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Evaluation of Diagnostic Value of Adenosine Deaminase in Diagnosis of Tuberculous Pleural Effusion

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Abstract

Background: Many studies suggest that adenosine deaminase is a marker for tuberculous pleurisy, while controversy exists as to its diagnostic value, accurate diagnosis is essential for tuberculous effusion for initiation of treatment. So evaluation of diagnostic value of adenosine deaminase in diagnosis of tuberculous pleural effusion is important.

Aim of the Work: To evaluate the diagnostic value of adenosine deaminase in diagnosis of tuberculous pleural effusion from non tuberculous pleural effusion.

Subject and Methods: Forty patients with pleural effusion were admitted to Giza chest hospital, Bab El-Sha'eria and Al-Hussein Al- Azhar University Hospital and after taking a written informed consent from the patients during period between April 2014 and May 2016.

All patients were subjected to full history and clinical examination, Plain chest X-ray, Sputum examination for Acid Fast Bacilli, ADA in pleural fluid, pleural biopsy and Culture and sensitivity of pleural fluid. Those patients were classified into (2) groups: Group (I): Twenty (20) patients with tuberculous pleural effusion. Group (2): Twenty (20) patients without tuberculous pleural effusion.

Results: Patients with tuberculous pleural effusion had significantly high ADA level in pleural fluid than patients with non-tuberculus effusion (parapneuomonic and malignant effusion) with $p<0.001$ with cutoff point in pleural fluid was 68.8 IU/l, sensitivity and specificity were 90% and Positive predictive value was 90%.

Conclusion: Adenosine deaminase ADA can be used in diagnosis tuberculous pleural effusion with significantly increase ADA level in pleural fluid than those with malignant, parapneumonic effusion. So Adenosine Deaminase ADA is a non invasive, inexpensive and repeatable test provides the results quickly which help to start early treatment.

Key Words: *ADA – Adenosine deaminase – Tuberculous effusion.*

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Introduction

PLEURAL effusion is fluid in the pleural space and categorized as either transudate or exudate [1].

Tuberculous pleurisy one of extra-pulmonary tuberculosis [2]. Diagnosed by pleural tissue biopsy and pleural fluid examination. And the culture for mycobacterium in pleural fluid has a relative lower success rate (36%) [3]. The diagnosis is difficult in as many as 20% of cases [4]. And cut-off value of ADA is still to be studied [5].

Aim of the work: To evaluate the diagnostic value of adenosine deaminase in etiological diagnosis of pleural effusion.

Subjects and Methods

This study was conducted on Giza chest hospital, Bab El-Sha'eria university Hospital and Al-Hussein University Hospital after recording written consent from the patients during period between April 2014 and May 2016 and includes 40 patients.

Forty patients classified into two groups:

Group (I) : (20) patients with tuberculous pleural effusion diagnosed clinical, radiological, bacteriological and also by pleural biopsy.

Group (2) : (20) Patients with Non tuberculous pleural effusion diagnosed clinical, radiological, bacteriological and also pleural biopsy, and include 15 cases with malignant effusion and 5 cases with parapneumonic effusion.

ALL patients underwent the following:

History taking clinical examinations, plain chest X-ray, Sputum for AFB, Laboratory investigations ,Complete Blood Picture, kidney function, liver function, random blood sugar, Tuberculin

skin test, ADA in pleural fluid, Histopathological examination (pleural biopsy either open or Abram's) and Culture of pleural fluid.

50mL of pleural fluid or more was collected in a syringe. Portion of the sample was taken for cytological examination, and measurement of protein, lactate dehydrogenase (LDH), and glucose, while the other portion for measurement ADA and early morning sputum sample from each patient on 3 successive days.

Closed pleural biopsy has been carried out for patients with tuberculosis and patients with malignancy by Abraham's or Coop's needle with a trocar and cannula at 3, 6, 9 o'clock (but not 12 to avoid injury of neurovascular bundle) or by thoracoscope.

