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Research article

COMPARISION OF ADENOSINE DEAMINASE [ADA] LEVELS WITH CYTO-CHEMICAL ANALYSIS OF PLEURAL FLUIDS TO DIFFERENTIATE TUBERCULAR AND NON – TUBERCULAR EFFUSIONS.

[1]Choukimath M Sharanabasav, [2]Channamma S Pattanshetti and [3]Vaishali Choukimath

[1]Department of Pathology. Karnatak Institute of Medical Sciences.
Consultant [2]Biochemist, [3]Pathologist, Sumukh Prayogalaya, Hubli.
Address for correspondence: Email: choukimathsm@gmail.com

ABSTRACT: This study is intended to utilize biochemical parameters like ADA and protein levels in comparison with cell count and cell type in pleural fluid to differentiate tubercular and non-tubercular effusions. We have analyzed a total of 208 cases and among them 59.61% cases were ADA positive and 40.39% cases were ADA negative, and 156 cases were exudates and 52 cases were transudates. Categorized these effusions into 4 groups taking consideration of ADA, cell count, lymphocyte and protein levels as exudate with ADA positive, exudate with ADA negative, transudate with ADA positive and transudate with ADA negative. This study has shown promising results to diagnose tuberculosis with immediate and cost effectiveness that can be undertaken by any basic laboratory, in a endemic areas and developing countries like India.

Key words: Pleural effusion, ADA (Adenosine Deaminase), Tuberculosis

INTRODUCTION

Tuberculosis is one of the commonest chronic infectious disease, which is highly endemic in India and five lakh patients die every year (Tandon,P.N et al.,1999).Delay in diagnosis and in initiating treatment results in poor prognosis and sequlae in upto 25% of cases (Gecia-monco J.C et al.,1999). Diagnosis of tubercular pleural effusion is difficult and remains a common clinical challenge. ADA has gained increasing popularity as a diagnostic test for tubercular pleuritis since 1978, especially in endemic areas. Adenosine deaminase (also known as adenosine aminohydrolase and abbreviated ADA) is an enzyme involved in purine metabolism, catalyzing the irreversible hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively (Bhaumik D et al.,1993, Hovi T et al.,1975). It is present in most mammalian tissues with its highest activity observed in organs with numerous lymphoid cells (Hovi T et al.,1975). The enzyme itself contains a parallel alpha-beta barrel motif, found in roughly 10% of known enzyme structures, with a zinc ion co-factor bound to the innermost region of the active site (Reardon D et al., 1995, Farber et al.,1990). It plays important role in differentiating lymphoid cells and is present in abundance in active T – lymphocytes whose concentration is inversely proportional to the degree of differentiation (Sharma S K et al.,1996). The measurement of ADA was initiated by Giusti in 1981 and applied extensively in clinical practice (Giusti G et al.,1974).Tuberculosis effusion is the result of cell mediated immune response to the presence of mycobacterium tubercular bacilli. This study is an attempt to utilize ADA values along with protein, cell count and cell type to make diagnosis of tubercular pleural effusions.

MATERIALS AND METHODS

A total of 208 patients with pleural effusions, received from different hospitals, nursing homes and medical colleges between august 2011 to December 2012, were included in the study and the patients age ranged between 7-84 years. Pleural fluids were subjected to biochemical analysis i.e. Protein, sugar and ADA, and microscopic examination for total cell count and differential Count. The fluid is centrifuged and the supernatant is used for the biochemical analysis. Protein estimation was done by Biuret Method and sugar by Hexokinase method. ADA estimation was done by Enzymatic-Kinetic Method.

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The entire enzymatic reaction scheme is shown below;

Adenosine + H_2O $\xrightarrow{ADA}$ Inosine +NH_3

Inosine +Pi $\xrightarrow{PNP}$ Hypoxanthine + Ribose-1-phosphate

Hypoxanthine +$2H_2O$+20_2 $\xrightarrow{XOD}$ Uric acid + $2H_2O_2$

H_2O_2 +4-AA +EHSPT $\xrightarrow{POD}$ $2H_2O$ +Quinone dye

(PNP-Purine nucleoside phosphorylase. XOD-Xanthine oxidase.

