Thoracoscopic pleural cryobiopsy versus conventional forceps biopsy in diagnosis of exudative pleural effusion of unknown etiology

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Background Rigid forceps is commonly used for pleural biopsies during medical thoracoscopy in undiagnosed pleural effusion, and recently, the use of cryoprobe for pleural biopsies was encouraged, as the procedure is effective and safe.

Objective This study compared between rigid forceps and cryoprobe pleural biopsies regarding biopsy characteristics, diagnostic yield, and tissue viability in patients with undiagnosed exudative pleural effusion who underwent medical thoracoscopy.

Patients and methods A total of 30 patients with undiagnosed exudative pleural effusion were selected for medical thoracoscopy, and pleural biopsies were taken by rigid forceps and cryoprobe in the same setting. All biopsies were processed for histopathology examination.

Results Of the 30 patients, 18 (60%) were males and 12 (40%) were females, with mean age of 51.03 years. The most frequent diagnosis was mesothelioma (43.3%) followed by chronic nonspecific inflammation (23.3%), metastatic carcinoma (16.6%) and tuberculosis (16.6%). Biopsies of rigid forceps (mean: 0.8193 cm^2) were larger than cryoprobe (mean: 0.3377 cm^2) but with less depth. Tissue viability of cryoprobe biopsies was better than rigid forceps biopsies, and the diagnostic yield of both techniques was the same.

Conclusion Cryobiopsies obtained during medical thoracoscopy is technically feasible and safe with high diagnostic value. Biopsies of cryoprobe were smaller than that of rigid forceps but were deeper and with better preserved cellular architecture. These results will encourage the use of cryotechnique for diagnosis of undiagnosed exudative pleural effusion.

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Introduction

Recurrent persistent exudative pleural effusion is common in clinical practice. Thoracentesis or blind pleural biopsy may not provide definitive diagnosis [1]. To reach diagnosis in pleural effusion, one should follow a stepwise approach [2]. Medical thoracoscopy is useful in diagnosis of pleural diseases, as it is safe with low incidence of complications [3]. Thoracoscopy is a minimally invasive procedure that allows visualization of pleural space with obtaining pleural biopsies under direct vision, therapeutic drainage of effusion, and pleurodesis in the same setting [4]. Thoracoscopy is the gold standard for diagnosis and treatment of pleural effusion; its diagnostic yield is ~95% in malignant pleural disease, and ~90% successful pleurodesis for malignant pleural effusion [5].

Cryotechnique was initially used for therapeutic management of airway tumors. Since then, it is used routinely through bronchoscopy as a diagnostic and therapeutic tool. Cryotechniques were not associated with increased incidence of complications [6]. Although, thoracoscopy is a well-known technique used for the diagnosis of pleural diseases, the use of freezing techniques in chest medicine is more recent. The important properties of ice including hemostatic, analgesic, and anti-inflammatory effects have been recognized for several years. The introduction of the new mini-cryoprobe opened the field to more applications of cryotechniques when biopsies are needed. The interest in using thoracoscopy for diagnosis and therapy of pleural diseases gives opportunity to combine cryotechniques with thoracoscopy to take biopsies [7].

This study compared between rigid forceps and cryoprobe pleural biopsies regarding biopsy characteristics, diagnostic yield, and tissue viability in patients with undiagnosed exudative pleural effusion who underwent medical thoracoscopy.

Patients and methods

A prospective interventional study was conducted that included 30 cases with pleural effusion exudative in

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nature, of unknown etiology, after being evaluated by thoracentesis. Patients were recruited from the chest departments in both Cairo and Beni-Suef university hospitals. The duration of the study was from February 2017 to March 2018. Cases that had bleeding tendency, respiratory failure, transudative effusion, pleural thickening without effusion, and unstable angina were excluded. Human Ethical Committee of Kasr Alainy, Cairo University, approved this study. Informed written consent was obtained from patients before inclusion in the study.