Statistics: Statistical presentation and analysis of the present study was conducted by IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 13 (MedCalc© Software bvba, Ostend, Belgium). Normally distributed numerical variables presented as mean and SD and intergroup differences were compared by the unpaired *t*-test. The Welch test was used in place of the *t*-test when equality of variance of the two groups could not be assumed. Skewed data presented as median and interquartile range and differences compared by the Mann-Whitney test (for two-group comparisons) or the Kruskal-Wallis test (for multiple-group comparisons). Categorical data were presented as ratio or number and intergroup differences were compared by the Pearson chi-squared test. Receiver-operating characteristic (ROC) curve analysis was used to examine the value of ADA and other biomarkers in pleural aspirate. The DeLong method used to compare the area under ROC curves (AUC). And when p-value <0.05 was considered statistically significant.

Diagnostic criteria:

1- Patient considered a case of tuberculous effusion if any one of the following present histopathology showing Caseating granuloma or positive sputum culture for M.tuberculosis without other cause for exudative pleural effusion.

2- Patient considered with malignant pleural effusion when malignant tissue in the pleural cavity was shown by pleural biopsy or Cytopathology of the pleural fluid.

3- Parapneumonic effusion by clinical suspicion, laboratory diagnosis, culture and sensitivity for effusion.

Results

Forty patients included in the study twenty of them with tuberculous effusion and twenty with non tuberculous effusion as follows parapnemonic 5 cases and malignant effusion 15 cases (Table 2).

There is high statistical significant difference between groups in age. Tuberculous pleural effusion cases were younger than the other nontuberculous pleural effusion group (p 0.001 high significant). Tuberculosis cases were younger than the other groups. (Malignant effusion versus Tuberculous effusion p=0.001 high significant). (Parapneumonic effusion versus Tuberculous effusion p=0.001 high significant). (Malignant effusion versus Para-pneumonic effusion p=0.752 non-significant) in (Table 3). Sex distribution among cases male 18 and female 22 in (Table 4). There is significant difference between groups in SGOT and SGPT. Cancer cases recorded the highest readings with (p=0.008) (p=0.024) for both in (Table 5).

There is high significant difference between two groups in ADA pleural fluid (p=0.0001) also there was statistical significant difference between two groups in protein level of pleural fluid (p= 0.006) in (Tables 6,7)

There is statistically elevated Mean ADA level in pleural aspirate, IU/l 96.5 IU/l in tuberculosis cases and 13.0 IU/l in parapneumonic cases and 48 IU/l in malignant cases with p-value <0.001 in (Table 8).

There was high statistical significant difference between two groups in ADA (p<0.0001) tuberculous effusion cases recorded higher levels of ADA than the other groups. There was no difference between cases with cancer and pneumonia in ADA. And low significant difference in protein (p=0.001) between two groups in (Table 8).

ROC (Receiver Operating Characteristic) curve was used to obtain the cut-off points and the likelihood ratios (LRs) of ADA and show the cut-off value at >68.8 IU/l for diagnosis of cases with tuberculous pleural effusion (Table 9).

There was statistical significant difference between ADA cases Versus LDH in diagnosis of tuberculus effusion in (Table 10).

Discussion

Pleural effusion is the presence of fluid in the pleural space and categorized as either transudate or exudate [1]. And the exudative pleural effusions

Table (1): Characteristics of the whole study population.

Variable	Metric
Age, yr	42.0 (15.0)
Gender, M/F	18/22
Smokers, n (%)	16 (40%)
Fasting blood sugar, mg/dl	96.5 (88.0–116.5)
Blood urea nitrogen, mg/dl	29.7 (10.1)
Serum creatinine, mg/dl	0.82 (0.17)
SGOT, IU/l	14.5 (9.0–23.0)
SGPT, IU/l	14.5 (9.0–21.5)
Total serum bilirubin, mg/dl	0.71 (0.11)
LDH level in pleural aspirate, IU/l	562.5 (476.5-683.5)
Protein level in pleural aspirate, mg/dl	4.2 (3.8–4.9)
ADA level in pleural aspirate, IU/l	69.9 (31.1–96.5)

Data presented as mean (SD), ratio, number or median (interquartile range).

Table (2): Distribution of cases in the study groups

Distribution of cases in the study groups			
Tuberculous effusion	20 (50%)		
Non tuberculous effusion:			
Para-pneumonic	5 (12.5%)		
Malignant effusion:			
Metastatic adenocarcinoma	4 (10%)	15 (37,5%)	20 (50%)
Epithelial mesothelioma	11 (27.5%)		

Data presented as mean (SD), ratio, number, or median (interquartile range).

Table (3): Age distribution among the study group.

