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Research Article

A clinical study of diagnostic efficacy of interferon gamma and adenosine deaminase in exudative pleural effusion

Lira Hakani*, Anila Mitre

Department of Biology, Faculty of Natural Sciences, University of Tirana, Albania

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***Correspondence:**
Dr. Lira Hakani,
E-mail: lirahakani@yahoo.com

ABSTRACT

Background: The aim of the study was to investigate the clinical utility of biomarkers, interferon gamma (INF- γ) and adenosine deaminase (ADA) in the differential diagnosis between tuberculosis and non-tuberculosis pleural effusion.
Methods: The present study enrolled 130 patients with pleural effusion that were admitted to hospital between 2012-2015. From the patients were obtained pleural fluid and serum which were administrated to test analyses within 24 hours.
Results: Based on Light's criteria, biochemical, cytological etc. analyses were established the pleural effusion as exudative and the etiology of the effusion, 40 malign, 48 tuberculosis, 42 parapneumonic. Pleural fluid, serum and pleural fluid/serum ADA and INF-γ pleural fluid results differ significantly between the different types of exudative effusion. For the difference of tuberculosis and non-tuberculosis pleural effusion ADA pleural fluid, ADA serum and INF- γ pleural fluid showed significance for this difference, meanwhile ADA pleural fluid/serum do not contribute in the difference.
Conclusions: The study conducted that ADA and INF-γ markers can be used for the differential diagnosis of exudative pleural effusion and tuberculosis from non-tuberculosis. INF-γ even if it has a higher specificity than ADA, due to its cost we recommend that it cannot be promoted as the only solution that facilitate the different diagnosis, other markers need to be taken into consideration to assist the results.

Keywords: Pleural effusion, Exudative effusion, Adenosine deaminase, Interferon gamma

INTRODUCTION

Exudative pleural effusion is an abnormal accumulation of fluid in the pleural cavity influencing the respiratory process and causing difficulties in the normal movement of the lungs.[1] In this case the pleural fluid formation is over passing its rate of absorption, and the pleural cavity has an exaggerated amount of pleural liquid in compare to its normal state. Based on Light's criteria on biochemical, cytological and microbiological analyses can be possible in evaluating a possible exudative pleural effusion.[2] Once the possible exudative pleural effusion is set up it is needed to determine the etiology of the effusion. The common causes for an exudative pleural effusion are malign, parapneumonic and tuberculosis. Tuberculosis meanwhile is ranked as the second for the number of death worldwide, after HIV/AID, caused from a single infective agent and in Albania the incidence rate of tuberculosis is considered 19/100000 and tuberculosis is a common cause of pleural effusion.[3-5] *Mycobacterium tuberculosis* grows very slowly and is needed from 2 to 6 weeks for the culture and the treatment often starts before the confirmation of the culture.[6] Further more is needed a rapid and accurate diagnosis and the measurement of the biomarkers can provide a reliable result for the estimation of the etiology of the pleural effusion. Detection of

tuberculosis and differentiation of pleural effusion from non-tuberculosis effusion still poses diagnostic challenges. Alternative methods and biomarkers for diagnosis and differentiation of tuberculous pleurisy from non-tuberculosis pleurisy effusion are being proposed as INF-γ and ADA. Adenosine deaminase (ADA) is an enzyme associated with T-lymphocyte activity and is produced from all the cells of human body but its level are higher in lymphocytes. ADA plays an important role in the differentiation and maturation of the lymphoid system.[7] INF-γ is secreted from T cells and natural killer mostly and is influencing in augmenting the microbial function of macrophages, it stimulates the differentiation of naive T helper in Th1, activate the polimorphonucleare leucocytes and T cell cytotoxic.[8-10] INF-γ is the crucial factor for the activation of the macrophages. The aim of the present study is to define the role ADA and INF-γ in the differential diagnosis of pleural effusion, and as *Mycobacterium tuberculosis* isolation rates, in tuberculosis are relatively low these two markers are proposed to diagnose tuberculous pleurisy from non-tuberculosis pleurisy.

