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Cytological Study of Effusions and the role of Morphometry in Cytodiagnosis

[1]Dr. Akshay Dhotre, Department of Pathology, NKP Salve Institute of Medical Sciences and Research Centre, Digdoh Hills, Nagpur, Maharashtra.

[2]Dr. Archana Joshi, Associate Professor, Department of Pathology, NKP Salve Institute of Medical Sciences and Research Centre, Digdoh Hills, Nagpur, Maharashtra

[3]Dr. Sadhana Mahore, Professor & HOD, Department of Pathology, NKP Salve Institute of Medical Sciences and Research Centre, Digdoh Hills, Nagpur, Maharashtra.

Corresponding Author: Dr. Akshay Dhotre, Department of Pathology, NKP Salve Institute of Medical Sciences and Research Centre, Digdoh Hills, Nagpur, Maharashtra.

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Abstract

Cytological study of effusions helps in determining the etiology of effusion as well as in certain cases can help to determine the prognosis of disease. However, the distinction of reactive mesothelial cells from malignant cells can sometimes be difficult for the cytopathologist. The aim of this study was to examine the cytomorphological features of effusions and to assess the utility the of morphometry in differentiating reactive mesothelial cells from malignant cells in effusion smears. This was a cross sectional study involving 190 participants, carried out for a period of two years from January 2016 to December 2017 in the Department of Pathology of a tertiary care center. All the exudative serous effusions of pleural, peritoneal and pericardial cavities were included in the cytological examination. Morphometric analysis was performed on H&E and PAP stained slides prepared by conventional smear and cytospin techniques. Photomicrographs were taken and morphometry by computer assisted digital software was done in suspicious cases. Qualitative data expressed in percentages and quantitative data in mean and standard deviation. For calculation of p values ANOVA test was used. A p-value of < 0.05 was considered significant. Out of total 190 cases, 160 were non neoplastic (84.21%) while 30 were neoplastic (15.79%). p value was <0.01 between the morphometric parameters of reactive mesothelial cells and malignant cells. The routine microscopic evaluation on effusion cytology many times is not able to discriminate between reactive mesothelial cells, atypical cells and malignant cells due to overlapping morphological features. Morphometry by computer aided image analysis can be a simple, relatively less expensive and helpful in diagnosis of malignant cells by evaluation of various parameters like nuclear and cytoplasmic variables of atypical or suspicious cells.

Keywords: Cytology, effusion, morphometry

Introduction

Cytological assessment of effusion fluid is far better than the biopsy of the serous cavity lining for the diagnosis of malignancy affecting any of the cavities, as focal lesions on a serous malignant cells accumulate from all surfaces lining representing the entire serous cavity and are simple to collect.[1]A frequent complication of malignant tumours is accumulation of fluid in the body cavities. Sometimes it may be the common presenting sign of malignancy.[2]

The general cytological examination can be performed easily, quickly, and inexpensively by conventional smears. Distinction between benign and malignant cellular changes requires meticulous screening, careful visualization of cellular features and an understanding of the range of changes in reactive process. [3]

Morphometry by computer aided image analysis can be a relatively less expensive ancillary technique in differentiating reactive mesothelial cells from malignant cells by evaluation of various nuclear and cytoplasmic parameters.[4]

Henceforth this study was carried out to study the cytomorphological features of effusions and to assess the utility of morphometry in differentiating reactive mesothelial cells from malignant cells in effusions.

Materials and methods

This was a cross sectional study involving 190 participants, carried out for a period of two years from January 2016 to December 2017 in the Department of Pathology of a tertiary care center. All the exudative serous effusions of pleural, peritoneal and pericardial cavities were included in the cytological examination. All the participants were included the study after obtaining consent. Transudate effusions were excluded from the study.

Data was collected using pretested, semi-structured proforma which included information regarding socio-demographic factors, clinical diagnosis and biochemical investigations. Each effusion sample was given unique identification number. For cytological examination. The samples were classified as transudative or exudative based on predefined criteria.

Samples were processed by routine conventional smear technique and cytospin technique. In case of delay in submitting after collection beyond working hours, samples were received and stored in refrigerator at temperature of 2-6℃. Afterwards, stored samples were processed as early as possible.