All patients were subjected to history taking, clinical examination, and laboratory investigations, including blood picture, serum albumin and creatinine, bleeding profile, and pleural fluid analysis with respect to lactate dehydrogenase, protein, and cytology. Chest computed tomography was done before medical thoracoscopy.

Pleural biopsies were obtained using rigid forceps and cryoprobe in the same setting during medical thoracoscopy. Rigid thoracoscopy (KARL-STORZ, Germany) was done in a well-equipped room with rigid thoracoscopy instruments (Fig. 1a), including trocar (8 mm in inner diameter), light source, biopsy forceps, rigid telescope made of stainless steel (27 cm in length, 7 mm in diameter), and cold (Xenon) light source with camera (Telecam) attached to eyepiece of the telescope. Cryo machine (ERBE, Germany) (Fig. 1b) consists of console, cryogen, and flexible cryoprobe (diameter 2.4 mm/length 900 mm) (Fig. 1c). Carbon dioxide was used as a cooling agent (−78°C) [8].

Technique

All cases were performed under local anesthesia (lidocaine 2%) and analgesia using pethidine 100 mg. Patient is positioned in lateral decubitus position, with the affected side up. Puncture site is usually at fifth orsix6th intercostal space in the mid axillary line. The single port entry technique was used in all patients. Skin incision of ~1 cm was made followed by blunt dissection of intercostal muscles until the costal pleura is reached. The rigid trocar was introduced through the chest wall, with its inner part then withdrawn, and thoracoscope was introduced inside the trocar. Rigid forceps was introduced at first through the working channel of the rigid thoracoscopy to obtain biopsies directly from abnormally visible areas. Then cryoprobe is introduced through the working channel of the thoracoscope and applied to the area of pleura to be biopsied through direct vision. After freezing for 20 s, the pleura in contact with the ice was frozen; this is confirmed by an increased electric resistance measured at the tip of the cryoprobe (Fig. 2a). The cryoprobe with the adherent pleural tissue was extracted together with the thoracoscopy. Then specimens were thawed in normal saline at ambient temperature. Multiple biopsy samples were obtained from visible abnormal areas in the parietal pleura with the rigid forceps and cryoprobe (Fig. 2b). After obtaining satisfactory biopsy specimens, the thoracoscope and trocar were removed, and chest tube (32F) connected to an underwater seal was inserted in place. All biopsies were fixed in formalin 10% and sent for histopathology.

Pathological evaluation

Specimens were described grossly (Fig. 2b) and put into a cassette that holds tissue while it is prepared to a paraffin block. Processing of tissues was completed in the same laboratory using consistent processor protocol. Tissue prepared on the slide was stained by hematoxylin and eosin stain for histopathological evaluation.

Statistical analysis

Data were statistically described in terms of mean±SD, median, range, or frequencies and percentages when appropriate. Comparison of numerical variables between study groups was done using Student's *t*-test for independent samples in comparing two groups of normally distributed data and Mann–Whitney *U*-test for independent samples for comparing non-normal data. For categorical data, comparison was done using χ^2-test. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (statistical package for the social sciences; IBM Corp, Armonk, New York, USA) release 22 for Microsoft Windows.

Results

Age range of studied cases was 30–61 years, with mean value of 51.03±7.518 years. The cases included 12 (40%) female and 18 (60%) male patients, and 19 were smokers (63.33%). Among the studied patients, five (16.67%) were diagnosed as metastatic carcinoma, including three patients with metastatic adenocarcinoma and two with squamous cell carcinoma; 13 (43.33%) patients were diagnosed as mesothelioma (Fig. 3); seven (23.33%) patients were diagnosed as chronic nonspecific inflammation; and five (16.6%) were diagnosed as *Tuberculous pleuritis* (Fig. 4). The characteristics of the studied patients in relation to final diagnosis are shown in Table 1. Biopsies obtained either by rigid forceps or cryoprobe were compared regarding surface area, tissue depth, and tissue viability, as shown in Table 2. The procedure was generally safe with no reported complications.

