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Evaluation of Diagnostic Value of Urinary Deep Stick in Differentiating Different Types of Exudative Pleural Effusions and Differentiating Exudative Pleural Effusions From Transudates

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Abstract **Objective:** Rapid diagnosis is one of the key factors in pleural effusion management. Dipstick has been suggested for use in other body fluids, while its role in pleural effusion has not yet been confirmed. This study was conducted with the aim of investigating the diagnostic value of dipstick in differentiating types of pleural effusion. **Methods:** This prospective study was conducted on 70 patients diagnosed with pleural effusion requiring thoracentesis in Ahvaz teaching hospitals in 2022. Microbiological and cytological laboratory tests were performed on pleural fluid samples. At the same time, pleural fluid sample was evaluated with urine dipstick. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of dipstick parameters in differentiating types of pleural effusion were calculated in comparison with the standard diagnostic method. **Results:** Dipstick protein test has the sensitivity 100%, specificity 96.23%, PPV 89.5%, and NPV 100% for differentiated exudative from transudative effusions and sensitivity, specificity, PPV and NPV 40%, 100%, 100% and 61.5% respectively in detecting infectious exudative effusions. Sensitivity, specificity, PPV and NPV of Dipstick leukocyte esterase test in differentiating exudative from transudative effusions were 100%, 56.6%, 42.5% and 100%, respectively, and in detecting different types of exudative effusions, sensitivity, specificity, PPV and NPV were all 100%. The Dipstick glucose test effectively differentiated exudative from transudative effusions (sensitivity, specificity, PPV and NPV 82.35%, 79.25%, 56.0% and 93.3% respectively) and detected infectious exudative effusions (sensitivity, 93.33% specificity, 93.30%; PPV, 93.3%; NPV, 91.3%). Sensitivity, specificity, PPV and NPV of dipstick pH test in differentiating exudative from transudative effusions were 100%, 64.15%, 76.9% and 100% respectively and for different types of exudative effusions were 86.67 % was 65.22%, 76.5% and 78.9%, respectively. **Conclusion:** Dipstick strip was effectively accurate in detecting transudative from exudative and infectious from noninfectious exudative effusions. Therefore, this accurate, rapid, easy to use, and inexpensive tool could be used to for distinguishing different types of pleural effusion in bedside which especially could be helpful in resource limited setup.

Key Words dipstick, reagent strip, pleural effusion, exudative, transudative

1. Introduction

Pleural effusion is caused by the accumulation of fluid in the pleural cavity, which can occur by itself or due to the disease of the surrounding parenchyma, such as heart failure, infection, malignancy, pulmonary embolism, and inflammatory conditions [1]. Pleural effusion is one of the main causes of mortality and pulmonary morbidity [2], [3].

Pleural effusion is divided into two types, transudative and exudative, and the first step in the etiological diagnosis of pleural effusion is the distinction between exudative and transudative types [4] which are distinguished in the clinical context using Light's criteria [5].

Based on this criterion, there is at least one of the following findings in exudative fluids: the ratio of pleural fluid protein to serum protein is more than 0.5, the ratio of pleural fluid lactate dehydrogenase (LDH) to serum LDH is more than 0.6,

the level of fluid LDH pleural more than two thirds of the highest normal limit of serum LDH; None of these criteria exist in transudative fluids [6], [7].

Pleural effusion can occur in a wide variety of complex conditions and there are more than 60 known causes for it [1]. Also, many cases of pleural effusion do not have a specific cause, so in addition to physical examination and radiological evaluations, a detailed analysis of pleural fluid is necessary to diagnose the cause and treat it [1], [8], such as the evaluation of protein level [8], [9], pH and glucose [10], LDH, cytology and pleural fluid microbiology are included in common clinical guidelines for the diagnosis and treatment of pleural effusion [1], [8].

Delay in the diagnosis of pleural effusion can be associated with significant complications and mortality [2]. Therefore, rapid diagnosis is a key factor in the management of pleural effusion. Rapid tests help in early diagnosis, immediate treatment, prevention of complications and reduction of transmission of infectious diseases.

Dipstick or leukocyte esterase reagent (LER) strip for use in other body fluids, including ascites and cerebrospinal fluid, for rapid diagnosis of spontaneous bacterial peritonitis (SBP) [11], [12], meningitis [13], [14] and urinary tract infection [15], [16] is proposed.