After staining, the slides were evaluated for cytomorphological features like cellularity, architectural pattern, cell population and individual cell morphology. The clinical findings were correlated with cytological diagnosis. The samples were categorized as non-neoplastic and neoplastic effusions. Non neoplastic effusions were further sub classified into inflammatory and reactive. Neoplastic effusions were further sub divided into suspicious and malignant. Histopathological confirmation was done wherever available. For retrospective cases, data and slides were collected from archives. Morphometry was done only in cases showing reactive mesothelial cells, suspicious cells, and malignant cells.

Morphometric analysis was performed on H&E and PAP stained slides prepared by conventional smear and cytospin techniques. A computerized digital photomicrograph system (Motic B1-223 ASC digital microscope with built-in 1.3 megapixel camera and Motic Images Plus 2.0 image analysis software) was used for image analysis. The measuring scale of the image analysis software was properly calibrated. Each image

had a resolution of 720x576 pixels and was saved in a tagged image file format. A digital picture was obtained under high power (400X) from ten different fields for cases showing malignant, suspicious and reactive mesothelial cells. Ten representative cells (one each from 10 different fields) per case were evaluated. Mean values for all morphometric parameters for each case was calculated. Only nuclei of non-overlapping well-preserved cells with sharp nuclear boundaries were chosen. Nuclei of multinucleated cells were not used for measurements. The nuclei and cytoplasm of the cells was manually outlined using mouse attached to computer. After measurement, the data was transferred to a Microsoft Excel sheet for further analysis. The morphometric parameters analyzed were nuclear diameter, nuclear area, nuclear perimeter, cytoplasmic diameter, cytoplasmic area and cytoplasmic perimeter.

Statistical analysis

Data was analyzed using software SPSS version 20.0. ANOVA was used to see difference among the groups. The diagnostic ability of various morphometric parameters to predict malignant effusion was assessed using Receiver Operating Characteristic (ROC) curves analysis. Overall diagnostic value is given by the area under the curve (AUC). A perfectly accurate test would yield a ROC of 1.0 and a ROC of 0.5 indicates a predictive efficacy no better than chance. A p value less than 0.05 was considered statistically significant for all tests.

Results

Out of 190 participants, there were around 104 females. Proportion of females was more among peritoneal fluid samples and proportion of males was more among samples of pleural fluid. There was only one person with pericardial fluid. Out of all samples 160 were non-neoplastic and 30 were neoplastic effusions. Among non-neoplastic fluids 119 were inflammatory and 41 were reactive effusion. Out 30 neoplastic effusions, 26 were malignant fluids and 4 were suspected malignant fluids. Maximum proportion of reactive and malignant effusions was found among 41-50 age-group while maximum proportion of inflammatory effusions was in 31-40 age group. Out of total 84 peritoneal effusions, 29 were due to ovarian masses followed by 9 because of cirrhosis. Most common cause of pleural effusion was LRTI which was present in 38 effusions. This was followed by tuberculosis in 28 effusions and lung malignancy in 10 effusions. By cytological examination it was found that, most common cause of effusion was chronic inflammation (40.53%). This was followed by reactive effusion (21.58%), malignant effusion (15.79%) and mixed inflammation (14.21%). [Table-1]. Out of total 190 cases of effusions, histopathological diagnosis was available only in 45 cases. All the malignant and suspicious cases were confirmed on histopathology. The most common cause of malignant effusion was Serous carcinoma Ovary (11cases) followed by Lung adenocarcinoma (8 cases). Out of total 30 malignant effusions, 28 were due to adenocarcinoma and remaining 2 were due to squamous cell carcinoma of lung. [Table-2].

Mean nuclear area, Mean nuclear diameter and Mean nuclear perimeter was significantly more in cells of malignant as well as suspicious effusions as compared to cells of reactive effusions and this difference was statistically significant.