Variable	Non-tuberculous pleural effusion (n=20)		Tuberculous pleural effusion (n=20)	*p*-value
	Malignant effusion	Para-pneumonic effusion		
Age, yr	52.73±9.41	45.4±10.78	30.85±10.49	<0.001¶
	53.2±9.5		30. 8±10.4	<0.001

Data are presented as mean (SD), ratio, or number (%), ¶Unpaired *t*-test and §Pearson chi-squared test.

Table (4): Sex distribution among the study group.

Variable	Non-tuberculous pleural effusion (n=20)		Tuberculous pleural effusion (n=20)		*p*-value
Male	8	40%	10	50%	0.525§
Female	12	60%	10	50%	

Table (5): Laboratory results of patients with tuberculous or non-tuberculous pleural effusion.

Variable	Non-tuberculouspleural effusion (n=20)	Tuberculous pleural effusion (n=20)	*p*-value
Fasting blood sugar, mg/dl	108.5 (90.0–121.0)	92.0 (85.5–107.0)	0.088¶
Blood urea nitrogen, mg/dl	32.1 (13.0)	27.4 (5.1)	0.142§
Serum creatinine, mg/dl	0.82 (0.20)	0.81 (0.13)	0.912¥
SGOT, IU/l	17.5 (12.5–29.0)	9.0 (8.0–18.0)	0.008¶
SGPT, IU/l	17.5 (12.0–28.0)	10.0 (8.0–16.5)	0.024¶
Total serum bilirubin, mg/dl	0.71 (0.12)	0.72 (0.11)	0.781#

Data are presented as median (interquartile range) or mean (SD), ¶Mann-Whitney test, §Welch test, Unpaired *t*-test, Unpaired *t*-test.

Table (6): Result of analysis of pleural fluid obtained from patients with tuberculous or non-tuberculous pleural effusion.

Variable	Non-tuberculouspleural effusion (n=20)	Tuberculous pleural effusion (n=20)	*p*-value
LDH level in pleural aspirate, IU/l	544.0 (428.0–696.5)	562.0 (490.0–666.0)	0.695¶
Protein level in pleural aspirate, mg/dl	3.9 (3.4–4.3)	4.7 (3.9–5.1)	0.006¶
ADA level in pleural aspirate, IU/l	44.1 (21.68–59.5)	96.5 (88.3–135.0)	<0.000 1¶

Data are presented as median (interquartile range), ¶Mann-Whitney test.

Table (7): Result of analysis of pleural fluid obtained from patients with tuberculous effusion, Para-pneumonic, metastatic adenocarcinoma, or mesothelioma.

Variable	Non-tuberculous pleural effusion (n=20)	Tuberculous pleural effusion (n=20)			*p*-value
		Pneumonia (n=5)	Metastatic effusion (n=4)	Mesothelioma (n=11)	
LDH level in pleural aspirate, IU/l	562.5 (490.0–666.0)	650.0 (551.5–674.3)	603.0 (426.5–789.0)	510.0 (424.0–864.5)	0.842¶
Protein level in pleural aspirate, mg/dl	4.7 (3.9–5.1)†‡	3.2 (3.1–3.7)	3.7 (3.3–4.1)	4.2 (3.8–4.5)†	0.003¶
ADA level in pleural aspirate, IU/l	96.5 (88.3–135.0) †‡§	13.0 (10.3–75.0)	21.68 (17.0–25.4)	55.0 (42.2–60.3)	<0.001¶

Data are presented as median (interquartile range) and ¶Kruskal-Wallis test.
†*p*-value <0.05 vs. pneumonia (Conover test).
‡*p*-value <0.05 vs. Metastatic effusion (Conover test).
§*p*-value <0.05 vs. Mesothelioma (Conover test).

Table (8): Result of analysis of pleural fluid obtained from patients with tuberculous, Para-pneumonic, or malignant effusion including both (metastatic adenocarcinoma and mesothiloma).

