Diagnostic Approach of Atypical Cells in Effusion Cytology Using Computerized Image Analysis

Zainab A J Al-Obaidi MBChB, FIBMS (pathology)

Abstract

Background: Cytology is one of the important diagnostic tests done on effusion fluid. It can detect malignant cells in up to 60% of malignant cases.

The most important benign cell present in these effusions is the mesothelial cell.

Mesothelial atypia can be striking andmay simulate metastatic carcinoma. Many clinical conditions may produce such a reactive atypical cells as in anemia,SLE, liver cirrhosis and many other conditions.

Recently many studies showed the value of computerized image analysis in differentiating atypical cells from malignant adenocarcinoma cells in effusion smears. Other studies support the reliability of the quantitative analysisand morphometric features and proved that they are objective prognostic indices.

Methods: Sixty three cases of pleural and peritoneal smears, previously reported as benign (19) cases, malignant (21) cases or atypical (23) cases, were retrieved from the files. In each of these smears; nuclear area, perimeter, and roundness coefficient of 80-100 cell were determined at x400 magnification by the use of image analysis system.

Statistical analysis was performed using analysis of variance and Tukey's HSD test.

Results: The mean values of nuclear roundness, nuclear perimeter and nuclear area vary between the three groups (benign, atypical and malignant cells) by using analysis of variance (p > 0.01).

The value of nuclear roundness, perimeter and area did not differ significantly between benign and atypical cells (Tukey's test: p<0.01).

On the other hand, the value of nuclear roundness, perimeter and area showed a significant difference between malignant and atypical cells(Tukey's test: p> 0.01).

Conclusion: In conclusion, our data suggest that cytomorphometry performed on effusion smear cells may provide important information for the differentiation of atypical cells from malignant cells, in which the values of atypical cells are closer to those of benign cells during the examination of pleural and peritoneal smears by the use of image analysis system.

Key words: Effusion cytology, Atypical cells, Cytomorphometry.

Introduction

Cytology is one of the important diagnostic tests done on effusion fluids [1]. Pleural fluid cytology is positive for malignancy in 60% of malignant effusions [2], This value increases in the subsequent samples obtained by thoracocentesis [3].The common site of metastasis is bronchogenic carcinoma in male and breast carcinoma in female[1].While a malignant peritoneal effusion is most commonly caused by gastrointestinal malignancy in male and ovarian carcinoma in female [1].

The most important benign cell present in these effusions is the mesothelial cell [1]

Mesothelial atypia can be striking and may simulate metastatic carcinoma [1].Many clinical conditions may produce such atypical cells as in anemia, cirrhosis, systemic lupus erythematosus, pulmonary infarction, renal failure or any condition which produce long standing fluid accumulation [1].Irritation of the mesothelium may provoke a reactive changes like the appearance of conspicuous nucleoliand the formation of pseudo glandular structures [4].

The differential diagnosis between carcinomatous and atypical cells in ascetic fluid is a difficult point [5]. Much of the pleural fluid literatures have focused on the use of immunocytochemistry to differentiate the mesothelial cells (benign or malignant) from malignant epithelial cellsand most commonly metastatic carcinoma [6].

So in order to ascertain the exact nature of the atypicalcells, flowcytometry & mmuno-cytochemistry can be performed [7].

Recently many studies showed the value of computerized image analysis in the differentiation of atypical cells from malignant adenocarcinoma cells in effusion smears [4,8,9].Other studies showed that the computed features enables a good discrimination between normal and cancerous cells, that it allows the distinction between normal andabnormal population in a range of 60-90% during the examination of serous fluid cytology specimens [9].

In addition it has been proved that the use of quantitative methods can reliably support the differential diagnosis between ovarian carcinoma cells and mesothelial cells in ascitic fluid [4].Many other studies support the reliability of the quantitative analysisandmorphometric features and onsider them as objective prognostic indices [10].

In the present study we attempted to assess the atypical cells present in the pleural and peritoneal spirates using a computerized nuclear image analysis.

Methods

Sixty three cases of pleural and peritoneal effusion smears were studied from routine cytological material , collected during one year period (January 2004 – january2005) from the files of the pathological department at Al-Yarmouk Teaching Hospital and were reevaluated separately by three different senior pathologists.

All samples had previously been fixed in 95% ethyl alcohol, stained with Hematoxyline and Eosin and were reported as either benign (19) cases, malignant (21) cases or atypical (23) cases.

In each case (80-100) nuclei were examined at X400 (X40 objective magnification, X10 camera ocular), by the use of image analysis system run by global lab image 2 software GLI2 (data translation Inc., USA).