Table 1: Distribution of study subjects according to cytological diagnosis

Cytological diagnosis	Frequency	Percent
Acute inflammation	13	6.84%
Chronic inflammation	77	40.53%
Mixed inflammation	27	14.21%
Eosinophilic effusion	2	1.05%
Reactive effusion	41	21.58%
Adenocarcinoma	25	13.16%
Squamous cell carcinoma	1	0.52%
Suspicious of epithelial malignancy	4	2.11%
Total	190	100.00%

Table 2 Correlation between cytological and histopathological diagnosis

Histopathological diagnosis	Cytological diagnosis				Total
	Non-neoplastic		Neoplastic		
	Inflammatory	Reactive	Suspicious of malignancy	Malignancy	
Adenocarcinoma colon	0	0	1	2	3
Adenocarcinoma endometrium	0	0	0	1	1
Adenocarcinoma lung	0	0	2	6	8
Adenocarcinoma stomach	0	0	0	2	2
Corpus luteal cyst	2	1	0	0	3
Dermoid cyst	1	1	0	0	2
Mucinous carcinoma ovary	0	0	0	2	2
Mucinous cystadenoma	1	3	0	0	4
Ovarian fibroma	0	1	0	0	1
Seromucinous carcinoma ovary	0	0	0	1	1
Serous carcinoma ovary	0	0	0	11	11
Serous cystadenoma	1	4	0	0	5
Squamous cell carcinoma lung	0	0	1	1	2
Total	5	10	4	26	45

Table 3 Comparison of mean nuclear area according to cytological diagnosis

Mean nuclear area (μm^2)	Cytological diagnosis			P Value
	Reactive mesothelial cells	Suspicious cells	Malignant cells	
Mean	63.33	140.92	143.51	<0.01

SD	10.67	21.01	21.91	
Mean nuclear diameter (µm)				
Mean	8.78	13.33	13.47	<0.01
SD	0.77	0.97	1.04	
Mean nuclear perimeter (µm)				
Mean	27.86	42.61	42.69	<0.01
SD	2.49	3.29	3.26	
Mean cytoplasmic area (μm^2)				
Mean	158.59	281.73	290.42	<0.01
SD	32.76	14.22	44.58	
Mean cytoplasmic perimeter (µm)				
Mean	15.77	18.93	18.92	<0.01
SD	4.11	1.50	6.09	
Mean n/c ratio				
Mean	0.30	0.49	0.49	<0.01
SD	0.02	0.44	0.05	

Mean cytoplasmic area, Mean cytoplasmic diameter and Mean cytoplasmic perimeter was significantly more in cells of malignant as well as suspicious effusions as compared to cells of reactive effusions and this difference was statistically significant. Mean n/c ratio was significantly more in cells of malignant effusions as well as suspicious effusions as compared to cells of reactive effusions and this difference was statistically significant.

The cutoff values of various morphometric measurements made, and their sensitivity, specificity and area under the curve has been shown in table no 4.

Table-4: Sensitivity and specificity of various parameters of morphometry

Parameter	Sensitivity	Specificity	AUC	Cut off value	p value
Nuclear area	88.24%	83.2%	0.951	≥ 116.46 μm^2	<0.001
Nuclear diameter	82.35%	82.9%	0.882	≥ 12.45 µm	<0.006
Nuclear perimeter	88.24%	83.4%	0.902	≥ 38.24 µm	<0.004
Cytoplasmic area	82.35%	82.88%	0.922	≥ 254.78 μm^2	<0.003
Cytoplasmic diameter	88.24%	83.1%	0.892	≥ 17.66 µm	<0.005
Cytoplasmic perimeter	88.24%	83.33%	0.892	≥ 55.46 µm	<0.005
N/C ratio	82.35%	83.33%	0.911	≥ 0.42	<0.003

Discussion

A total of 190 effusions of pleural, peritoneal and pericardial effusion were included in our study. The purpose of this study was to evaluate the utility morphometry in differentiating reactive mesothelial cells from malignant cells. Out of total 190 study subjects,

maximum i.e. 48 (25.26%) were present in 41-50 years age group. Similar findings were seen in a study by Mahajan S et al.[1] Chakrabarti PR et al in their study found that the maximum number of cases (25.6%) were observed in the 4th decade. As inferred from various studies and our study the overall the incidence of serous effusions is reported more in middle age group.[5]

