Original Article

Role of adenosine deaminase and biochemical analysis of body fluid to differentiate the tubercular from non-tubercular effusion

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Background: Examination of serous body fluids is extremely important for the right and timely diagnosis of tubercular effusion. Adenosine deaminase (ADA) along with biochemical analysis of body fluid is valuable tool in the patient management process. **Aims and Objectives:** The objective of this study was to assess the activity of ADA with biochemical parameter in body fluid of patients to differentiate tubercular from non-tubercular effusion. **Materials and Methods:** Our study was performed in the Department of Pathology, Rohilkhand Medical Collage and Hospital from January 1, 2014, to December 31, 2016. Body fluids included in the study were pleural, peritoneal, and CSF. Hemorrhagic samples were excluded from this study. The clinical history and relevant parameters were noted and correlated clinically. **Results:** Total 249 cases of fluid specimen were included, out of which 98 cerebrospinal fluids, 77 were pleural fluid and 74 were ascitic fluid. Out of these effusions, 30, 21, and 9 were tubercular, respectively, and 68, 56, and 65 were non-tubercular, respectively. **Conclusion:** In the lower age groups, benign effusions are typical; in later age groups, malignant effusions are more common. The May–Grunwald–Giemsa (MGG) and Papanicolaou (PAP) combined approach to morphology assisted in better interpretation than any method employed alone. In settings with limited resources, preliminary fluid analysis for cytology is still the most practical and economical way to make a diagnosis, avoiding the need for invasive investigations and the difficulties that go along with them. Malignant cells' presence or absence can occasionally be the only indicator of malignancy, which can have an impact on the patient's prognosis and course of therapy.

KEY WORDS: Serous effusion, Transudate, Exudates, Adenosine deaminase

INTRODUCTION

The pleural cavities, pericardial cavity and peritoneal cavity are three serous cavities of the body. They have a common embryologic origin from the mesenchymal embryonic layer. The visceral and parietal layer surfaces of each cavity are lined by mesothelial cells and are supported by appropriate connective tissue, vascular, and nervous apparatus. In the absence of disease, a thin layer of lubricating fluid that facilitates the passage of two membranes against one another separates the parietal and visceral layers of the mesothelium.[1] A number of situations can cause the abnormal buildup of serous fluids in the serous cavities. Serous effusion develops due to two processes. Exudates form as a result of increased permeability, which is typically brought on by inflammation, whereas transudates form as a result of a physical disruption of the circulation, typically a rise in venous pressure or a fall in oncotic pressure. The diagnosis of tuberculosis in body fluids (Pleura, pericardium, peritoneum, and CSF) is still a common clinical problem. Traditional diagnostic methods are very useful but do not provide enough

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Table 1: Age-wise distribution tubercular and non-tubercular cases of body fluids

Age	CSF		Ascitic		Pleural	
	TB, *n* (%)	NTB, *n* (%)	TB, *n* (%)	NTB, *n* (%)	TB, *n* (%)	NTB, *n* (%)
0–20	11 (36.77)	33 (48.53)	2 (22.22)	5 (7.69)	10 (47.62)	6 (10.71)
21–40	12 (40.00)	19 (27.94)	3 (33.33)	24 (36.92)	7 (33.33)	11 (19.64)
41–60	6 (20.00)	13 (19.12)	4 (44.45)	28 (43.08)	1 (4.76)	29 (51.79)
>60	1 (03.33)	3 (04.41)	-	8 (12.31)	3 (14.29)	10 (17.86)
Total	30	68	9	65	21	56

CSF: Cerebrospinal fluid, TB: Tubercular, NTB: Non-TB

Table 2: Gender-wise distribution of cases

Type of fluid	TB			NTB		
	Male, *n* (%)	Female, *n* (%)	Total, *n* (%)	Male, *n* (%)	Female, *n* (%)	Total, *n* (%)
CSF	14 (46.67)	16 (53.33)	30 (100.00)	41 (60.29)	27 (39.71)	68 (100.00)
Ascitic	7 (77.78)	2 (22.22)	9 (100.00)	43 (66.15)	22 (33.85)	65 (100.00)
Plerual	16 (76.19)	5 (23.81)	21 (100.00)	45 (80.36)	11 (19.64)	56 (100.00)
Total	37 (61.67)	23 (38.33)	60 (100.00)	129 (68.25)	60 (31.75)	189 (100.00)
P		0.0046*			0.0521	

*Statistical significant. CSF: Cerebrospinal fluid, TB: Tubercular, NTB: Non-TB

sensitivity and specificity. For diagnosis of tubercular effusions, ADA has been used since 1978.[2-5] ADA is an enzyme that increases in TB due to the stimulation of T-cell lymphocytes by mycobacterial antigens.