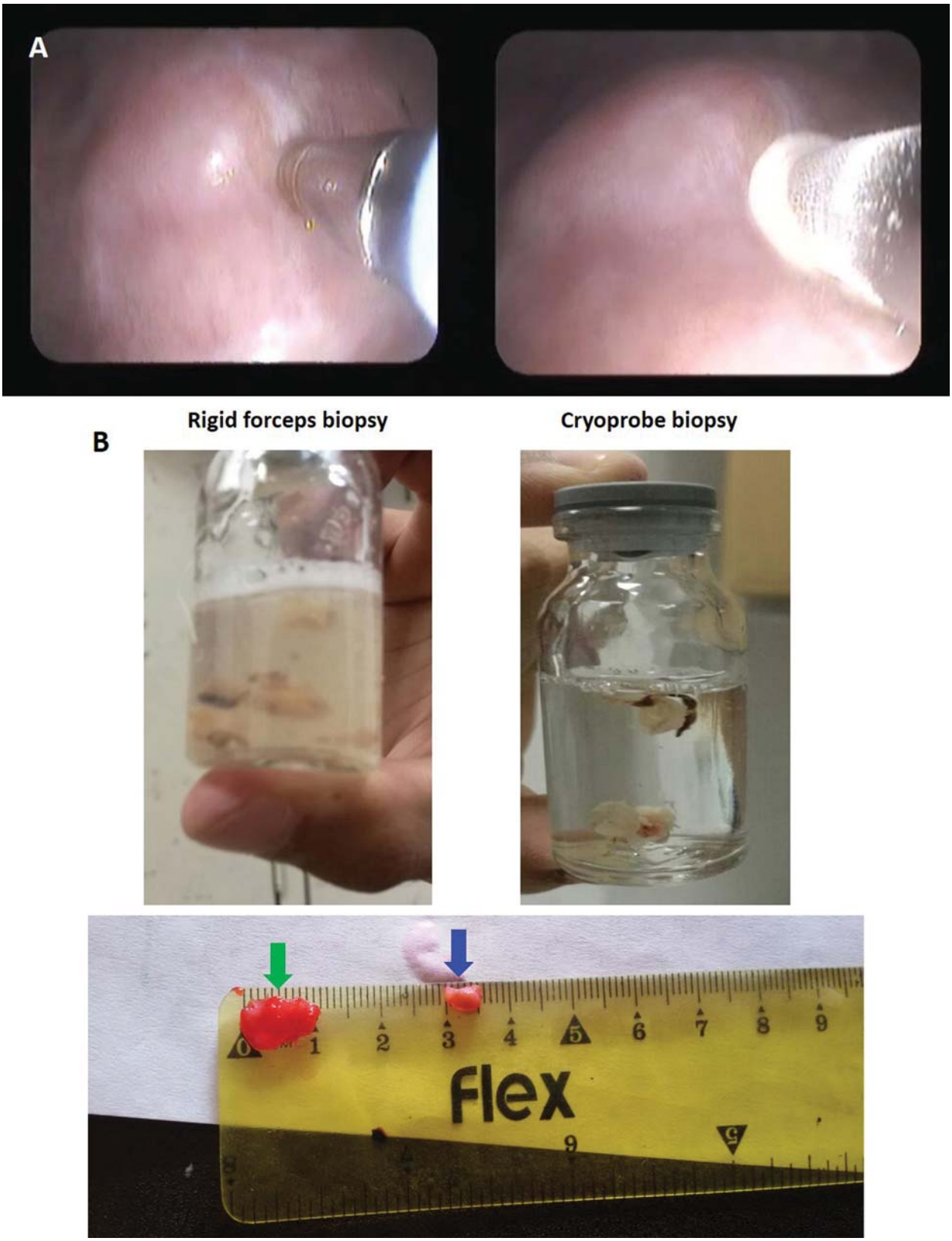
Fig. 1

(a) Thoracoscopy instruments (Karl-Storz, Germany) including trocar and cannula; rigid biopsy forceps and single-entry rigid telescope for adults. (b) Erbe Cryo unit (Germany). (c) Flexible cryoprobe (Erbe, Germany).