In the present study out of 190 samples most common type of fluid was pleural 105 (55.26%) followed by Peritoneal fluid 84 (44.21%). Similar findings were seen in a study by Agrawal T et al found that out of 100 samples most common type of fluid was pleural (43%).[2] Similarly Jadhav AB et al in their study found that Pleural fluid was the most common type of fluid received (172 cases- 60.56%) followed by ascitic fluid (110 cases (38.73%).[6]

Out of 190 total subjects maximum were females i.e. 104 (54.74%). Males were 86 (45.26%). Proportion of pleural effusions was more among males. Proportion of peritoneal effusions was more among females. Gojiya P et al in their study found that 295 cases of pleural effusion evaluated in which 29% are female and 71% are male.[7] Out of total 190 cases, 26 cases were diagnosed as malignant effusions. 25 cases revealed tumor cells organized predominantly in large three dimensional clusters. Out of 26 cases, 25 were diagnosed as adenocarcinoma and one case as squamous cell carcinoma on cytology and all 26 cases were confirmed on histopathology. Oba et al did a study and found that the most common cause of EPEs was malignancy (26%) followed by idiopathic (25%) and parapneumonic (13%) effusions.[8]

Out of 190 study subjects 160 (84.21%) were non-neoplastic effusions and 30 (15.79%) were neoplastic effusions. There were 119 (74.38%) inflammatory and 41 (25.62%) reactive effusions out of total 160 non-neoplastic effusions. Out of total 30 neoplastic effusions 4 (13.33%) were suspicious of malignancy and remaining 26 (87.67%) were malignant effusions.

Similar findings were seen in a study by Gojiya P et al**,** found that on cytological examination, 95% of pleural fluids were non-malignant, out of them 13% were acute inflammations and 11% effusions had reactive mesothelial cells. Malignant pleural effusions were 15 (5%) out of which 60% pleural effusions have non-specific malignant cells effusions.[7] Similarly, Saha R et al 32% cases were diagnosed as non-neoplastic pleural lesion among them tuberculosis (18%) was the most common followed by nonspecific inflammation of pleura (10%).66% cases showed neoplastic involvement of pleura.[9]

Out of total 84 peritoneal effusions 29 because of ovarian masses followed by 9 because of cirrhosis. Most common cause of pleural effusion was LRTI which was present in 38 effusions. Biswas B et al revealed that Tuberculosis was the commonest non-neoplastic lesion followed by chronic nonspecific pleuritis comprising 60% and 33.3% of the non-neoplastic cases respectively.[10] Dowerah et al found that cirrhosis was the commonest cause of ascites comprising 40 % of the total cases.[11]

According cytological finding most common cause of effusion was chronic inflammation (40.53%). This was followed by reactive effusion (21.58%), mixed inflammation (14.21%) and malignant effusion (15.79%). Jadhav AB et al in their study found that out of total 172 pleural effusions chronic nonspecific inflammation was accounted for 160 cases (93.02%) which showed predominantly a chronic inflammatory infiltrate composed of lymphocytes and macrophages.[6]

In this study, the most common type of morphological pattern in malignant effusions was adenocarcinoma. Out of total 30 malignant effusions, 25 cases were diagnosed as adenocarcinoma on cytology. The studies done by Pradhan et al[15], Awasthi et al[16], Gupta et al[17], Kumavat et al[12]and Jadhav et al[6] showed similar findings.

Mean nuclear area was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Similar findings were seen in a study by Scott N et al.[18] Similar findings were seen in a study by Arora B et al[19] on morphometry.