EHSPT- N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline 4-AA – 4-aminoantipyrine. POD-Peroxidase).
We have correlated ADA values with protein, total count, lymphocyte percentage. An attempt was made to differentiate exudates versus transudates, in that tubercular and non-tubercular effusions .Protein levels above 3g/dl were considered as exudates and below 3g/dl were transudates. Diagnostic cut off for ADA level for tubercular effusions was taken > 40 U/L.

RESULTS

Among the 208 cases, ADA levels were above cut off value in 124(59.61%) cases with mean of 96.49U/L, below in 84(40.39%) cases with mean of 13.22U/L (Table 1). In ADA positive 124 cases cell counts were high with mean of 10139.0, negative 84 cases with mean of 6162.7.However the lower limit of total cell count was 410 cells/cmm in positive cases and it was only 34 cells/cmm in negative cases (Table 2). ADA positive cases along with total high cell count have shown predominance of lymphocytes (mean value 62.56%) (Table 3). Table 4 shows categorization of all 208 cases into exudates and transudates taking pleural fluid protein as 3g/dl as cut off. Among 124 ADA positive cases, 113(91.12%) cases were exudates and 11(8.87%) cases were transudates. In 84 ADA negative cases 43(51.19%)cases were exudates and 41(48.80%)cases were transudates.ADA positive exudates have shown high cell count (mean 10521.7) with lymphocyte predominance (64.3%). ADA negative exudates have shown high cell count (mean 10072.9) with neutrophil predominance (67.67%). ADA positive transudates have also shown high cell count (mean 6099) with lymphocyte predominance (67.18%). ADA negative transudates have shown low cell count (mean 1915.5) with lymphocyte predominance (66.17%). (Table 5 and 6). Further analysis was done correlating with exudates and transudates with the ADA levels of all 208 cases.

Table 1: Table showing range and mean of ADA.

S.No	ADA	Number of cases	Range(U/L)	Mean
1	Positive	124	41.0 – 436.0	96.49
2	Negative	84	0.1 – 38.0	13.22

Table 2: Table showing range and mean of Cell Count

S.No	ADA	Number of cases	Range	Mean
1	Positive	124	410– 1,90,000	10139.0
2	Negative	84	34 – 2,40,000	6162.7

Table 3: Table showing range and mean of Lymphocytes.

S.No	ADA	Number of cases	Range	Mean
1	Positive	124	2 – 97	62.56
2	Negative	84	1 – 58	31.47

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Table 4: Exudates and Transudates in comparison of ADA levels.

		EXUDATE: >3g/dl	TRANSUDATE: <3g/dl				
S.No	**ADA**	**Number of cases**	**Range**	**Mean**	**Number of cases**	**Range**	**Mean**
1	Positive	113	3.1-11.1	4.89	11	1.0-2.9	2.46
2	Negative	43	3.1-6.7	4.14	41	0.5-2.7	1.95

Table 5: Exudates.

S.No	**ADA**	**Number of cases**	**Range**	**Mean**				
			Cell count	**Lymphocyte**	**Neutrophil**	**Cell count**	**Lymphocyte**	**Neutrophil**
1	Positive	113	410-190000	2-97	1-56	10521.7	64.30	30.49
2	Negative	43	34-240000	1-96	1-98	10072.9	39	67.67

Table 6:Transudates.