Discussion

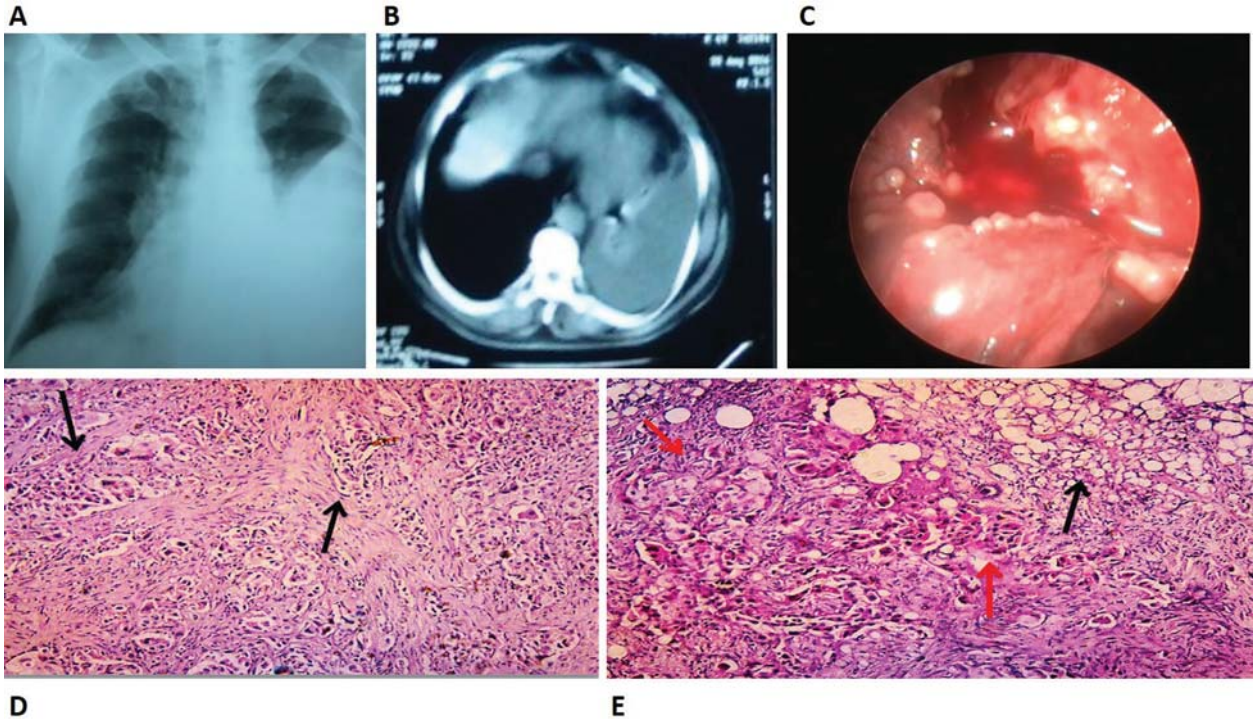
Thoracoscopy is the recommended diagnostic procedure for patients with exudative pleural effusions of unknown etiology [9]. Thoracoscopy helps to visualize the hemithorax, obtaining biopsies, and mechanical or chemical pleurodesis with improved distribution of the sclerosing agent [10]. Bronchoscopic cryotherapy has been used since 1970s in the management of obstructive endobronchial malignancy [11], endobronchial biopsy, and transbronchial lung

Fig. 2



(a) The flexible cryoprobe applied to the area of costal pleura to be biopsied through direct vision with ice ball formed at the tip of the probe after freezing for 20 s. The attached tissue was extracted together with the cryoprobe and thoracoscopy. (b) Image of some biopsy samples obtained by the rigid forceps (surface area of this biopsy was 1.2×1.2=1.44 cm^2; green arrow) and cryoprobe (surface area of this biopsy was 0.6×0.6=0.36 cm^2; blue arrow).

