kit. Mean age of tubercular pleural effusion patients was significantly lower [33.2±17.1 years, (p<.001)] as compared to malignancy [56.4± 13.3 years].Mean value of interferon-gamma (617.0pg/ml) was significantly high in tubercular effusion (p<.001). Cut off level of interferon-gamma was decided to 15 pg/ml by ROC curve analysis. About 80.6% patients of tubercular effusion hadinterferon-gamma above 15 pg/ml. Sensitvity was (80.65%), specificity was (96.77%), positive predictive value was (96.15%), negative predictive value was (83.33%) and the diagnostic accuracy was 88.71%.Thus our study concludes that Interferon-gamma concenteration in pleural fluid can be used as arapid precise diagnostic test in differentiating tubercular from malignant pleural effusion.

INTRODUCTION

There are many causes of pleural effusion. According to BhavsarKaushal M *et al*. November 2015,[1] In India most common cause of pleural effusion is tuberculosis followed by malignancy. Incidence of tubercular pleural effusion was 66%, and malignant Pleural effusion was 18%.(1)

In present study analysis is restricted to these two groups to mimic the common clinical situation.

The diagnosis of tuberculouspleuritis depends on the demonstration of tubercle bacilli in the sputum, pleural fluid, or pleural biopsy specimen, or the demonstration of granulomas in the pleura.[2]

However, sensitivity of these methods are sufficiently low that even when histopathology and culture are combined, the diagnosis can be uncertain or missed in ‘‘negative’’ cases.[3,4]While repeating invasive diagnostic procedures ultimately may yield positive results, such an approach places patients at increased risk of complications and also increases costs.

The inefficiency of conventional laboratory methods has resulted in the development and evaluation of alternative diagnostic strategies.

Interferon - gamma (IFNγ) is adimerized soluble cytokine that is the only member of the type II class of interferons. [5]

IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once antigen specific immunity develops.(6)

INF-gamma, produced by T-lymphocytes, is capable of activating macrophages, increasing their bactericidal capacity against M tuberculosis and is involved in granuloma formation.[7] Several studies have found elevated concentrations of Interferon-gamma in TB pleural effusions, which is related to increased production at the disease site by effector T cells. The sensitivity of an elevated level varies from 78 to 100% and specificity from 95 to 100%.[8-16]

A metaanalysis[17] that reviewed articles from 1978 to 2000 concluded that both ADA and IFN-gamma appeared to be reasonably accurate in detecting TB pleural effusions with a maximum joint sensitivity and specificity of 93% for ADA and 96% for IFN-gamma.

Hence the main aim of this study was to see the diagnostic value of IFN-gamma in differentiating tubercular from malignant pleural effusion.

MATERIAL AND METHOD

This observational and case-control study was conducted in Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi between period of June 2015 to August 2016 after approval from ethical committee.31 pleural fluid samples of tubercular pleural effusion and 31 samples of malignant pleural effusion were collected from chest OPD and ward of SSH, IMS-BHU.

First we evaluated all patients of pleural effusion by channeling through detailed history, through physical examination and a battery of relevant investigatons.

All Patients under went thoracocentes is after obtaining written consent. Pleural fluid was analysed for routine microscopy, Gram stain, and bacterial and TB culture, ZN stain for AFB, malignant cytology etc. Two sputum samples were collected from each patient and sent for Z.N. stain for AFB and culture for Mycobacterium T.B. At the same time, blood samples were taken for CBC, RFT, LFT, RBS etc. Effusions were classified as transudates or exudates, using Light's criteria. Still undiagnosed patients having exudative effusion underwent pleural biopsy with the Abram's needle. Pleural biopsy was sent to the microbiology and histopathology laboratories. Pleural fluid samples for IFN-γ measurements were centrifuged at 2000 revolutions per minute for 10 minutes, and the supernatant was frozen at -20°C until assayed for marker.

Inclusion criteria- Patients having tubercular or malignant pleural effusion.

Diagnostic criteria of tubercular and malignant pleural effusion

Tubercularpleural effusions- If at least one of the following criteria were fulfilled:(1) Caseous necrotic granulomas were found in pleural biopsy tissue samples;(2)Ziehl–Neelsenstains or Lowenstein cultures of pleural fluid or biopsy tissue samples were positive; or (3) A sputum culture finding was positive for TB or Z.N. stain positive for AFB in the presence of exudative pleural effusion.