However, the diagnostic function of dipstick in the assessment of pleural fluid has not yet been fully investigated, test strips to determine the amount of protein and leukocyte esterase in pleural fluid, two parameters that are of particular importance in the diagnosis of pleural types, have been less evaluated [6], [7].

Considering that the role of urinary dipstick in the diagnosis of pleural effusion has not yet been confirmed, this study was conducted with the aim of investigating the diagnostic value of urine dipstick in quickly differentiating exudative pleural effusion from transudate, as well as infectious exudative pleural effusion from non-infectious.

2. Method and Materials

This prospective study was conducted on patients diagnosed with pleural effusion requiring thoracentesis in the pulmonary department of teaching hospitals under the supervision of Jundishapur University of Ahvaz (including Imam Khomeini, Golestan and Razi hospitals) in 2022. The ethical committee of Ahvaz Jundishapur University of Medical Sciences has approved this study (Ethics number: IR.AJUMS.HGOLESTAN.REC.1401.182). The Declaration of Helsinki outlines basic ethical principles to protect human research subjects.

A. Sample size and sample characteristics

The sample size is based on the same article (17) in which the dipstick sensitivity for detecting infectious pleural effusion was reported to be 90% compared to the gold standard (P=0.9), taking into account the confidence factor of 95% and the accuracy of 0.5 0 was calculated using the following formula equal to 70 individuals.

$$N = \frac{Z^2_{1-\frac{\alpha}{2}}\ P(1-P)}{d^2}$$

At first, the pleural fluid samples of 88 patients with pleural effusion were included in the study by purpose-based sampling method. But based on the inclusion and exclusion criteria, 70 patients were included in the final analysis.

The inclusion criteria were: clinical diagnosis of pleural effusion by a lung specialist and confirmed by imaging findings and requiring thoracentesis, normal coagulation tests (INR less than or equal to 2), absence of thrombocytopenia (platelet level less than 100,000 per microliter) and patient consent to participate in the study. Also, patients with coagulopathy, frequent pleural effusion, antibiotic use in the recent past (one month) and any defects in file information and laboratory results were excluded from the study.

B. Etiological diagnosis of pleural effusion

After obtaining informed consent, history and clinical examination, routine examinations were performed in all patients. Epidemiological and clinical information of the patients, including sex, age, co-morbidities at the time of thoracentesis were recorded. Pleural effusion was diagnosed by a pulmonologist based on the absence of breathing sounds on auscultation, dullness on accuracy and reduction of tactile fremitus and confirmed on the basis of radiological findings.

Based on the type of pleural effusion, the patients were divided into 3 groups: infectious exudative pleural effusion, non-infectious exudative pleural effusion, and transvasive pleural effusion. The cause of pleural effusion was determined based on clinical findings and laboratory results of pleural fluid and serum.

Exudative and transudative effusions were defined based on Light's criteria. The ratio of pleural fluid protein to serum protein, lactate dehydrogenase ratio less than 0.6 was used to differentiate exudative from transudative [5].

C. Thoracentesis and assessment of pleural fluid by dip stick

All patients underwent ultrasound-guided pleural aspiration (USG). Thoracentesis was performed using an 8 mm needle and a sample of pleural fluid was sent to the laboratory for biochemical evaluations (protein, glucose and lactate dehydrogenase, pH) and microbiological and cytological evaluations. Simultaneously, pleural fluid was evaluated using a leukocyte esterase reagent strip designed for urine testing to measure protein, leukocyte esterase, pH, and glucose.

A drop of non-centrifuged pleural fluid collected in heparinized tubes was placed on the leukocyte label of the strip. After exactly 2 minutes, the color change in the strip was visually read. The results of the reagent strip were read using a calorimeter: on a scale of 6 degrees (grade 0 to +4) for protein evaluation, on a scale of 5 degrees (grade 0 to +3 based on the density of purple color) for leukocyte esterase level, on a scale of 5 degrees (grade 0 to +3) for glucose level, as values for pH (range 8.50-6, with 0.5 intervals) and

numerical values for specific gravity (dark green 1.005, light green 1.015, brown 1.02, orange 1.03).

All analysis of the dipstick results was done by a researcher who was unaware of the results of the biochemical and microbiological tests. A dip stick protein grade higher than +3 was considered exudative and a leukocyte grade higher than +2 was considered infectious. Finally, the diagnostic performance of the leukocyte esterase strip test was compared with the standard diagnostic method (laboratory and microbiological findings).

