Accuracy of pleural effusion cytopathology

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Abstract: Introduction: Pleural effusions are pathological processes defined by the accumulation of fluid in pleural cavity. Different etiology causes this problem and occurred in different degrees of clinical severity. The main aim of present study was to determine the accuracy of cytopathology in the diagnosis of pleural effusions, according to histomorphologic features, in the patients that referred to the Rajaee and Kowsar Hospitals. **Methods and materials:** For this cross-sectional study data were evaluated from 100 patients who underwent pleural tap at the Rajaee and Kowsar Hospitals between April 2008 and April 2011. Each subject underwent general physical and radiologic examinations. The smears prepared from the pleural tap specimens and stained by the Papanicolau and Giemsa methods. We classified the cytopathologic results in five groups as following: Malignant mesothelioma (MM), metastatic malignancy, Malignant cells with unknown origin (primary or secondary), Benign, Suboptimal for further diagnosis. All the cases were confirmed by Immunohistochemistry (IHC) and clinical and histopathological follow-up. Both descriptive and statistical analysis methods were applied. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated. **Results:** The total number of patients was 100. The overall mean age was 60 ± 9.48 (ranging from 39 to 80) years. 57 preparations were benign effusions, 8 of these were malignant mesothelioma and 35 of these were metastatic carcinomas. In our study, sensitivity, specificity, positive predictive value and negative predictive value and efficiency of cytopathology in diagnosis of malignant pleural effusion, were 83%, 100%, 100% 79.7% and 90%, respectively. **Conclusion:** In this study, cytopathology is a safe, useful and reliable procedure in discrimination between malignant and benign pleural effusions, and has not sufficient power for identification between primary and secondary pleural malignancy, based on histomorphologic findings. However in these situation uses of cell blocks, IHC studies is highly mandatory.
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1. Introduction

Pleural effusions are pathological processes defined by the accumulation of fluid in pleural cavity due to disruption of balance between hydrostatic and oncotic pressures in the visceral and parietal pleural vascular channels and occlusion of lymphatic vessels (Light, 2006). Different etiology causes this problem and occurred in variable degrees and different clinical situations (Light, 2006; Lee and Light, 2004).

In the developed countries, the main cause of pleural effusions are as following: end organ failure including heart, kidney, liver, bacterial and viral infections (parapneumonic effusions), malignant involvements (primary or secondary including: lung, breast, ovary, gastrointestinal tract and lymphoma), collagen vascular diseases and asbestos inhalation. In other part of world, especially in developing countries, tuberculosis infections are popular and prevalent (Light, 2006; Lee and Light, 2004; Marel, 1993; Batungwanayo, 1993).

In the unknown primary site pleural effusions, the discrimination between benign effusions from Malignant Mesothelioma (MM) and metastatic carcinoma, is necessary and this differentiation should be perform based on a accurate tool for discovery of lesion nature. Because, the correct and exact treatment and prognosis followed by definite diagnosis (Lee and Light, 2004; Barreiro and Katzman, 2006). By using the cytopathology, in the more than 70% of patients, differential or definite diagnoses are confirmed (Fiegl, 2003; Maskell and Butland, 2003).

The cytopathology is a safe, rapid, simple, less invasive procedures and cost effective tool for pleural effusions evaluation and malignancy ruled out.

Neoplastic conditions including about 20% causes of pleural effusions (Ammon, 1993).

The most pathogenesis of malignant pleural effusions are permeation of pleura by malignant cells, angiolymphatic invasion and occlusion of sub mesothelial lymphatic channels by tumoral cell embolism, however in some patients, reactive pleural effusions secondary to mass or immunological mediated form were developed. In these conditions, the cytopathological investigations can't be identify atypical cells. The most important ultimate goal in cytopathologic studies of pleural effusions are

diagnosis of the malignancy-related effusion (Johnston, 1985; Menard, 1993; Salyer, 1975; Escudero Bueno, 1990).