S.No	**ADA**	**Number of cases**	**Range**	**Mean**				
			Cell count	**Lymphocyte**	**Neutrophil**	**Cell count**	**Lymphocyte**	**Neutrophil**
1	Positive	11	120-48000	5-94	1-69	6099	67.18	35.81
2	Negative	41	22-14000	2-98	1-72	1915.5	66.17	23.21

DISCUSSION

Tuberculous pleural effusion is thought to result from delayed hypersensitivity reaction that occurs in response to the presence of mycobacterial antigens in pleural space (Leibowitz S et al.,1973). The problem in diagnosing tuberculosis is that no symptom or sign is exactly typical of it in many cases.It has been proved that the patients with tuberculosis can have a negative tuberculin skin test(Berger,H.W et al .,1973) and pleural fluid culture results are positive in <25% cases(Scharer,L et al.,1968).Other techniques have been reported to help make the diagnosis of tuberculosis are :,interferon gamma and polymerase chain reaction(PCR). Polymerase chain reaction has relatively low sensitivity in body fluids(42 to81%)(De Lassence,A et al.,1992 ,Querol J.M et al.,1995 ,Villena V et al.,1998) and is expensive. The sensitivity of an elevated interferon level appears better (89-99%) (Kataria Y.P et al.,2001, Villena V et al.,1996)but relatively only few studies of its use have been reported and the assay is expensive and cannot be done in routine laboratory. It was in 1978, when ADA was found to be useful in diagnosing tuberculous pleurisy (Piras,M.A et al.,1978, Ocana, I et al.,1983, Pettersson, T et al.,1984, Porcel,J.M et al.,2002).ADA is an enzyme involved in purine catabolism. There are several isoforms of ADA; ADA-1 and ADA-2,which are located on different gene loci(Hirschhorn,R et al.,1980) and have different optimal pH, michaelis constant and relative substrate specificity pattern.ADA-1 isoenzyme is found in all cells with lymphocytes and monocytes showing high concentration ,whereas ADA-2 isoenzyme appears to be found only in monocytes(Ungerer,J.P.J et al.,1992). In our study, ADA levels were increased in 124 cases with more than cut off of 40U/L for tuberculosis and in 84 cases the ADA levels were below cut off of 40U/L for non-tuberculosis.

Depending on protein values and ADA levels we have categorized fluid analysis into 4 categories.

1.Exudate with ADA positive. 2.Exudate with ADA negative.

3.Transudate with ADA positive. 4.Transudate with ADA negative.

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1) In this category there were 113 cases with high total count which ranged from 410-1,90,000, lymphocytes were predominant showing mean of 64.30.There was a conclusive diagnosis of tubercular effusion in these cases.

2)There were 43 cases which were exudates with ADA negative, showing total count ranging from 34-2,40,000 with neutrophil predominance with mean of 67.67,so these cases are diagnosed as acute inflammatory effusions.

3)There were 11 cases of transudates with ADA positive, these cases were also concluded as tubercular effusions, the reason being there was pre-existing hypoproteinimea in these cases which has led to fluid protein levels <3g/dl.

4) Fourty one cases were transudates with ADA negative, with cell count ranging 22-14,000 and lymphocyte with the mean of 66.17, these cases were categorized as non-tubercular and non-bacterial effusions. In our study we found that, in ADA positive cases, cell count was high wih lymphocyte predominance, these cases were well correlated with exudates and transudates. Similar results were seen with Jadhav and Bardapurkar who concluded that ADA is a useful biochemical marker to evaluate exudative pleural effusions (Jadhav AA et al.,2007).

Rafael concluded that the ADA assay should be considered as a screening test to guide further diagnostic procedures in cases of exudative pleural effusion (Laniado-Laborin R 2005).While Kataria and Imtiaz concluded that ADA testing can be an integral part of diagnostic workup in lymphocyte rich exudative body fluids both in countries with high and low prevalence of tuberculosis (Kataria Y P et al.,2001).

CONCLUSION

In developing countries like India, where tuberculosis is endemic and resources are the constraints in the evaluation and diagnosis of tuberculosis, simple lab investigations of pleural fluid in co-relation with protein levels, cell count, lymphocyte percentage compared with ADA values will help to diagnose tuberculosis. However false positive and false negative results have to be kept in mind. These controversial cases have to be co-related clinically and evaluated with expensive diagnostic modalities like PCR, gamma interferon etc.

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