Malignant pleural effusion- If at least one of the following criteria were fulfilled: 1. Demonstrating malignant cells in the pleural fluid, or 2. Demonstrating malignant cells in the pleura.

Exclusion criteria

1. Patients not giving consent.
2. Patients with hemodynamic instability
3. Patients with low platelet counts and known history of bleeding disorder.
4. Patients with other causes of pleural effusion than tuberculosis and malignancy.

Study size

31 Tubercular pleural effusion + 31 Malignant pleural effusion. Malignant pleural effusions were considered as control.

Measurement of IFN-gamma levels in pleural fluid

IFN-gamma levels were measured using a Human IFN-gamma ELISA Kit (Gen-Probe Diaclone, BesanconCedex France) according to the manufacturer's instructions.

Statistical Analysis

Observations were recorded and analysis was done using SPSS v.16. For continuous variables Independent Simple t-test, Mann Whitney U test were used to compare two groups. For categorical variables Chi square test and Fisher's – exact test were used.

Receiver operator characteristic curve for determining optimal Interferon-gamma cut off value for diagnosing tubercular pleural effusion. P value<0.05 considered as statistically significant.

RESULTS

In this study total 62 patients were taken, out of which 31 patients were of tubercular pleural effusion and 31 were of malignant pleural effusion.

Mean age of tubercular patients was 33.2±17.1 years whereas of malignant was 56.4±13.3 years, this was statistically significant(p< .001).In biochemical parameters, there were no statistically significant differences between these two groups for effusion concentrations of protein, sugar, LDH and total cell count (/Cu.mm.), lymphocyte (%).[Table 1]

Table 1 Mean age and biochemical findings of pleural fluid in tubercular and malignant pleural effusion (n=62)

	Tubercular pleural effusion Mean ± Sd (n = 31)	Malignant pleural effusion Mean ± Sd (n = 31)	t-value	p-value
Age(yr)	33.2±17.1	56.4±13.3	5.983	<.001
Sugar(mg/dl)	69.9±31.4	74.6±28.4	0.615	0.541
Protein(gm/dl)	4.2±0.4	4.3±0.5	0.620	0.537
Total_Cell_count (/Cu. mm)	1847.0±3749.2	1032.1±819.2	-1.182	0.242
Lymphocyte(%)	86.7±16.6	82.2±21.3	- .916	0.363
LDH(IU/L)	325.2±53.7	291.2±39.1	-2.856	0.066

Age wise distribution of patients revealed that 67.8% patients of tubercular effusion were children and young adults between 10 to 40 years of age. Contrary to this 90.4% patients of malignant effusion were above than 40 years of age.[Table 2]

Table 2 Showing comparison of age group in tubercular and malignant pleural effusion

Age Group (yrs)	Tubercular pleural effusion (n=31)		Malignant pleural Effusion (n=31)		χ^2	P
	No.	%	No.	%		
10-20	11	35.5	1	3.2		
21-30	7	22.6	0	0.0		
31-40	3	9.7	2	6.5		
41-50	3	9.7	7	22.6		
51-60	5	16.1	7	22.6	26.467	<0.001
>60	2	6.5	14	45.2		

Sex wise distribution revealed that tubercular patients were mostly males (67.7%) while in malignant group female predominated over male (p=.041)[Table 3]

Table 3 Showing sex wise distribution of tubercular and malignant pleural effusion

Sex	Tubercular pleural effusion		Malignant pleural effusion		χ^2	P
	No.	%	No.	%		
Female	10	32.3	18	58.1		
Male	21	67.7	13	41.9	4.168	0.041
Total	31	100	31	100		

Median of IFN-gamma levels were significantly higher in patients with tubercular pleural effusion than in patients with malignant pleural effusion (617.0 pg/ml Vs. 3.0pg/ml, p<0.001). [Table 4]

Table 4 Median (interquartile range) of IFN-gamma levels for patients with tubercular and malignant pleural effusion (n=62).