Variable	Tuberculous effusion (n=20)	Non-tuberculous pleural effusion (n=20)		*p*-value
		Para-pneumonic effusion (n=5)	Malignant effusion (n=15)	
LDH level in pleural aspirate, IU/l	562.5 (490.0–666.0)	650. (551.5–674.3)	510.0 (424.0–831.3)	0.718
Protein level in pleural aspirate, mg/dl	4.7 (3.9–5.1)†‡	3.2 (3.1–3.7)	4.0 (3.7–4.3)†	0.002¶
ADA level in pleural aspirate, IU/l	96.5 (88.3–135.0) †‡§	13.0 (10.3–75.0)	48.0 (27.2–57.5)	<0.001

Data are presented as median (interquartile range)and ¶Kruskal-Wallis test.
†*p*-value <0.05 vs. Post- pneumonic effusion (Conover test).
‡*p*-value <0.05 vs. Malignant effusion (Conover test).

Table (9): Receiver-operating characteristic (ROC) curve analysis for the diagnosis of tuberculous pleural effusion using ADA, LDH, or protein level in pleural aspirate.

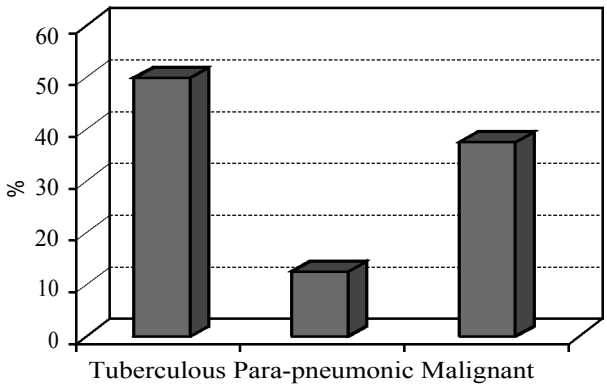
ROC curve parameter	ADA	LDH	Protein
Area under the ROC curve (AUC)	0.895	0.536	0.755
95% CI for AUC	0.764 to 1.000	0.348 to 0.724	0.598 to 0.912
p-value (AUC0=0.5)	<0.0001	0.705	0.001
Youden index J	0.8	0.25	0.45
Best cutoff criterion	>68.8 IU/l	>436 IU/l	>4.5 mg/dl
Sensitivity, %	90	95	55
95% CI for sensitivity	68.3–98.8	75.1–99.9	31.5–76.9
Specificity, %	90	30	90
95% CI for specificity	68.3–98.8	11.9–54.3	68.3–98.8
PPV, %	90	57.6	84.6
95% CI for PPV	68.3–98.8	39.2–74.5	54.6–98.1
NPV, %	90	85.7	66.7
95% CI for NPV	68.3–98.8	42.1–99.6	46.0–83.5

PPV:Positive predictive value, NPV: Negative predictive value.

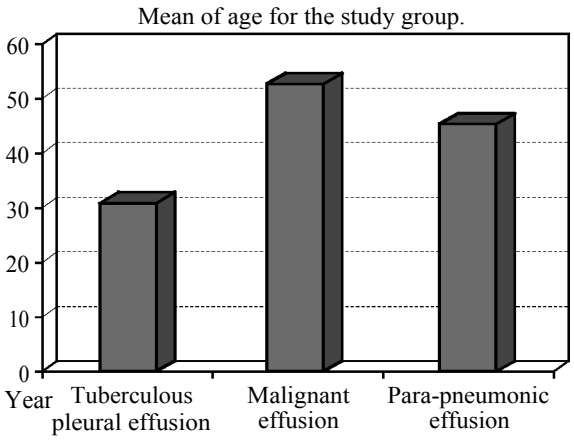
Table (10): Comparison of the receiver-operating characteristic (ROC) curves for the diagnosis of tuberculous pleural effusion using ADA, LDH, or protein level in pleural aspirate.

Comparison	ΔΔAUC	95% CI for ΔΔAUC	*p*-value
ADA vs. protein	0.140	–0.053 to 0.333	0.154
ADA vs. LDH	0.359	0.117 to 0.601	0.004
Protein vs. LDH	0.219	–0.018 to 0.456	0.071

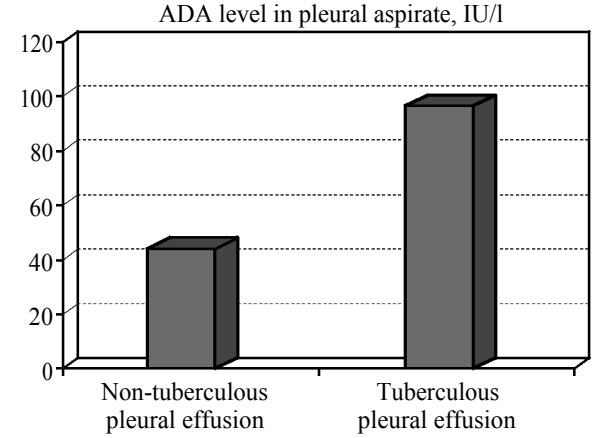
ΔΔAUC: difference between areas under the ROC curve.