Mean nuclear diameter was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Scott N et al found that on morphometry, values for mean nuclear diameter in benign cases was found to be 8.0 ± 1.3 µm, in suspicious cases was 8.4 ± 0.6 µm and in malignant cases was 8.9 ± 1.9 µm. ($p<0.05$, difference was significant).[18] Bisht B et al in their study found that the mean nuclear diameter of the benign group was 9.51 ± 3.94 µm and that of malignant cases was 18.67 ± 2.84 µm.($p<0.05$, difference was significant)[20]

Mean nuclear perimeter was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Similar findings were seen by Scott N et al and they found that on morphometry, values for mean nuclear perimeter in benign cases was found to be 25.4 ± 4.3 µm, in suspicious cases was 26.3 ± 1.8 µm and in malignant cases was 28.1 ± 6.0 µm, ($p<0.05$, difference was significant).[18] Similar findings were seen in a study by Al-Obaidi ZAJ[21], Similarly Ambroise MM et al in their study found that significant differences were found between benign and malignant effusions for the nuclear perimeter. No significant difference was found for circularity, a shape descriptor. [22]

Mean cytoplasmic area was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Our findings were similar to Arora B et al[46] but not consistent with the studies done by Scott N et al. [18]and Sen R et al[4].

Mean cytoplasmic diameter was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Our findings were not consistent with the study done by Scott N et al.[18]

Mean cytoplasmic perimeter was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Our findings were not consistent with the study done by Scott N et al.[18]

Mean n/c ratio was significantly more in cells of malignant effusions as compared to cells of reactive effusions and this difference was statistically significant. Similar findings were seen in a study by Arora B et al[19] Similarly Sen R et al in their study found that N:C ratio of mesothelial cells in benign effusions were 0.31 ± 0.01 and in malignant effusions were 0.34 ± 0.01.[4]

Out of total 190 cases of effusions, histopathological diagnosis was available only in 45 cases. All the malignant and suspicious cases were confirmed on histopathology. 12 cases were diagnosed as benign neoplasms on histopathology out which 9 were reported as reactive and 3 as inflammatory on cytology. Similar findings were seen in a study by Saha R et al.[9] They found that Pleural fluid cytopathology – histopathology correlation statistic showed moderate agreement (Cohen's kappa = 0.5).

In the current study nuclear area has very strong association in predicting the malignancy (p value<0.001, AUC=0.951). It has a sensitivity of 88.24% and specificity of 83.33%. The nuclear area ≥ 116.46 μm^2 is derived as a cut off value for prediction of malignancy. Similar findings were seen in a study by Ambroise MM et al.[22] In this study, nuclear diameter has very strong association in predicting the malignancy (p value<0.006, AUC=0.882). It has a sensitivity of 82.35% and specificity of 83.33%. The nuclear diameter ≥ 12.45 μm is derived as a cut off value for prediction of malignancy. Similar findings were seen in a study by Ambroise MM et al.[22]

In the current study nuclear perimeter has very strong association in predicting the malignancy (p value<0.004, AUC=0.902). It has a sensitivity of 88.24% and specificity of 83.33%. The nuclear perimeter ≥ 38.24 μm is derived as a cut off value for prediction of malignancy. Similar findings were seen in a study by Ambroise MM et al.[22] In this study N/C ratio has very strong association in predicting the malignancy (p value<0.003, AUC=0.911). It has a sensitivity of 82.35% and specificity of 83.33%. The N/C ratio ≥ 0.42 is derived as a cut off value for prediction of malignancy. Similar findings were seen in a study by Sen R et al**.** They found that using ROC (Receiver Operating Characteristic) curve N/C ratio 0.345 [sensitivity (93%) and specificity (94%)] considered as cut off values.[4]

Conclusion

Cytological examination of effusions is an inexpensive and simple procedure useful in finding the etiology and understanding the course of disease. However, there is a considerable overlap of cytomorphological features between reactive mesothelial cells and malignant cells in effusions. Computerised Image Morphometry can serve as a sensitive and specific tool to differentiate reactive mesothelial cells from malignant cells in effusion cytology. Thus, morphometry can aid in cytological diagnosis quickly where resources like immunocytochemistry are not available.