D. Statistical Analysis

Statistical analysis was done by SPSS software version 26 (SPSS Inc, Chicago, IL, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for the normality of data. Central and descriptive statistics were reported for quantitative. Analytical analyzes were performed using Kruskal-Wallis non-parametric and chi-square test tests. P value<0.05 was considered statistically significant.

To check the diagnostic performance of urinary dipstick parameters, ROC curve was drawn and areas under the curve (AUC) were determined in 95% confidence interval (CI). To determine the diagnostic performance of the parameters of the reagent strip in the diagnosis of pleural effusions at the optimal cut-off point, sensitivity percentage, specificity, positive predictive value (PPV), negative predictive value (NPV) were reported. ROC analysis was performed using MedCalc software (MedCalc Software Bvba).

3. Results

In this study, 70 patients diagnosed with pleural effusion requiring thoracentesis, with mean age of 58.64 ± 23.04 years (range 16 to 96 years), including 37 males (52.9%) and 33 females (47.1%) participated (Table 1).

Most of the patients (84.29%) were hospitalized in ICU due to acute respiratory failure. According to laboratory reports, 17 patients (24.29%) had transudative pleural effusion and 53 patients (75.71%) had exudative pleural effusion.

The underlying causes of transudative effusion included kidney failure, pulmonary embolism, heart failure, and liver cirrhosis. Patients with exudative pleural effusion included 30 cases (56.60%) of infectious exudative (pneumonia, tuberculosis, empyema) and 23 cases (43.40%) of non-infectious exudative (including malignancies, lupus erythematosus, kidney disease).

The results of laboratory evaluations showed that the number of leukocytes and protein level of pleural fluid were the highest in patients with pleural effusion and infectious exudate and the lowest in transudative patients (P< 0.0001 and P= 0.001, respectively) (Table 2).

The results of pleural fluid evaluation by dipstick showed that bilirubin (P=0.092) and ketone (P=0.799) tests were not related to the type of pleural effusion (Table 3). Also, the result of nitrite test was negative in all pleural effusion patients (100%). Protein test of reagent strip in all patients with transudative pleural effusion showed +1 and +2 results.

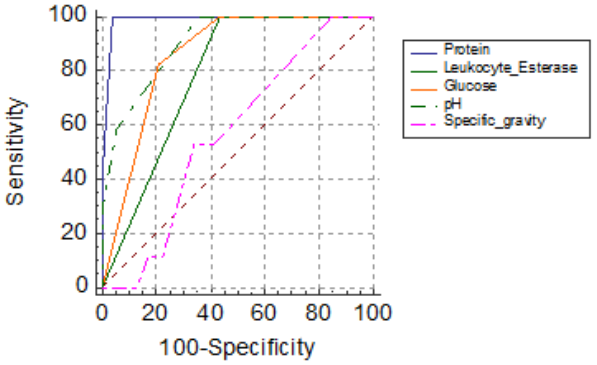

Figure 1: Comparing the diagnostic performance of different urinary dipstick parameters in differentiating exudative pleural effusion from transudation

Also, in 96.22% of patients with exudative pleural effusion, it was grade +3 or more.

Leukocyte esterase test was negative in all patients with transudative pleural effusion as well as patients with non-infectious exudative effusion. In all patients with infectious exudative pleural effusion, LER grade was +2 and +3. Glucose test strip was +2 and +3 in all patients with transudative effusion. The pH test of the reagent strip showed values greater than or equal to 8 in all patients with transudative pleural effusion and greater than or equal to 7 in patients with non-infectious exudate. In infectious exudative patients, the result of pH test was between 6 and 8.

The results of the analysis of the area under the ROC curve showed that protein tests (AUC: 0.989; P < 0.0001), leukocyte esterase (AUC: 0.783; P < 0.0001), glucose (AUC: 0.858; P < 0.0001) P<0), and the pH of the reagent strip (AUC: 0.906; P<0.0001) were effective in differentiating types of exudative pleural effusions from transudates compared to the standard method (laboratory findings and microbiological culture) (Table 4 and Figure 1). However, pleural fluid specific gravity test was not effective in differentiating types of exudative pleural effusion from transudate (AUC: 0.568; P=0.312).