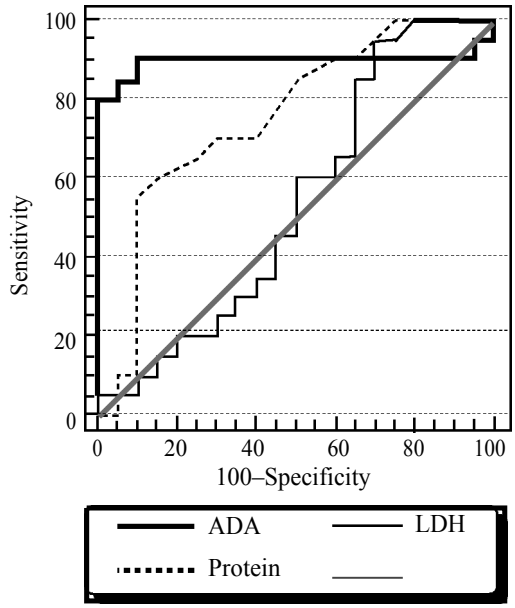
Histogram (1): Distribution of cases in the study groups.



Histogram (2): Age distribution among the study group.



Histogram (3): ADA level in pleural aspirate, IU/l.



Histogram (4): Receiver-operating characteristic (ROC) curves for the diagnosis of tuberculous pleural effusion using ADA, LDH, or protein level in pleural fluid.

are serious and difficult to treat. So there is a need for simpler test to help in diagnosis of pleural effusion [6] . There is a long list of causes including: Infection (Para pneumonic) with its causative organisms (bacterial, viral, parasitic), malignancy (pleural tumors, metastatic effusion), pulmonary embolism, abdominal diseases (sub phrenic abscess hepatic amebiasis), collagen vascular diseases (SLE, rheumatoid arthritis) and others (drugs, sarcoidosis, asbestosis [7] .

And aim of this study is to assess role of adenosine deaminase enzyme level in pleural fluid to differentiate between cases with tuberculous pleural effusion and cases with non tuberculous pleural effusion.

This study was carried out on (40) patients, selected from chest department in Al-Hussein University Hospital, Bab El-Sha'eria university Hospital and & Giza chest hospital after recording written consent from the patients during period between April 2014 and May 2016.

In the present study there 40 patients with mean age 30.8± 10.4 years for tuberculous pleural effusion, 53.2±9.5 years for non tuberculous pleural effusion including 45.4± 10.78 years for Para-pneumonic pleural effusion and 52.73 ±9.41 years for malignant pleural effusion in Table 3 this shows tuberculous pleural effusion common in young age and malignant pleural effusion present in mean

age 52,7 years and his in agreement with Valdes et al study who found that the mean age of tuberculous group was 33.9±13.2 years and that of malignant group was 45.5±16.8 years **[8]**.

In the present study there is 18 patients were male and 22 were female, 10 male, 10 female in tuberculous effusion group, 8 male and 12 female in non tuberculous effusion group with no statistical significance in Table (4).

There is elevation in median level of LDH in tuberculous pleural effusion 562 IU/l less than Para-pneumonic effusion 650 IU/l, Metastatic effusion 603.0 IU/l without statistical significance (Table 8).

There is elevation in median level of protein in tuberculous pleural effusion 4.7 mg/dl than the median level of protein in Para-pneumonic, metastatic effusion and pleural effusion due to mesothelioma with statistical significance and *p*-value 0.003 (Tables 7,8).

There is elevation in median level of ADA in tuberculous pleural effusion 96.5 IU/l than the median level of ADA in Para-pneumonic, metastatic effusion and pleural effusion due to mesothelioma with statistical significance *p*-value <0.001 Tables (7,8). These results agree with values obtained by Petterson t et al study who reported that the ADA activity increases in tuberculous pleural effusion in average 90 and 100 IU/I **[9]**.