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Legend Figures

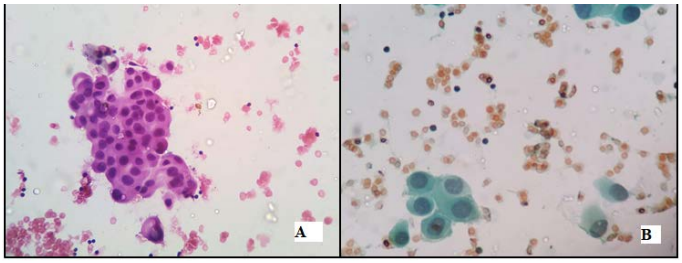

Figure 1: A) Reactive mesothelial cells showing anisonucleosis (H & E, 400X) B) Reactive mesothelial cells showing windows(PAP, 400X)

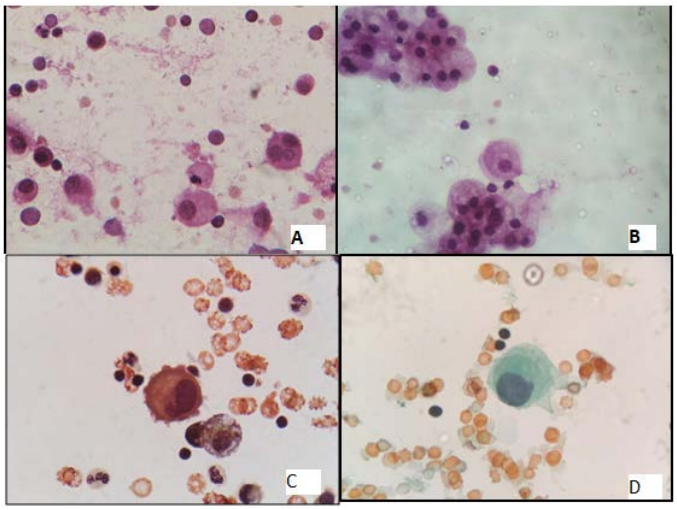

Figure 2: Cytomorphology of reactive mesothelial cells

A) Trinucleated reactive mesothelial cell, H & E, 400X
B) Reactive mesothelial cell showing peripheral vacoulation, H & E, 400X
C) Reactive mesothelial cell showing cytoplasmic blebs and microvilli, PAP 1000X
D) Reactive mesothelial cell showing biphasic staining, PAP, 1000X.

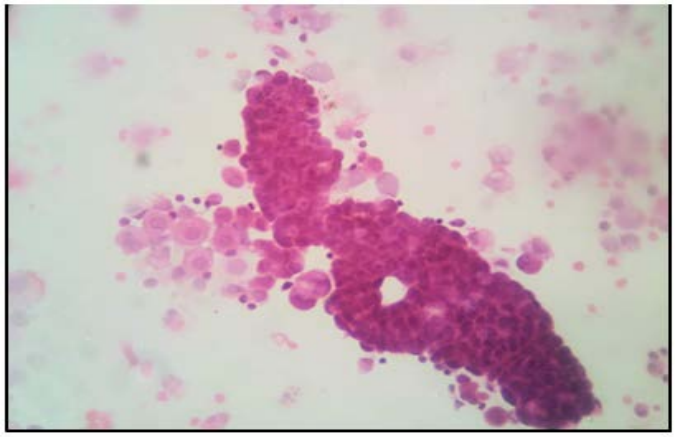

Figure 3: Pleural fluid showing malignant cells arranged in acinar pattern (Cytocentrifuged smear, H & E, 400X) 400X)

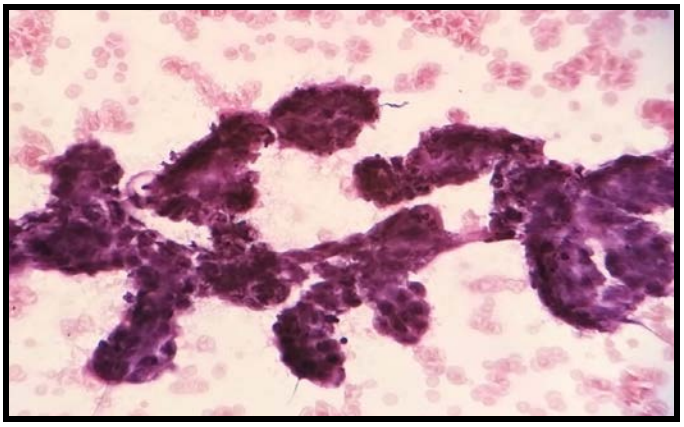

Figure 4: Peritoneal fluid showing malignant cells arranged in papillary pattern (Conventional smear, H & E, 400X)