MATERIALS AND METHODS

This study on body fluid was undertaken in the Department of Pathology, RMCH Bareilly for 2 year. Clinical information and other data of patient collected from the departmental records. Every sample (CSF, pleural, and ascitic fluid) were process as early as possible. The fluid was divided into three parts, one part was used for cell count, for this fluid is mixed with turk's diluting fluid and counted in an improved Neubauer chamber and second part was poured into the centrifuge tubes and centrifuged for 10 min at 2000 rpm. The supernatant was poured off. After that, sediment was transferred with the help of a Pasteur pipette to three slides coated with albumin. One was air dried and stained with Leishman and Giemsa stain for differential count; the other two were fixed in 95% alcohol for a minimum period of 15 min and stained with Papanicolaou stains. Third parts of sample send for biochemistry analysis for protein, sugar, and ADA. The value of protein, sugar, and ADA determines by Autoanalyzer of ERBA-EM360 System by the use of commercially prepared reagent. For glucose estimation, GOD–POD method is used. Pleural fluid, ascitic fluid, and pericardial fluid protein estimated by Biurate method. Adenosine deaminase was estimated by kinetic method employing xanthine oxidase, whereas albumin was estimated by BCG method. Reference range of ADA for diagnosis of tuberculosis in CSF is >5U/L (0-5), pleural fluid >24 U/L (0–24), and ascitic fluid ≥30 U/L. All the cases of CSF were included in tubercular effusion with lymphocytic predominance and high protein 100–200 mg/dL and low sugar, for pleural and peritoneal fluid with lymphocytic predominance and high protein >3 g/dL and low sugar <60 mg/dL. Hemorrhagic samples were excluded from this study.

Table 3: Distribution of cases according to different types of body fluid

Type of fluid	Total	TB cases, *n* (%)	NTB cases, *n* (%)	*P*
CSF	98	30 (30.61)	68 (69.39)	0.0146*
Ascitic	74	9 (12.16)	65 (87.84)	
Pleural	77	21 (27.27)	56 (72.73)	
Total	249	60 (24.10)	189 (75.90)	

*Statistical significant. CSF: Cerebrospinal fluid, TB: Tubercular, NTB: Non-TB

RESULTS

Table 1 shows the age-wise distribution of case of different body fluid. Tubercular meningitis more common in 21–40 years age group (40.00%), tubercular peritonitis is more common in 41–60 years age group (44.45%), and tubercular pleural effusion is more common in 0–20 years of age group (47.62%).

Table 2 shows the total 30 cases of tubercular origin of CSF, out of which 14 (46.67%) were males and 16 (53.33%) were female, nine cases of ascitic fluid were of tubercular origin out of which 07 (77.78%) were male and 02 (22.22%) were female and 21 cases were of pleural effusion of tubercular origin, out of which 16 (76.19%) were male and 05 (23.81%) were female.

Table 3 shows out of total 249 cases of fluid specimen, 98, 77, and 74 were cerebrospinal fluid, pleural fluid, and ascitic fluid, respectively. Out of total 98 cases of cerebrospinal fluid, 30 (30.61%) cases were of tubercular effusion and 68 (69.39%)

Table 4: Different parameter of body fluid to differentiate tubercular from non-tubercular effusion

Parameter	Ascitic fluid (*n*=74; 100.00)		Pleural fluid (*n*=77; 100.00)		CSF (*n*=98; 100.00)	
Total number of sample, *n* (%)	09 (12.16)	65 (87.84)	21 (27.27)	56 (72.73)	30 (30.61)	68 (69.39)
Type	TB	NTB	TB	NTB	TB	NTB
ADA (U/L)	>30	<30	>24	0–24	>5	0–5
Lymphocyte count (%)	>50	<50	>50	<50	>50	<50
Protein	>3.0 g/dL	<3.0 mg/dL	>3.0 g/dL	<3.0 g/dL	100–200 mg/dL	<100 mg/dL
Sugar (mg/dL)	<60	>60	<60	>60	<40	>40

CSF: Cerebrospinal fluid, TB: Tubercular, NTB: Non-TB, ADA: Adenosine deaminase

were non-tubercular effusion. Among 74 cases of ascitic fluid, 9 (12.16%) were tubercular and 65 (87.84%) were non-tubercular. In 77 cases of pleural effusion, 21 (27.27%) were tubercular and 56 (72.73%) were non-tubercular.