METHODS

The study included 130 patients with pleural effusion in a period from January 2013-September 2015. The patients underwent thoracentesis and venous blood samples that were analyzed within 24 hours. Pleural fluid samples of the patients were classified as exudative effusion based on Light's criteria, biochemical, cytological analyses etc. The exudative pleural effusion is diagnosed as tuberculosis, malignant and parapneumonic pleural effusion. Based on the following criteria was made available the possible diagnosis of tuberculosis: *Mycobacterium tuberculosis* can be isolated from the pleural fluid of tissue and granuloma are present in tissue that shows the presence of Acid Fact *Bacili*, malignant: the cytology of the pleural fluid is positive or it is known a malignant disease after the exclusion of alternative causes of pleural effusion, parapneumonic: patients with empyema, as pus in the pleural cavity were included in the parapneumonic group. To measure the level of ADA and to evaluate INF-γ were used the Giusti and Galanti method for ADA and INF-γ was measured using commercial enzyme linked immunosorbent assay (ELISA) kits. To carry out statistical analyses and to present the results were used the program of Microsoft office Excel (2007), SPSS version 20 (IBM statistics 2011). The data were presented as mean±standard deviation (SD). The test used in this study for the intergroup comparison of more than two groups of non-parametric data was Kruskal-Wallis H, for intergroup comparison of two groups was Mann-Whitney U test. Post-hoc tests were used in the assessment of data established to be significant in Kruskal-Wallis H. Receiver operating characteristic (ROC) was used to investigate the diagnostic value. The Spearman coefficient was used for the correlation of the markers. For the tests used in this study, the differences are considered significant for $p<0.05$, $\alpha=5\%$.

RESULTS

The present study was carried out in 130 patients, 81 female (62%); 49 males (38%); age 47.58±17.47. Based on the diagnostic criteria were identified, 40 malign, 42 parapneumonic and 48 tuberculosis pleural effusion. The 130 patients' distribution of ADA level in pleural fluid, serum and pleural fluid/serum are presented in (Table 1) and INF-γ positive and negative results are presented in (Table 2). ADA has higher value in pleural fluid than in serum and tuberculosis pleural effusion has higher value of ADA than the other diagnostic groups of pleural effusion. ADA and INF-γ were evaluated for their distributions between the diagnostic groups and the possibility that these biomarkers might have in differentiating the groups between them. The statistical test Kruskal-Wallis H results for ADA pleural fluid (2)=50.755, p=0.0005, for ADA serum (2)=37.957, p=0.0005 and for ADA pleural fluid/serum (2)=0.085, p=0.985. The post hoc test results of the Kruskal Wallis H statistical test are presented in (Table 3). ADA biomarker is a significant marker for the diagnosis of the different types of exudative pleural effusion. ADA pleural fluid/serum does not contribute in this diagnosis significantly. The post hoc analyses demonstrate that ADA pleural fluid and serum differ significantly when comparing malign-tuberculosis and malign-parapneumonic effusion. ADA value in pleural fluid and serum do not contribute significantly in the value differences between tuberculosis and parapneumonic effusion. ADA level were evaluated for their correlation with gender and age. The Spearman correlation for ADA serum-ADA pleural fluid rs=0.364, p=0.0005; ADA pleural fluid-gender rs=0.144, p=0.103; ADA pleural fluid-age rs=-0.114, p=0.195; ADA serum-gender rs=0.163, p=0.064; ADA serum-age rs=-0.076, p=0.195. ADA serum and ADA pleural fluid are significantly correlated with each other but the correlation is not strong, the other correlation are not significant in this way ADA pleural fluid and serum is not correlated with age and gender.

Table 1: ADA level distribution in the three types of exudative pleural effusion.

	ADA pleural fluid	ADA serum	ADA pleural fluid/ serum
Malign (40)	52.35±21.30	30.33±15.37	2.44±2.08
Parapneumonic (48)	89.29±22.93	51.14±16.12	1.89±0.68
Tuberculosis (42)	108.65±44.14	59.77±23.73	2.16±1.29

Table 2: INF γ test results in the three types of exudative pleural effusion.

	INF-γ pleural fluid	INF-γ serum	INF-γ pleural fluid	INF-γ serum
	(+)	(-)	(+)	(-)
Malign (40)	10	30	20	20
Parapneumonic (48)	14	28	20	22
Tuberculosis (42)	30	18	26	22

Table 3: Post hoc analyses of ADA biomarker.