	Median(Interquartile Range)		P-value
	Tubercular pleural effusion (n=31)	Malignant pleural effusion (n=31)	
Interferon-gamma (pg/ml)	617.0 (23.20 - 653.0)	3.0 (2.35-6.48)	<0.001

By ROC curve analysis optimal cut-off point for IFN- gamma was determined at 15 pg /ml.[Table 5]

Table 5 Results of ROC curve analysis for interferon-gamma in diagnosis of Tubercular pleural effusion-

Area under the ROC curve (AUC)	0.873
Std. Error	0.052
95% Confidence Interval	0.770 to 0.975
p-value	<0.001

About 80.6% patients of tubercular effusion had IFN-gamma> 15 pg/ml. Contrary to this in malignant pleural effusion 96.8% patient had IFN-gamma below15 pg/ml.[Table 6]

Table 6 IFN-gamma in tubercular and malignant pleural effusion

Interferon-gamma (pg/ml)	Tubercular pleural effusion	Malignant pleural effusion	Total
≥15	25(80.6%)	01(3.2%)	26
<15	06(19.4%)	30(96.8%)	36
Total	31(100%)	31(100%)	62

For diagnosis of tubercular pleural effusion, sensitivity of interferon-gamma was (80.65%), specificity was (96.77%), Positive Predictive Value was (96.15%), Negative Predictive Value was (83.33%) and the diagnostic accuracy was 88.71%. [Table 7]

Table 7 Showing sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy for the diagnosis of tubercular pleural effusion-

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	80.65%	(63.72, 90.81)
Specificity	96.77%	(83.81, 99.43)
Positive Predictive Value	96.15%	(81.11, 99.32)
Negative Predictive Value	83.33%	(68.11, 92.13)
Diagnostic Accuracy	88.71%	(78.48, 94.42)

DISCUSSION

In India, most common cause of pleural effusion is tuberculosis followed by malignancy. [1]Differential diagnosis between tubercular and malignant pleural effusions represents a critical clinical problem. The diagnosis of tuberculous pleuritis depends on the demonstration of tubercle bacilli in the sputum, pleural fluid, or pleural biopsy specimen, or the demonstration of granulomas in the pleura.[2]

Conventional methods generally have poor sensitivity and are time consuming because the cultures require time to grow. Although a biopsy sample of the pleura is more sensitive, it is also more invasive, which increases the possibility of associated morbidity and is subject to sampling error.[18]

The inefficiency of conventional laboratory methods has resulted in the development and evaluation of alternative diagnostic strategies.

Interferon-gamma, secreted by antigen-triggered CD4+ lymphocytes, is a key lymphokine that activates macrophages, increasing their bactericidal activity against M. tuberculosis.[19]Therefore, INF-gamma detected in pleural fluid may be the result from stimulation of T lymphocytes by tubercular antigens.[20]

The aim of the present study is to see the diagnostic value of IFN-gamma in differentiating tubercular pleural effusion from malignant pleural effusion.

In this study 31 patients of tubercular pleural effusion and 31 patients of malignant pleural effusion were taken.

In the present study the mean age of tubercular pleural effusion patients was 33.2±17.1 years while those of malignant pleural effusion patients were 56.4± 13.3 years. This study is similar to study of Valdes *et al* (1998), [21] who found that the mean age of tubercular group was 33.9±13.2 years and that of malignant group was 45.5± 16.8 years.

In this study, IFN-gamma levels were significantly higher in patients with tubercular pleural effusion than in patients with malignant pleural effusion (617.0 pg/ml Vs. 3.0pg/ml, p<0.001).This result is supported by Yung-Ching Liu *et al* (2011).[22]

In our study the level of IFN-gamma was measured in the pleural fluid. The ROC curve for interferon-gamma was plotted. The optimal cut-off point for the diagnosis of tubercular pleural effusion was determined at 15pg/ml by ROC curve analysis.

At this cut-off point, the sensitivity of interferon-gamma was (80.65%), specificity was (96.77%), positive predictive value was (96.15%), negative predictive value was (83.33%) and the diagnostic accuracy was 88.71%.

This result was agreed with Poyraz *et al*. (2004),[16] using 12 pg/mL as cut-off point had a sensitivity of 87%and specificity of 95%.

We reviewed the literatures and found a difference in the diagnostic capacity of the pleural fluid IFN –gamma between different study, this might be due to the different cut off point level were used and also may be due to inter- laboratory variability.

CONCLUSION

This study shows that measurement of interferon-gamma concentration in pleural effusion fluid has high diagnostic accuracy (88.71%),sensitivity (80.65%) , specificity (96.77%) ,positive predictive Value (96.15%), and negative Predictive Value (83.33%) and can be used as a rapid precise diagnostic test in differentiating tubercular pleural effusion from malignant pleural effusion.

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