The results of the analysis of the area under the ROC curve also showed that protein tests (61.5%; AUC: 0.726; P<0.0001), leukocyte esterase (AUC: 1.000; P<0.0001), glucose (AUC: 0.935; P<0.0001) and reagent strip pH (AUC: 0.838; P<0.0001) were effective in differentiating infectious exudative effusion from non-infectious (Table 5 and Figure 2). However, pleural fluid specific gravity test did not have a significant diagnostic function in differentiating infectious exudative from non-infectious (AUC: 0.616; P=0.122).

4. Discussion

The results of the present study showed that the leukocyte esterase test was negative in all patients with transudative pleural effusion and non-infectious exudative effusion, and cases with infectious pleural effusion had a leukocyte esterase grade of more than 2.

Variable		Result
Age (years), S.D± mean		58.64±23.0
Gender, frequency (%)	female	37 (52.9)
	Male	33 (47.1)
Symptoms at the time of visit, frequency (%)	shortness of breath (dyspnea)	59 (84.29)
	Chest pain	52 (74.29)
	Dry cough	21 (31.43)
*Cause for hospitalization in ICU, frequency (%)	Acute respiratory failure	59 (84.29)
	Cardiogenic pulmonary edema	12 (17.14)
	Acute renal failure	14 (20.0)
	Pulmonary embolism	13 (18.57)
	Shock	17 (24.29)
	Coma	5 (7.14)
Clinical infection, frequency (%)		30(42.9)
Need for mechanical ventilator, frequency (%)		41(58.57)
Type of pleural effusion, frequency (%)	transuda (non-infectious)	17 (24.3)
	Infectious exudate	30 (42.9)
	Non-infectious exudate/malignancies	23 (32.9)
* There may be more than one reason for each patient.		

Table 1: Basic characteristics of the studied patients

P-value*	Transudative	Infectious exudative	Non-infectious exudative	Laboratory Test	
<0.0001	675.68±163.87	4674.44±685.87	2450.00±424.57	mean±SD	WBC(µL-1)
	700 (400-1200)	5270 (2800-7400)	2100 (1880-3200)	median (IQR)	
0.001	1.94±0.36	3.62±0.28	3.39±0.29	mean±SD	Protein (g/dl)
	1.20 (1.0-1.8)	2.70 (2.50-4.30)	3.70 (2.40-4.40)	median (IQR)	
<0.0001	258.06±13.09	70.91±18.19	201.76±25.46	mean±SD	Glucose (mg/dl)
	280 (250-292)	77 (19-91)	200 (96-300)	median (IQR)	
WBS: White blood cells; IQR: Interquartile range (25th–75th percentile).					
*: Kruskal-Wallis test					

Table 2: The results of laboratory tests in types of pleural effusion

P-value*	Transudative	Infectious exudative	Non-infectious exudative	Laboratory Test	
0.092	9 (52.9)	22 (73.3)	20 (87.0)	Negative	Bilirubin
	2 (11.8)	2 (6.7)	0	+1	
	4 (23.5)	6 (20.0)	3 (13.0)	+2	
	2 (11.8)	0	0	+3	
0.799	4 (82.4)	24 (80.0)	20 (87.0)	Negative	Ketone
	3 (17.6)	6 (20.0)	3 (3.0)	Positive	
<0.0001	7 (41.2)	0	0	+1	Protein
	10 (58.8)	0	2 (8.7)	+2	
	0	18 (60.0)	21 (91.3)	+3	
	0	12 (40.0)	0	+4	
<0.0001	17 (100)	0	23 (100)	Negative	Leukocyte esterase
	0	0	0	+1	
	0	12(40.0)	0	+2	
	0	18 (60.0)	0	+3	
<0.0001	0	20 (66.7)	0	Negative	Glucose
	0	8 (26.7)	2 (8.7)	+1	
	3 (17.6)	0	12 (52.2)	+2	
	14 (82.4)	2 (6.7)	9 (39.1)	+3	
<0.0001	0	8 (26.7)	0	≤6	PH
	0	4 (13.3)	0	6.5	
	0	14 (6.7)	8 (34.8)	7	
	7 (41.2)	4 (13.3)	12 (52.2)	8	
	10 (58.8)	0	3 (13.0)	8.5≤	
* Chi-square test					

Table 3: The results of different urinary dipstick parameters in the diagnosis of pleural effusions