	ADA pleural fluid		ADA serum	
Exudative pleural effusion	Test statistic	p** value	Test statistic	p** value
Malign-parapneumonic	-44.761	0.000*	-38.464	0.000*
Malign-tuberculosis	-54.915	0.000*	-47.594	0.000*
Parapneumonic-tuberculosis	10.153	0.606	9.129	0.754

p<0.05 significance; *significance test, **Adjusted p value

Results of INF-γ in pleural fluid and serum were as well compared between the diagnostic groups of exudative pleural effusion. The test results of Chi-square test for INF-γ in serum (2)=0.398, p=0.820, showing in this way that INF-γ in serum is not significant in the differentiation of the diagnostic groups of exudative pleural effusion. The test results of INF-γ in pleural fluid (2)=14.355, p=0.001, showing in this way INF-γ in pleural fluid present significance in differentiating the diagnostic groups of exudative pleural effusion.

The ADA and INF-γ were evaluated for their significance in the diagnosis and differentiation of tuberculosis from non-tuberculosis pleural fluid. The Mann-Whitney U result test for ADA are presented in (Table 4) and was used the ROC analyses for the ADA accuracy test. The AUC of ADA pleural fluid (0.746, p=0.0005) is higher than the AUC of ADA serum (0.715, p=0.0005) for a cut off of 79.3 IU/L with specificity 62%. Chi square result for INF-γ in pleural fluid (1)=13.769, p=0.0005; for INF-γ serum (1)=0.351, p=0.553, with a specificity of INF gamma pleural fluid 71%.

Table 4: Mann-Witney U test result for ADA in tuberculosis and non-tuberculosis group.

	ADA pleural fluid	ada serum	ADA pleural fluid/serum
Mann-Whitney U	999.5	1123.5	1948.5
p	0.000*	0.000*	0.925

p<0.05 significance; *significance test

It is shown that ADA serum, pleural fluid, and INF-γ pleural fluid are directly associated in differentiating tuberculosis from non-tuberculosis. ADA pleural fluid/serum and INF-γ serum have no significant association (p=0.925 and p=0.553 respectively).

DISCUSSION

Pleural effusion might be easy to be determined but to establish the etiology is often complicated. Nowadays different biomarkers are proposed that can facilitate the diagnosis. Furthermore, a specific interest the tuberculosis pleural effusion diagnosis shows in relation to non-tuberculosis pleural effusion. In the present study ADA pleural fluid and serum shows significance for the differentiation of the different types of exudative effusion. Tuberculosis test is not specific and its results might be negative.[11] The ADA activity in the tuberculous patients was significantly higher than in the other groups of exudative effusion, this is in line with other study.[12] In the present study ADA don't show a correlation with age or gender but ADA in pleural fluid and in serum shows a correlation between them. In other studies it is seen a correlation between age and ADA level, with is negative which is negative or positive.[12,13] Pleural fluid ADA has been shown to be a useful biochemical marker of tuberculosis and provides a reliable basis for a treatment decision. However, the elevation may be limited in early stages of disease, and in addition, high levels of ADA can also be found in patients with neutrophilic effusions such as parapneumonic effusions or empyema.[14] Pleural fluid ADA can be utilized for differentiating tuberculosis effusions from those of non-tuberculosis etiology, this is confirmed in other studies.[15] ADA level in the present study more than 79.3 IU/L are interpreted for the difference of tuberculosis and non-tuberculosis pleural effusion. In similar studies the ADA cut off value are proposed as above 40 IU/L, 72 IU/L, 77.5 IU/L.[15-17] Even more are proposed studied with cut off 100 IU/L.[18] High levels of ADA are seen to be increase in different body conditions not related strictly with pleural effusion, in this way this marker might be considered with reserves as a very good options for the difference. Pleural fluid ADA estimation is quick and relatively inexpensive.

INF-γ test is relatively new it its implementation but it is estimated that INF-γ pleural fluid has more accurate results.[19] INF-γ pleural fluid is considered as an additional biomarker in tuberculosis diagnosis, but this test needs further scientific researches to be confirmed furthermore as such[20] INF-γ due to its cost it is not promoted as the only test available for tuberculosis diagnosis, in this was ADA test is being proposed as well, due its large availability and cost.[21] In other studies was evaluated the cost for performing and INF-γ test in comparison to ADA for pleural fluid and the cost for detecting one additional patient using INF-γ was equivalent of the cost to complete a tuberculosis treatment for six patients.[22]

CONCLUSION

In conclusion, the present study provides useful information on the effectiveness of the biomarker tests ADA and INF-γ. ADA and INF-γ pleural fluid help in interpretation of the diagnostic groups of exudative pleural effusion and the tuberculosis and non-tuberculosis. We recommend that other biomarkers can help in the differential diagnosis and INF-γ cannot be strictly related as the only biomarker for the differentiation.

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