Johnston et al shown that the specificity of cytopathology in evaluation of pleural effusion was sufficient and its sensitivity was less than optimal for diagnosis. They found, in unknown origin pleural effusions, application of cytopathology in the identification benign conditions from Malignant Mesothelioma (MM) and metastatic neoplasms has significant accuracy (Johnston, 1985). In the most pleural effusions cases, before any medical or surgical investigations, for better evaluation, pleural tap and cytopathological examination, was performed. It is important, that precision and accuracy of this tool are challenging diagnostic problems. According to results of Bonito et al study, the sensitivity of the cytopathology in diagnosis of MM varies from 31.9% to 86.3% and for malignancy with unknown origin were 11.7% to 75.3% for an accurate evaluation of primary origin (Di Bonito, 1993).

The study results of Jha et al revealed, serosal fluid cytopathology had sensitivity: 56.7%, specificity: 100%, positive predictive value: 100% and negative predictive value: 63.6%, respectively for malignant cells identification (Jha, 2006).

However, definite diagnosis performed by application of pleural biopsy through thoracoscopy or thoracotomy procedure. Both of tools were invasive, aggressive, high instrument cost and intensive training (Johnston, 1985). Confirmatory method for identification of benign from malignant effusions was histopathologic examination of pleural biopsy specimen (Kuralay, 2000).

The main aim of present study was to evaluation the accuracy of cytopathology in the diagnosis of pleural effusions, according to histomorphologic features, in the patients that referred to the Rajaee and Kowsar Hospitals.

2. Material and Methods

For this cross-sectional and descriptive study, data were evaluated from 100 patients who underwent pleural tap at the Rajaee and Kowsar Hospitals between April 2008 and April 2011. Each subject underwent taking medical history including patient demographics, general physical and radiologic examination. Pleural fluid obtained during thoracentesis. Pleural fluid samples were centrifuged at 2000 (speed of revolutions) rev /min for 10 min to detect cellular components. The smears prepared from the collected specimens and stained by the Papanicolau and Giemsa methods.

The gold standard and confirmatory method was the pleural biopsy for histopathological study, perform with Abrams needle in distinguishing malignant from benign pleural fluid.

Cytopathologic features in favor of malignancy are as following: Richly cellular smear, marked pleomorphic and enlarged cells with high nucleo to cytoplasmic (N/C) ratio, hyperchromatic nuclei, occasional multinucleated, prominent nucleoli, vacuolated cytoplasm associated with blebs formation and distinct cell borders, in strongly cohesive cell groups pattern(three- dimentional fragments) including : papillary, tumor ball and tight clusters structures. Mitotic activites were increased ncluding atypical form (Robinson and Lake, 2005; Whitaker, 2000).

We classified the cytopathologic results in five groups as following: Malignant mesothelioma (MM), metastatic malignancy, Malignant cells with unknown origin (primary or secondary), Benign, Suboptimal for further diagnosis (Fassina, 2008).

All cytopathologic diagnosis was established by Immunohistochemistry (IHC) studies and also by clinicopathologic correlations. For ruled out of possible malignancy, total false negative results, were re-examined, and in this situation no evidence of malignancy are found.

We were applied both descriptive and statistical analysis methods. The statistical evaluation was performed by computer analysis with SPSS Software. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated. Also we calculated ROC (Receiver Operating Characteristic) based on recommended formulas (Hanley and McNeil, 1983; Hanley and McNeil, 1982; Park, 2004). The area under the ROC curve (AUC) was calculated with 95%confidence intervals (CIs).

3. Results

The one hundred patients studied had a median age of 60 ± 9.48 (ranging from 39 to 80) years. 74 patients were men, and 24 patients were women. The Most prevalent symptom was dyspnea (81%). The malignancy risks factors including: tobacco abuse in 69 patients, a family history of lung cancer in 5 patients, radiation exposure in 2 patients and asbestos fibers exposure in 4 patients. Unilateral pleural effusions were observed in 78 cases. In the 38 patients, radiologic findings were characteristic of malignancy. Simultaneous ascites was found in 23 patients. Among the participants, 47 were no malignancy patients which included 11 with tuberculosis (TB) pleurisy, 20 with parapneumonic effusion, 5 with liver cirrhosis, 5 with end stage renal disease, and 6 with congestive heart failure.