These results are in agreement with the results obtained by Mohd et al., who found that protein concentration in pleural fluid of more than 5 gm/dl was seen in 64% and found that elevated lactic acid dehydrogenase (LDH) of more than 400 U/L was seen in 77% patients with pleural fluid TB **[10]**.

In the present study the Best cutoff criterion for ADA >68.8 IU/l is highly suggestive to tuberculous pleural effusion with sensitivity 90%, specificity 90%, positive predictive value 90% and negative predictive value90% with Area under the ROC curve AUC 0.895 and *p*-value <0.0001 (Table 9).

These results are in agreement with results obtained by Orphanidou et al., study who reported that sensitivity (87.3%) and specificity (91.8%) **[11]** and also are in agreement with Banales et al., study they reported sensitivity was found as 98%, specificity 96%, PPV 94% and NPV 99% for the cutoff value of ADA 70 U/L with a mean±SD 123.25±39.4 **[12]** and with Reechaipichitkul et al., study who found sensitivity 98%, specificity 96%, PPV 94% and NPV 99% for the cutoff value of ADA 70 U/L with a mean±SD 123.2±39.4 **[13]** and with Jimenez et al., study who reported that elevated pleural fluid ADA level predicts tuberculous pleural effusions with a sensitivity of 90% and a specificity of 89%. They reported cutoff value for ADA varies from 47 to 60 UI/L **[14]**.

Perez-Rodriguez, et al., found that pleural fluid ADA activity has a valuable biochemical marker that has a high sensitivity and specificity for pleural tuberculosis diagnosis **[15]**.

In a review study by Krenke and Korczyn´ski on adenosine deaminase and interferon gamma in diagnosis of pleural effusion, they found an important role for both in diagnosis of tuberculous pleurisy **[16]**.

Conclusion:

Patients with tuberculous pleural effusion had higher ADA level in pleural fluid than patients with non-tuberculous effusion (para-pneuomonic and malignant effusion) $p<0.001$) with cutoff point was 68.8 IU/l, with sensitivity and specificity were 90% and positive predictive value was 90%.

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تقييم القيمة التشخيصية للأدينوزين دى أمينيز فى تشخيص الانصباب البلورى السلى

إن الانصباب البللورى يعد مشكلة شائعة كثيرا ما تواجه اطباء الأمراض الصدرية وهو لا يعد مرضا فى حد ذاته ولكنه علامة لكثير من الأمراض، و من الضرورى البحث عن طريقة دقيقة وسريعة لتشخيص أسباب الانصباب البللورى.

ومن هنا جاءت هذه الدراسة لتقييم إحدى هذه الطرق وهى استخدام إنزيم الأدينوزين دى أمينيز لتشخيص الانصباب البللورى السلى واللا سلى عن طريق قياس معدله فى السائل البللورى.

وقد أجريت هذه الدراسة على أربعين مريضاً تم دخولهم قسم الأمراض الصدرية بمستشفى الحسين الجامعى، ومستشفى باب الشعرية الجامعى ، ومستشفى صدر الجيزة بالعمرانية في الفترة من أبريل ٢٠١٤ وحتى مايو ٢٠١٦ وبعد أخذ موافقة كتابية من المرضى. وتم تقسيمهم إلى مجموعتين :

المجموعة الأولى : عشرون مريضاً مصابون بالانصباب البللورى السلى (الدرنى) .

المجموعة الثانية : عشرون مريضاً مصابون بالانصباب البللورى اللا درنى.

وقد أشارت الدراسة إلى :إرتفاع معدل إنزيم الأدينوزين دى أمينيز بشكل إحصائى ملحوظ فى السائل البللورى فى المجموعة الأولى.

ولهذا أوضحت النتائج أنه : من الممكن استخدام قياس معدل الأدينوزين دى أمينيز فى السائل البللورى فى تشخيص وتفريق الانصباب البللورى السلى واللاسلى.

وقد أوصت هذه الدراسة : باستخدام قياس الإنزيم كإجراء روتينى لتشخيص الدرن الموجود فى الغشاء البللورى لعدة أسباب منها : انخفاض سعر تكلفة قياس الإنزيم ، وسهولة استخدامه ، بالإضافة الى وجود بعض الحالات التى يصعب فيها سحب عينة من الغشاء البللورى للفحص الباثولوجى.