Table 4 shows relationship between ADA levels, lymphocyte count, protein, and sugar to differentiated tubercular effusion with non-tubercular effusion. Out of 74 cases peritoneal effusion, 09 (12.16%) cases show increased ADA activity and lymphocytes count is more than 50% and 65 (87.84%) cases shows normal ADA activity and lymphocytes count<50%. Out of 77 cases pleural effusion, 21 (27.27%) cases show increased ADA activity and lymphocytes count >50%, whereas 56 (72.73%) cases show normal ADA activity and lymphocytes count <50%. Out of total 98 cases of CSF specimen, 30 (30.61%) cases show increased ADA activity and lymphocytes count (>50%) and 68 (69.39%) cases show normal ADA activity and lymphocytes count (<50%).

DISCUSSION

Distribution of Demographic Profile

In our study, we found that out of 30 tubercular cases, maximum cases 40.00% were in 21–40 years age group, 66.67% males and 33.33% were female while similar findings by Sharma *et al.*[6] found maximum cases (35.38%) in 16–30 years age group, 61.29% males and 38.71% females. In contrast, Mehta *et al.*[7] found 44% males and 56% females and maximum cases were similarly found 35.38% in 16–30 years age group.

Accumulation of fluid in a serous cavity in excesses amount than normal is known as effusion. Body fluid aspiration is a simple and relatively non-invasive technique. The information received after body fluid analysis is a complete diagnostic modality. It aims to pointing out the etiology of effusion as well as prognostication of the disease process. Tuberculosis is a one of the major public health problem in developing countries.[1] Because particular medication is most effective when started early in the course of the illness, early and appropriate treatment is crucial for patients with tuberculosis meningitis, tuberculous pleural, and tuberculous ascites to have a satisfactory outcome. Sensitive biochemical indicators, such as ADA, INF-α, TNF-α, and IL-1, are present in bodily fluids such CSF, pleural fluid, and peritoneal fluid. It has been observed that the measurement of ADA activity in these bodily fluids is a useful complement in the diagnosis of extrapulmonary tuberculosis. A technique for the differential diagnosis of many extrapulmonary TB manifestations, including pleuritis, synovitis, meningitis, etc., is the ADA assay in biological fluids.[8]

Tubercular effusion is usually characterized by a majority of lymphocytes or other mononuclear cells. In our study, 9 (12.16%) samples of tubercular ascitic effusion, pleural effusion 21 (27.27%) and cerebrospinal fluid 30 (30.61%) had >50 % lymphocytes, increased ADA, protein, and decreased sugar, whereas non-tubercular effusions 65 (87.84%) cases had <50% lymphocytes, increased ADA, and decreased sugar. Similar findings of raised ADA and lymphocytes were found in 30% and 34% cases of menigitis in studies conducted by Shinde *et al.*[9] and Mehta *et al.*[7] respectively In contrast, Gupta *et al.*[10] found tubercular ascitic effusion 41.48 %, tubercular pleural effusion 51.72%, and tubercular cerebrospinal fluid 42.29%.

It has been advised to utilize the ADA assay as a diagnostic procedure for TB peritonitis.[11]. Furthermore, in line with earlier studies, we discovered that mean ADA levels were considerably greater in patients with TB peritonitis compared to patients with non-TB peritonitis.[12-15]

CONCULSION

Many tests are available for diagnosis of tuberculosis, but analysis of fluid ADA level along with other parameters of sample can provide the clinical information about the fluid etiology and refer him to final diagnosis and treatment options. In developing countries like India, where tuberculosis is endemic and resources are the limited in the evaluation and diagnosis of tuberculosis and low socioeconomic status of patient, simple laboratory investigations of body fluids in co-relation with lymphocyte percentage compared with ADA values, protein, and sugar value will help to diagnose tuberculosis. However false-positive and false-negative results may be there. These controversial cases have to be co-relate clinically and evaluated with expensive diagnostic modalities such as PCR and gamma interferon.

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