The most common causes of benign pleural effusion in male and female patients were parapneumonia and tuberculosis, respectively. The

pleural malignancy was diagnosed in 53 patients. Based on histopathologic and IHC studies, the final diagnosis in patients with malignant pleural effusions included: lung adenocarcinoma in 18 patients, squamous cell lung carcinoma in 9 patients, malignant mesothelioma in 8 patients, breast cancer 8 patients, gastrointestinal cancer 6 patients and 4 ovarian cancer patients (figure 1-5). Overall, most common primary source of malignancy associated with pleural effusions was lung 27(51%) followed by breast 8(15.1%). In female patients breast malignancy was the most common and in the male patients lung malignancy.

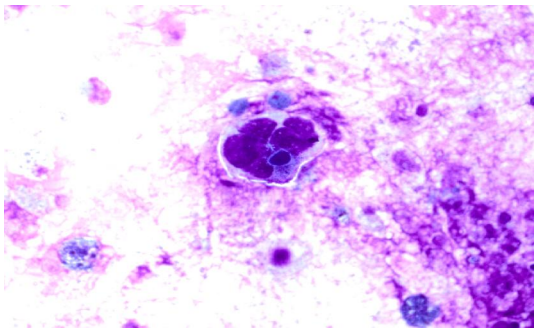

Figure 1.Cytopathology, squamous cell carcinoma, 40. Atypical cell with high N/C ratio, hyperchromatic multi-lobated nuclei, distinct cytoplasm. Pap staining, ×40

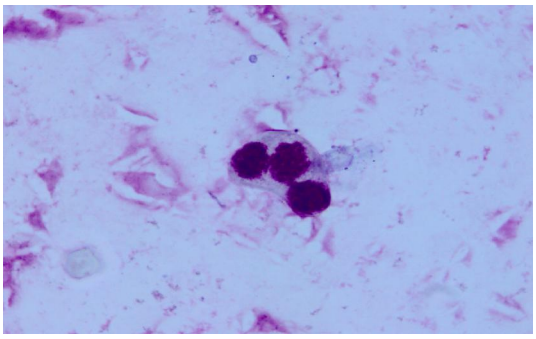

Figure 2.Cytopathology, squamous cell carcinoma. Atypical cell with high N/C ratio, hyperchromatic multi-lobated nuclei, distinc cytoplasm. Pap staining, ×40

Diagnosis comparison between cytopathology and histopathology methods and differential diagnosis of pleural effusions were present in the Table1. Among the 31 benign effusions that reported by cytopathology tool, malignant cells were observed in 2 cases by histopathology evaluation. Also among the 28 inadequate pleural effusions for further cytopathological diagnosis, in the 10 cases malignancy was confirmed by histopathology method. The overall, in malignant pleural effusion, sensitivity, specificity, positive predictive value and negative predictive value and efficiency were 83%, 100%, 100% 79.7% and 90%, respectively. All of 41 malignant effusion that reported by cytopathology tool, malignancies were confirmed, histopathology. In the evaluation of cytopathology by using of ROC, area under the curve (AUC) of 0.977 (0.940-1.014), the sensitivity and specificity were high in 95% confidence interval (figure 6).

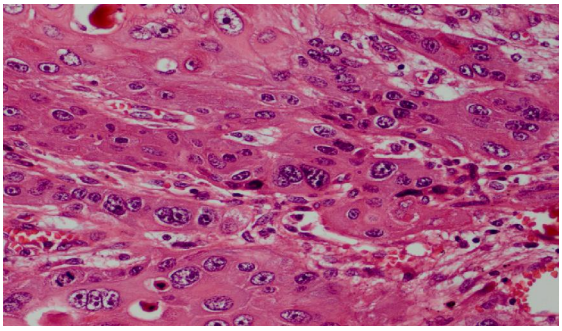

Figure 3. Histopathology of metastatic pleural squamous cell carcinoma. Marked pleomorphic cell with high N/C ratio, vesicular to hyperchromatic mono or multi nuclei,prominent nucleoli, eosinophilic cytoplasm with distinct cell borders. Hematoxylin & Eosin staining ×40