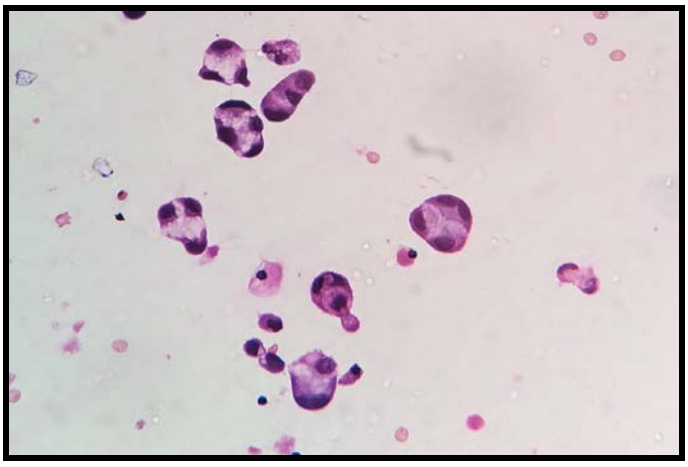

Figure 5: Peritoneal fluid showing deposits of mucinous carcinoma of ovary (H&E, 400X)

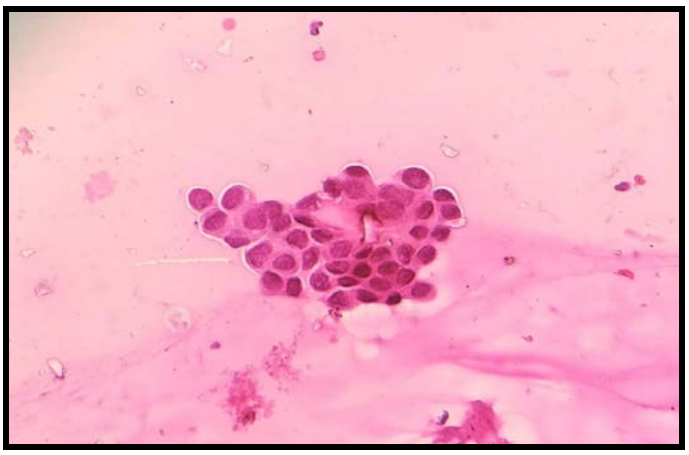

Figure 6: Pleural fluid showing deposits of squamous cell carcinoma of lung (H&E, 400X)

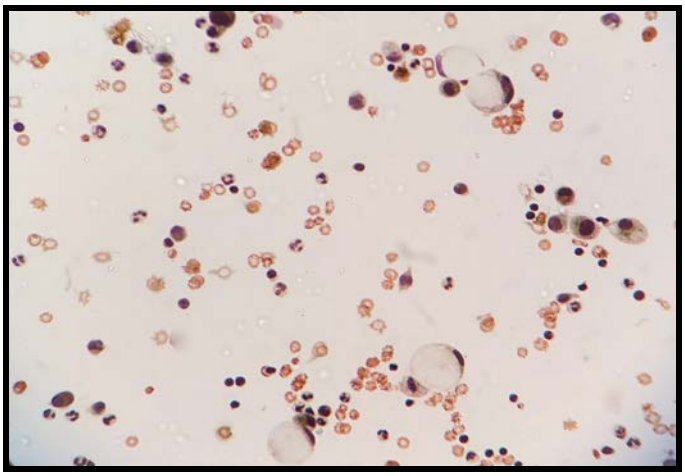

Figure 7: Signet ring cells of gastric adenocarcinoma in peritoneal fluid (PAP, 400X)