Fig. 3



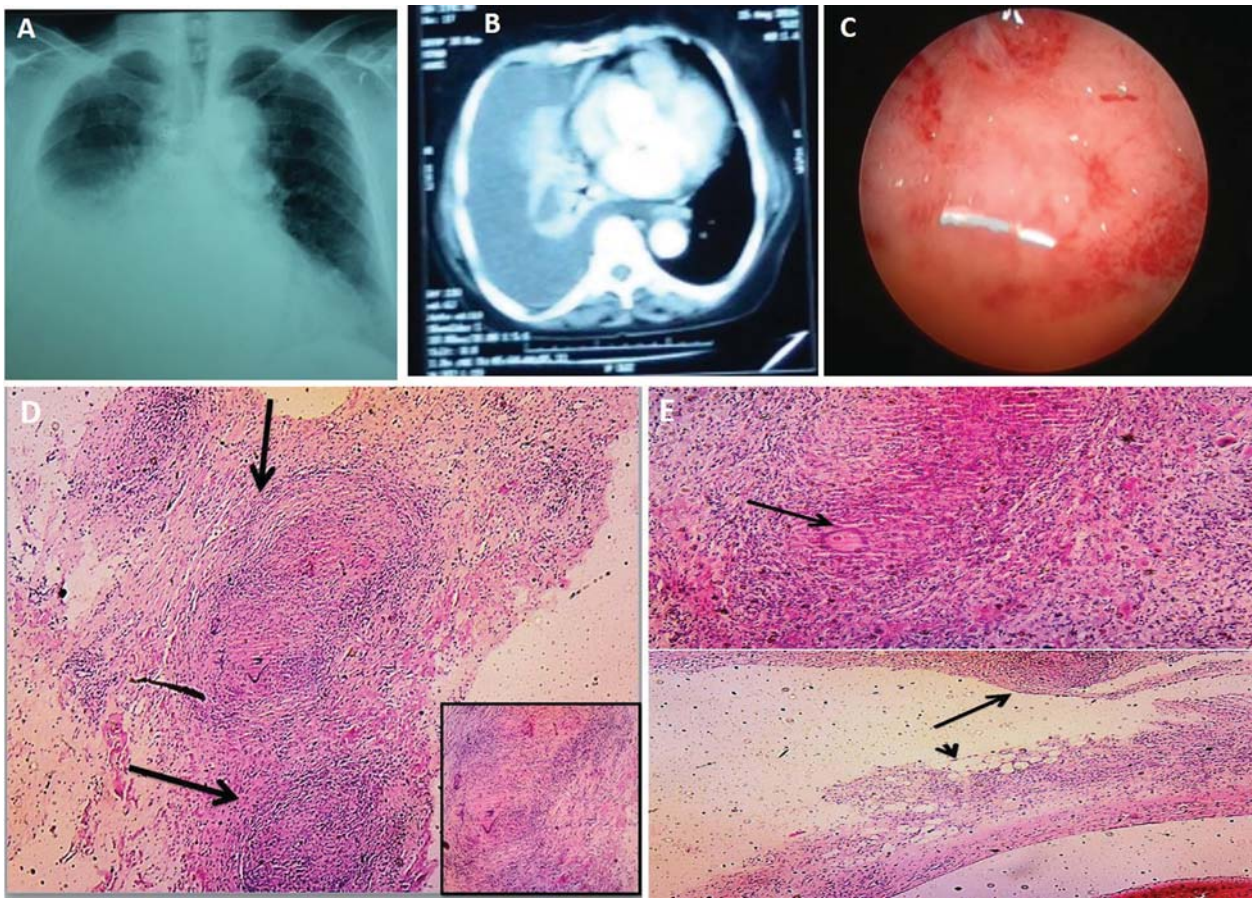
(a) Chest radiography showing left pleural effusion. (b) Computed tomography chest showing left pleural effusion. (c) Thoracoscopic image showing multiple nodules on costal and diaphragmatic pleura (d) Rigid forceps biopsy histopathology image (surface area 1.1×1.1=1.21 cm^2) showing sheets of malignant epithelioid cells consistent with mesothelioma (black arrows). (e) Cryoprobe biopsy histopathology image showing (surface area 0.9×0.9=0.81 cm^2) good biopsy depth evident by mesothelioma sheets of malignant epithelioid cells (red arrows) infiltrating too deep to the fat tissue (referred by black arrow).