The system composed of, personal computer PC with frame grabber (DT3120k-1data translation Inc., USA) attached to the PC, and a microscope (Olympus BH, Japan) with a video camera (KGB, cc-8603, Taiwan).

The images from the smears were obtained at 800X600 pixels resolution in BMP format.

The morphometric features assessed included:

• Nuclear area (a)

• Nuclear perimeter (p)

• Nuclear roundness coefficient calculated as follows: (r)

$r=4\pi a/p^2$ (where "1" value means a complete regular circle, while "zero" value mean a complete irregular shape)

The digitalized images of the nuclear profile were outlined on the monitor screen using a computer mouse.

The system was calibrated with a micrometer slide before each measurement.

The data were transferred to a Microsoft® excel work sheet and were expressed in terms of micrometers, the differences in terms of morphometric measurement between the three groups studied were statistically tested using analysis of variance, and Tukey's HSD test .

Results

The descriptive data obtained from the study expressed in terms of means ± one standard deviation are summarized in **Table-1.**

The mean values of nuclear roundness, nuclear perimeter and nuclear area vary between the three groups (benign, atypical and malignant cells) by using analysis of variance (p > 0.01) **Table 2, 3, and 4.**

The value of nuclear roundness, perimeter and area did not differ significantly between benign and atypical cells (Tukey's test: p<0.01).

On the other hand, the value of nuclear roundness, perimeter and area showed a significant difference between malignant and atypical cells (Tukey's test: p> 0.01).

Discussion

Since the differential diagnosis between adenocarcinoma cells and atypical cells has become an obstacle during the examination of pleural and peritoneal smears [5], many techniques were introduced such as immunohistochemistry, flowcytometry and morphometry[7] ,in order to decide the nature of these atypical cells in these smears.

Quantitative pathology methods have been used for the study of atypical cells [8] and they found to be a reliable, objective method for such a study [10].

In one previous study done on effusion smears using computerized image analysis, two morphometric variables (nuclear major axis length and nuclear area) of the nuclei were measured .Higher values for the area found for malignant rather than benign and atypical cells. The same result was extracted for the nuclear major axis length values. The values of the two parameters did not differ significantly between benign and atypical cells. The result shows that the ranges of the values for atypical cells are closer to the range of benign cells [8].

The present study was performed on cytological specimens of both pleural and peritoneal fluids and our aim was to check whether morphometric features may help in deciding the nature of atypical cells in these smears.

Statistically significant difference was found between the malignant and atypical cells concerning nuclear roundness, perimeter and area while there was no significant difference between benign and atypical cells regarding nuclear roundness, perimeter and area.

These results showed that the values of atypical cells are closer to those of benign cells during the examination of pleural and peritoneal smears by the use of image analysis system.

Although other more conclusive tests are needed for the final diagnosis of these atypical cells, yet our data suggest that cytomorphometry performed on effusion smear cells may provide important information for the differentiation of atypical cells from malignant cells.

(Table 1)

Pooled Data Showing the Values of the three Parameters
Used in the three Groups, Expressed as Mean ± S.D.

Parameter	Benign	Atypical	Malignant
Roundness	0.765 ± 0.042	0.773 ± 0.054	0.742 ± 0.053
Perimeter µm	22.62 ± 6.657	24.982 ± 3.958	28.780 ± 8.033
Area µm^2	32.672 ± 17.881	39.403 ± 13.380	53.519 ± 30.183

(Table 2)

One Way ANOVA for the Nuclear Roundness

Source of Variance	Sum of Squares	Degree of Freedom	Mean Squares	F
Among groups	0.065	2	0.032	12.564
Within groups	0.898	348	0.003	
Total	1.056	350		

f=4.666

(Table 3)
One way ANOVA for the Nuclear Perimeter

Source of variance	Sum of squares	Degree of freedom	Mean squares	F
Among groups	2027.529	2	1013.764	25.863
Within groups	13640.64	348	39.197	
Total	15668.17	350		

f=4.666

(Table 4)
One way ANOVA for the Nuclear Area

Source of Variance	Sum of Squares	Degree of Freedom	Mean squares	F
Among groups	24191.52	2	12095.76	26.375
Within groups	159594.7	348	458.605	
Total	183786.2	350		

f=4.666

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*From the Department of pathology, College of Medicine / Al-Mustansiryah University

Correspondence Address to:
Dr. *Zainab A.J Al-Obaidi*
E-Mail: zajobaidi@yahoo.com