Table 1.Diagnosis comparison between Cytopathology and Histopathology

Cytology / Pathology	Malignant mesothelioma	metastatic malignancy	malignant cells with unknown origin	Benign	suboptimal for further diagnosis	Total
Malignant mesothelioma	5	0	3	0	0	8
Adenocarcinoma, lung	0	9	4	2	2	18
Squamous cell carcinoma ,lung	0	3	2	0	5	9
Breast cancer	0	3	3	0	2	8
Gastrointestinal cancer	0	4	2	0	0	6
Ovarian cancer	0	2	1	0	1	4
Benign	0	0	0	29	18	47
Total	5	21	15	31	28	100

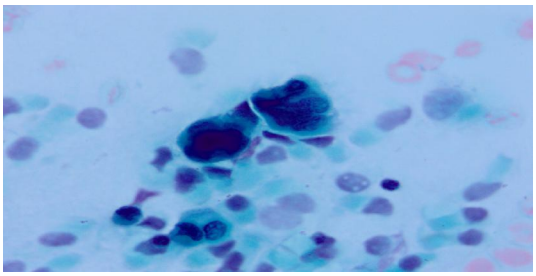

Figure 4.Cytopathology of lung adenocarcinoma.40. Atypical cell with high N/C ratio, hyper chromatic multi-lobated nuclei, distinct cytoplasm. Pap staining ×40

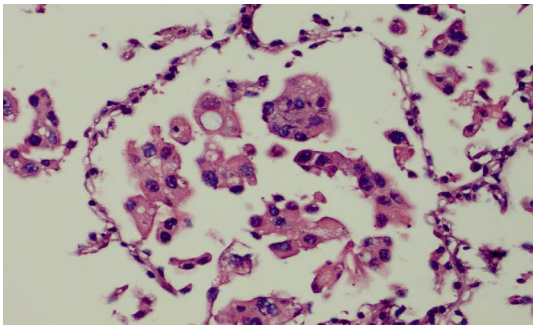

Figure 5. Histopathology of lung adenocarcinoma. The pleomorphic tumoral cells with high N/C ratio, hyperchromatic nuclei, eosinophilic cytoplasm. Hematoxylin & Eosin staining ×40

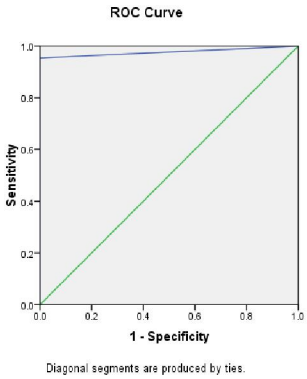

Figure 6. Roc curve for cytopathology tool in evaluation of pleural lesions. AUC: 0.977 (0.940-1.014)

4. Discussions

In the recent study, we found that pleural effusion cytopathology was good accuracy in the discrimination of benign from malignant cases. In pleural effusion cytology, determination of reactive mesotelial cells from metastatic carcinoma and MM is critical. The problem is compounded when neoplastic cells disclose only mild atypia and when reactive mesotelial cells reveal marked atypical and cellular pleomorphism (Ikeda, 2010).

In many situations, the diagnosis of MM, metastasis, or benign reactive mesothelial proliferation in effusion specimens is based on experienced cytopathologic. The other problems are as following: sampling error, failed tap, few malignant cells shedding, hemorrhagic or inflammatory effusion and interpretative errors (Fassina, 2008). Two other common situations that associated with diagnostic pitfalls are pleural lavage samples and samples from patients having had radiotherapy (Zimmerman, 2005).

By using of cytopathologic features, identification of reactive mesothelial hyperplasia from malignant effusion can be possible, but definite diagnosis of primary or secondary malignancies are not straightforward. Many cellular changes are seen in reactive processes that bleared accurate diagnosis, including: nuclear pleomorphism, macronucleoli and vacuolated cytoplasm (Fassina, 2008; Gupta and Dey, 2003).