biopsies [12]. Thoracoscopic pleural cryobiopsy is a new application of the technique [7]. Cryotechnique has many advantages including analgesic effects of ice, which allows obtaining several biopsies with no pain, and also specimen quality is better than that obtained using electrocoagulation [13].

The different diagnoses included in the study are metastatic carcinoma (16.67%), mesothelioma (43.33%), chronic nonspecific inflammation (23.33%), and tuberculous pleurisy (16.67%). Mesothelioma was the most frequent diagnosis, and this agreed with Thomas *et al.* [14] who had 11 (50%) of 22 patients with mesothelioma. Moreover, an Egyptian study of Mohamed and Shaban [15], showed mesothelioma in 47.01%. However, Wurps *et al.* [6] diagnosed mesothelioma in only three (4%) of 80 patients, and Bonniot *et al.* [13], diagnosed mesothelioma in two (11.1%) of 18 patients; this difference between the studies may be owing to the different residence and exposure risk. Metastatic carcinoma was less frequently diagnosed than mesothelioma (16.67%), and this was similar to Mohamed and Shaban [15] where they diagnosed metastatic adenocarcinoma in 22.22%. In contrast, Bonniot *et al.* [13], diagnosed metastatic adenocarcinoma in 72.2%. Chronic nonspecific inflammation was diagnosed in 23.33% which was less than the percentage obtained by Wurps *et al.* [6] (41%) but more than that obtained by Mohamed and Shaban [15] (13.68%).

The mean age of the studied cases was 51.03 years, which was lower than previous similar studies of Thomas *et al.* [14] (mean age of 72 years) and Wurps *et al.* [6] (mean age of 67.5 years). There was a significant correlation between age and final diagnosis. Malignant pleural effusion was more common in elderly patients (above 50 year), and this was similar to Kalaajieh [16] who diagnosed malignant effusions more frequently among older age groups, and Prabhudesai *et al.* [17], who diagnosed malignant pleural effusions in 64.47% of their patients who were over the age of 40 years. There was a significant correlation between sex and final diagnosis in this study as 77.77% of males (14 out of 18) were diagnosed as having malignant pleural effusion. However, only 33.33% of females (four out of 12) had malignant pleural effusion (33.33%). Increased malignant pleural effusion incidence in males may be explained by more exposure to risk factors. This result was similar to Anurag *et al.* [18] and Muharrem and Atilla [19] who found that the incidence of malignant pleural effusion was more in males than females. Mesothelioma is most frequently diagnosed in males (12 of 13 mesothelioma cases; 92.3%)

Fig. 4



(a) Chest radiography showing moderate right pleural effusion. (b) Axial computed tomography chest mediastinal window with IV contrast showing right pleural effusion. (c) Thoracoscopic image showing diffuse thickening and inflammation of the costal pleura. (d) Rigid forceps biopsy histopathology image showing multiple caseating granulomas (black arrows) consistent with tuberculosis. (e) Cryoprobe biopsy histopathology image showing caseating granuloma with Langhans giant cells (black arrows) and good biopsy depth was evident by the presence of vacuolated fat cells (black arrow head).