NPV	PPV	specificity	Sensitivity	P-value	AUC (95% CI)	Optimal Cut-off	Parameters of reagent strip
100	89.5	96.23	100	<0.0001	0.989 (0.928-1.000)	≤2	Protein
100	42.5	56.60	100	<0.0001	0.783 (0.668-0.873)	≤0	Leukocyte esterase
93.3	56.0	79.25	82.35	<0.0001	0-858 (0.754-0.930)	>2	Glucose
100	76.9	64.15	100	<0.0001	0.906 (0.813-0.963)	>7	PH
81.4	33.3	66.04	52.94	0.312	0.568 (0.444-0.686)	>1.008	Special Weight
AUC: Area under the ROC curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value							

Table 4: Diagnostic performance of dipstick parameters in differentiating exudative pleural effusions from transudates

NPV	PPV	specificity	Sensitivity	P-value	AUC (95% CI)	Optimal Cut-off	Parameters of reagent strip
65.1	100	100	40	<0.0001	0.726 (0.586-0.840)0	>3	Protein
100	100	100	100	<0.0001	1.000 (0.933-1.000)	>0	Leukocyte esterase
91.3	93.3	91.3	93.33	<0.0001	0.935 (0.832-0.984)	≤1	Glucose
78.9	76.5	65.22	86.67	<0.0001	0.838 (0.711-0.925)	7≥	PH
66.7	63.4	34.78	86.67	0.122	0.616(0.472-0.746)	≤1.010	Special Weight
AUC: Area under the ROC curve; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value							

Table 5: Diagnostic performance of dipstick parameters in differentiating infectious and non-infectious exudative pleural effusions

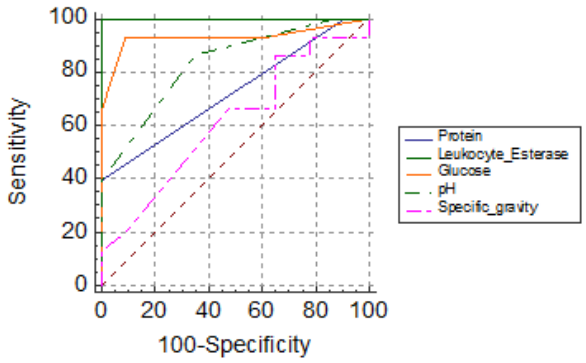

Figure 2: Comparing the diagnostic performance of different urinary dipstick parameters in differentiating infectious exudative pleural effusion from non-infectious

The analysis of the area under the ROC curve also showed that the leukocyte esterase test was effective in differentiating exudative pleural effusions from transudates (sensitivity and negative nasal value of 100%) as well as differentiating infectious from non-infectious exudatives. These results show that this test can be useful for quick and accurate diagnosis of infection in pleural effusion.

The leukocyte esterase dipstick test is designed and considered for the detection of leukocytes in urine, but it has also been used in other biological samples for rapid diagnosis of infection. This test uses the ability of esterase enzyme (which is present in polymorphonuclear leukocytes of pleural fluid) to break down heterocyclic carboxylates and form a pyrrole.

Then pyrrole reacts with diazonium salt and creates purple color in the reagent strip [17]. Since there are many causes of pleural effusion, the importance of ruling out infectious causes is essential for deciding on the use of antibiotics. If the cause of secondary effusion is due to reasons such as heart, liver or kidney failure, the use of diuretics is recommended and the unnecessary use of antibiotics should be avoided. Therefore, this test may be used as a bedside test, until the laboratory results are ready and available, it will be useful in making decisions and choosing the appropriate management strategy (treatment of heart failure or change in antibiotic treatment and pleural drainage).

In past studies, the high diagnostic power of evaluating ascites fluid by the LER reagent strip in diagnosing SBP infection has also been reported, so that false negative results were zero in most studies (specificity 100) [11], [18], [19].

However, the diagnostic performance of leukocyte esterase dipstick for identifying and differentiating types of pleural effusions has not been fully investigated and there are very few studies in this field; Among other things, in a study, Satheesnath et al. showed that the leukocyte esterase test has a good performance in detecting infectious exudative pleural effusion from non-infectious cases (sensitivity 75%, specificity 100%, positive predictive value 100% and negative predictive value 93.4 %) [20].