Architectural setting also is very critical in definite diagnosis in comparing to cytomorphologic features, because large number of the MMs pertaining to well differentiated categorized, and therefore these features are not significant. The most common growth patterns in MM are three-dimensional tissue fragments including morular structure. However keep minding, for correct diagnosis correlation between cytomorphologic features, architectural growth pattern, tumor necrotic or inflammatory background and clinical data are highly mandatory (Leiman, 2001).

In many conditions in obvious ill and serious individuals with repeated pleural fluid accumulation, only safe route for taking of adequate specimen for appropriate cytopathologic examination is pleural tap (Fassina, 2008).

The sensitivity (range and mean), specificity, positive predictive value and negative predictive value in the previous study of Fassina et al were (65.5% - 90.3% , mean: 81.2%), (64.3% - 100%, - mean: 81.3%),(84.4% - 100%, mean: 91.2%) and (54.5% - 76.9%, mean: 67.4%), respectively(Fassina, 2008).

Our results were agreement with Fassina et al´s results in sensitivity and specificity and were higher accuracy in PPV and NPV.

In Fassina study, the further subspecialization of malignant effusions, whether primary or secondary in origin, lead to an obvious decrease in diagnostic accuracy (Fassina, 2008).

The results of previous study shown, the sensitivity of the cytopathology in diagnosis of MM ranged between 31.9% to 86.3% and for metastatic

malignancy 11.7% to 75.3%, in order to accurate finding of primary site (Di Bonito, 1993).
Motherby et al believed that pleural effusion cytopathology has low sensitivity 5.8% to 50% for malignancy detection compared with high specificity power (Motherby, 1999).

The various studies reported different results, especially wide range of difference in sensitivity and specificity. Among them, choose and application of "gold standard" and confirmatory method is important cause. Optimally it should be detailed histopathological review; however some of authors often applied other tool including clinical conception or radiologic investigations (Michael, 1993).

Another cause for these variations was easy availability of expert pulmonary cytolopathologist, which is still challengeable concept in many areas of world (James, 2010).

The false negative result in our study was 20.3% and in other research ranged from 23%-42%. Johnsons et al shown, this problem was not resolved by re-examination of slides. He believed main cause of this discrepancy was due to lack of shedding of malignant cells into serosal fluid or due to error in procedure applied to convey the cells to the slides not of inability to recognize the malignant cells (Michael, 1993; Johnson, 1966).
The cellular type of neoplasm that causes a pleural effusion changes false negative rate when pleural effusion is processed by cytology techniques. Squamous cell carcinoma, adenocarcinoma and breast carcinoma were most frequently positive on cytopathologic analysis (Light, 1995; Light, 1973; Prakash and Reiman, 1985; Spriggs and Boddington, 1968; Naylor and Schmidt, 1964).

The application of cell blocks and smears in the evaluation of pleural effusions were associated with better isolation and identification of malignant cells than by either method alone(Sallach, 2002; Dekker and Bupp, 1978).

In the present study, we found satisfactory levels of sensitivity and specificity only in the discrimination of benign from malignant effusions that compared with results of Fassina study (Fassina, 2008).

Due to presence of prominent reparative atypia in reactive hyperplastic mesothelial cells that could mimic malignant conditions, false positive and false negative interpretations are found. With application of ancillary techniques, identification of reactive and malignant processes, and also, MM from unknown origin pleural malignancy was possible for the accurate isolation of reactive processes from malignant forms (Motherby, 1999; Attanoos, 2003).

Generally, Immunohistochemistry (IHC), have greatly enhanced the ability of the cytopathologic to resolve a major difficulty in the categorization of pleural effusions. However, proper interpretations of IHC results are crucial (Leiman, 2001).

We were not added cell blocks to conventional cytospin (smear) preparation of pleural effusions and it was a limitation in this study.

Conclusion:

In this study, Cytopathology is a useful and reliable tool in discrimination between malignant and benign pleural effusions, and has not sufficient power for identification between primary and secondary pleural malignancy, based on histomorphologic findings. However in this situation use of pertinent clinical history, cell blocks, Immunohistochemistry (IHC) studies are highly mandatory.

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