Table 1 Patient characteristics in relation to final diagnosis (number=30 patients)

Characters	Final diagnosis [*n* (%)]				*P* value
	Metastatic carcinoma (*n*=5) (16.67%)	Mesothelioma (*n*=13) (43.33%)	Tuberculosis (*n*=5) (16.67%)	Chronic nonspecific inflammation (*n*=7) (23.33%)	
Age (mean±SD)	52.25±5.682	53.62±7.869	44.40±7.301	50.375±3.936	0.012*
Sex					
Female	3 (60)	1 (7.7)	3 (60)	5 (71.42)	0.017*
Male	2 (40)	12 (92.3)	2 (40)	2 (28.5)	
Smoking history					
Yes	4 (80)	11 (84.61)	2 (40)	2 (28.57)	0.003*
No	1 (20)	2 (15.38)	3 (60)	5 (71.42)	
Pleural fluid protein (mean±SD)	3.67±0.21	3.85±0.711	3.92±0.610	3.725±0.8305	0.278
Pleural fluid LDH (mean±SD)	274.91±69.607	306.00±67.202	279.60±52.823	286.835±74.434	0.437

LDH, lactate dehydrogenase. *$P<0.05$, statistically significant.

and this was similar to McDonald *et al.* [20] who showed that proportion of MPM in women is low, and this was explained by the differences in occupational asbestos exposure, which is predominantly in job settings typically held by men. Historically, secondary exposure through spouses' clothing, low-level environmental exposure, and other sources (e.g. cigarette or powder talc) have been suggested as etiologic factors in women.

Regarding other potential risk factors for malignant pleural effusion in this study, 78.95% (15 out of 19) of smokers had malignant pleural effusion. The correlation

Table 2 Comparison between rigid forceps and cryoprobe biopsies (*N*=30 patients)

Characters	Rigid forceps biopsies [*n* (%)]	Cryoprobe biopsies [*n* (%)]	*P* value
Surface area (cm^2)			
Minimum	0.15	0.06	0.000*
Maximum	1.65	0.90	
Mean±SD	0.819±0.404	0.338±0.247	
Biopsy depth			
Fat	12 (40)	21 (70)	0.000*
No fat	18 (60)	9 (30)	
Tissue viability			
Crushed cells	9 (30)	0 (0)	–**
No crushed cells	21 (70)	30 (100)	

*$P<0.05$, statistically significant. **No statistics were computed because cryoprobe biopsies are constant.

between smoking and final diagnosis was significant (P=0.003). This agreed with West [21] who showed that chronic smoking is a risk factor for developing malignant pleural effusion, and Sophia *et al.* [22] who showed that cigarette-smoke promotes MPE formation by enhancing tumor-associated inflammation. Moreover, 84.61% of our patients with mesothelioma were smokers. This was confirmed by McDonald *et al.* [20] who mentioned that cigarette smoking has been suggested as an etiologic factor of mesothelioma in women, but Lopes *et al.* [23] mentioned that smoking is not risk factor for mesothelioma

Medical thoracoscopy is considered an effective and beneficial tool for cases with undiagnosed exudative pleural effusion. Biopsies obtained using flexible forceps through semirigid thoracoscopy were small compared with those obtained using rigid thoracoscopy forceps, but yield of diagnosis was nearly similar [24]. Cryotechnique during bronchoscopy has been used as an efficient procedure for diagnosis and therapeutic indications [25] without increased complications [26]. Moreover, obtained biopsies were large with maintained cellular architecture compared with crushed samples when using forceps [27].