In the study by Azoulay et al. on patients with pleural effusion hospitalized in ICU, the evaluation of pleural fluid protein by dipstick had a high accuracy for the diagnosis of exudative pleural effusion from transudate (sensitivity 93.1%, specificity 50%, positive predictive value 3 84.84% and negative predictive value 71.5%). The level of pleural fluid protein evaluated by the reagent strip was in 84.3% of exudative patients grade >3, however, 50% of transudative patients also had protein grade >3 [21].

In the present study, the glucose test strip is useful in differentiating types of exudative pleural effusion from transudate (sensitivity, specificity, positive and negative predictive value, 82.35% and 79.25%, 56.0% and 93.3%, respectively) and It was also quite effective in differentiating infectious from non-infectious exudates (93.33% sensitivity; 93.30% specificity; 93.3% positive predictive value; 91.3% negative predictive value). In other studies, the evaluation of pleural fluid glucose has been confirmed for the diagnosis of parapneumonic effusions and has shown a better diagnostic performance than other pleural fluid biomarkers [22], [23].

Assessment of pleural fluid pH and glucose is recommended in common clinical guidelines for the evaluation of pleural effusions [10], [24]. Also, these two parameters are closely related, so that the pH of the pleural fluid is determined by the acids produced from the glucose metabolism of the cells in the pleural space, as well as the exit of those acids and the entry of glucose from the pleural membrane. The release of acids in the pleural fluid can be observed in acute inflammatory conditions (such as infection), chronic fibrotic conditions and pleural malignancies [25], [26].

In the present study, the pH test of the reagent strip was effective in differentiating types of exudative pleural effusions from transudates and also in differentiating infectious from non-infectious exudates. In other studies, the performance of reagent strips to determine the pH of pleural fluid has been

confirmed [27], [28].

In Azoulay et al.'s study, pleural fluid pH assessed by urinary dipstick was significantly lower in patients with infectious pleural effusion than non-infectious ones, but it did not have a significant diagnostic function for differentiating types of pleural effusions and classifying exudative effusions as infectious or non-infectious [21]. Therefore, the low pH of the pleural fluid, which is determined through a reagent strip or laboratory tests, can be helpful in the discussion of chest tube drainage.

Another finding of this study was that pleural fluid specific gravity test was not effective in differentiating types of exudative pleural effusion from transudate and also in differentiating infectious exudative from non-infectious exudative. In the study of Abdollahi et al, the evaluation of the specific gravity of pleural fluid by dip stick compared to the standard diagnostic method (Lite criterion) did not have enough sensitivity and specificity to distinguish pleural effusion exudate from transudate [29]. These results show that the use of this method in distinguishing types of pleural effusion is not acceptable and is not recommended.

In total, according to the results of the present study, among the five investigated parameters, the protein strip test had the best diagnostic performance in differentiating exudative pleural effusion from transudate at the ideal cutoff ≥ 2. Also, leukocyte esterase test strip is the most effective and accurate parameter for rapid diagnosis of infection and differentiation of infectious pleural exudation from non-infectious, and leukocyte esterase grade more than 2 indicates infectious pleural effusion.

Since the value of a diagnostic test lies in its ability to distinguish between disorders that are commonly confused, it seems that the primary use of these reagent strips is the differentiation of infectious (bacterial) effusions.

Therefore, based on these results, the use of urine dipstick can be valuable for quick diagnosis, especially in areas with limited facilities (technical or economic) or there is a time limit to perform standard laboratory analyzes in order to manage and treat patients as best as possible.

Finally, it should be mentioned that the present study was conducted for the first time in examining the diagnostic value of various urinary dipstick parameters in pleural effusion patients and obtained valuable information. But it was also faced with limitations, such as the fact that very few similar studies were found in the literature review, which made a complete and accurate comparison of the results impossible. Other limitations of the study include the small number of samples examined. Therefore, more reliable results can be obtained by conducting more studies with larger sample size.

5. Conclusion

The results of this study showed that the four parameters of urinary dipstick test including protein, leukocyte esterase, glucose and pH are accurate compared to the standard diagnostic method for differentiating exudative pleural effusion from transudate and also differentiating infectious exudative effusion from non-infectious. Therefore, reagent strips can speed up the diagnosis of the type of pleural effusion at the bed side. As a result, pleural fluid analysis with reagent strips can be used as a quick, easy, practical and inexpensive method to differentiate pleural effusion types, which can be especially helpful in areas with limited resources.

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Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

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