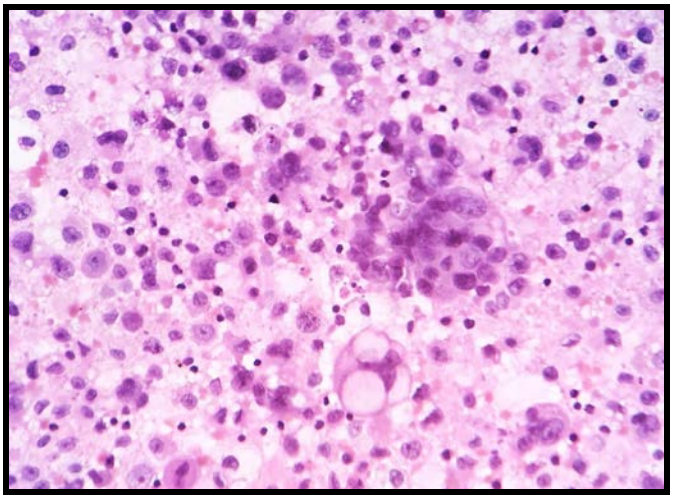

Figure 8: Peritoneal fluid showing deposits of Seromucinous carcinoma of ovary (H&E, 400X)

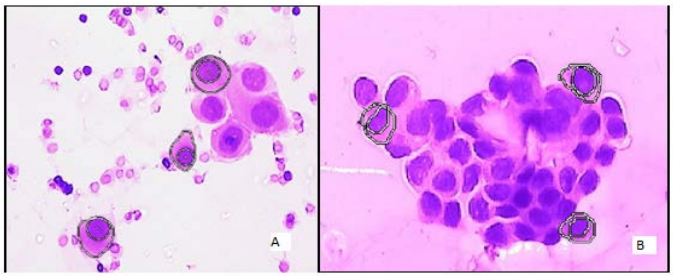

Figure 9: Manual cytoplasmic and nuclear markings for digital morphometry

A) Reactive mesothelial cells(H&E, 400X)

B) Malignant cells (H&E, 400X)

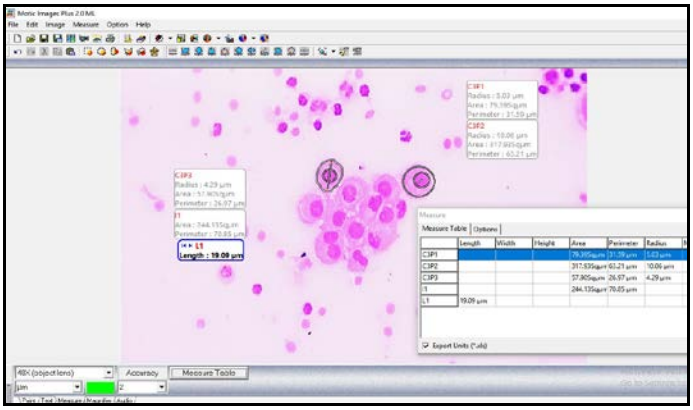

Figure 10: Morphometry of reactive mesothelial cells by image analysis software. (C3P1, C3P3- Measurements of nuclear parameters, C3P2- Measurements of cytoplasmic parameters, I1 – Cytoplasmic area and perimeter, L1- Cytoplasmic diameter)

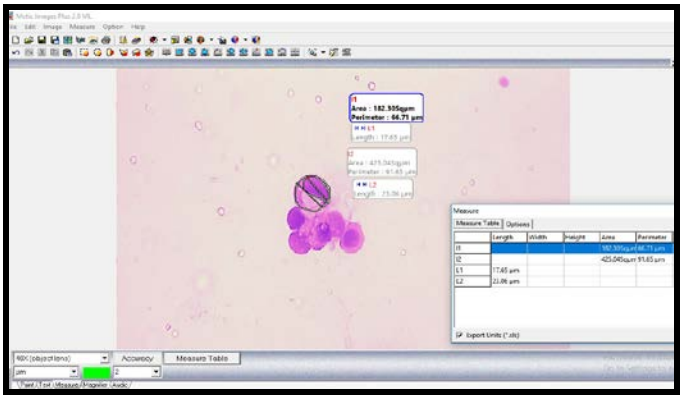

Figure 11: Morphometry of malignant cell by image analysis software. (I1- Nuclear area and perimeter, I2 – Cytoplasmic area and perimeter, L1 – Nuclear diameter and L2 – Cytoplasmic diameter)