In this study, the mean surface area of rigid forceps biopsies was 8.193 mm^2, whereas the mean surface area of cryoprobe biopsies was 3.377 mm^2. This was less than the mean surface area of rigid forceps and cryoprobe biopsies obtained by Wurps *et al.* [6] which was 22.6 and 14.4 mm^2, respectively. This difference may be attributed to the way of measurement as in this study, as the surface area of largest biopsy is only measured and not all obtained biopsies because the number of biopsies was not standardized. Mean surface area of rigid forceps biopsies was significantly larger than that of cryoprobe (P=0.000), and this was highlighted by Wurps *et al.* [6] who mentioned that the size of cryoprobe biopsies was larger than flexible forceps but smaller than rigid forceps biopsies. Moreover, Thomas *et al.* [14] and Pathak *et al.* [28] mentioned that the size of cryoprobe biopsies was larger than flexible forceps. This may be owing to the small cups of the flexible forceps. So cryoprobe can overcome the limitations of flexible forceps providing larger and deeper tissue samples.

Deep biopsies containing fatty tissue were significantly obtained in 70% of cryoprobe biopsies and in 40% of rigid forceps biopsies. Obtaining deeper tissue may be of much importance in establishing a histological diagnosis of mesothelioma where the pleura is extremely tough and thick. In contrast, Wurps *et al.* [6] showed that a deep biopsy containing fatty tissue was obtained in 63% of the rigid forceps biopsies, 39.5% of flexible forceps biopsies and in 49.5% of the samples harvested using cryoprobe. Thomas *et al.* [14], showed that deep biopsy containing fatty tissue was obtained in 63.6% of cryoprobe biopsies and in 22.7% of flexible forceps biopsies, which means that cryoprobe biopsies are deeper than flexible forceps biopsies.

Regard tissue viability, cryoprobe biopsies showed no crushed cells, but rigid forceps biopsies showed crushed cells in 30% of specimens. So cryoprobe preserves tissue integrity and preserves important molecular markers for immunohistochemical studies which are essential for confirming histopathological diagnosis especially in malignant cases. This agreed with Hatzel *et al.* [29] and Rozman *et al.* [30]. Moreover, the study by Thomas *et al.* [14], showed crushed cells in only 9.09% of cryoprobe biopsies (two out of 22) and in 95.4% of flexible forceps biopsies (21 out of 22). Biopsies taken by cryoprobe were in a better quality with preserved architecture in comparison with those obtained using electrocauterization [13].

The diagnostic yield of cryoprobe in this study was the same as that of the rigid forceps, and this was confirmed by Pathak *et al.* [28] who showed that the diagnostic yield was similar in both forceps and cryoprobe groups. Thomas *et al.* [14] showed that

cryoprobe has the same diagnostic yield as flexible forceps whereas Wurps *et al*. [6] found that the diagnostic yield of cryoprobe was inferior to that of rigid forceps but superior to flexible forceps.

No significant reported complications following cryoprobe biopsies were seen in this study, and this was confirmed by Pathak *et al*. [28] who reported no increased incidence of bleeding or pain using cryoprobe in any of their patients. Moreover, Thomas *et al*. [14] and Bonniot *et al*. [13] stated that there was no significant reported complication following cryobiopsy with reduced risk of hemorrhage or air escaping after using cryoprobe.

In conclusions, cryobiopsies of the pleura using medical thoracoscopy are technically feasible, with a diagnostic yield similar to that obtained using rigid forceps. Cryobiopsies are small in size than samples obtained using the rigid forceps but with a good depth and better preserved cellular architecture. This will favor the use of cryotechnique for undiagnosed exudative pleural effusion.

It is recommended to do this study on a larger number of patients. The technique of pleural biopsies should be standardized regarding number of biopsies obtained either by of rigid forceps or cryoprobe, cooling agent, and the time of freezing. Moreover, further studies should be done to evaluate the difference between cryoprobe biopsy and rigid forceps biopsy regarding preserved cellular architecture and its value in improving the outcome of immunohistochemical studies and the diagnostic yield. Applying cryobiopsies during medical thoracoscopy was encouraged as the procedure is effective and safe.

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Conflicts of interest
There are no conflicts of interest.

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