AGAR BASED CELL BLOCK PREPARATION; A NEW PROMISING METHOD FOR ACCURATE EVALUATION AND ANCILLARY STUDY OF BODY EFFUSION

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Abstract

Background: The evaluation of serous effusions is essential in cytopathology due to their diagnostic and prognostic significance. The lack of material for adjuvant diagnostic investigations, such as immunocytochemistry and cytogenetic study, is a major drawback of the standard cytological smear. The cell block method helps you avoid this limitation. It is made from sediment cells that are converted into solid compact pellets, which helps in studying tissue architecture and applying further immunohistochemical studies. The use of cell block (CB) as an adjunct to routine cytology smears of body fluids can increase the sensitivity to a considerable extent.

Aim: Aim of the work to apply different cell block techniques on effusion samples (pleural and peritoneum) and correlate them to reach the best cell block techniques which give the highest diagnostic scores and accuracy.

Patients and Methods: This was a prospective analytic comparative study conducted at the Department of Pathology, Qena University Hospital (in the period from March 2021 to December 2022).

Results: Agar-based cell block showed the highest accuracy (93.33%) followed by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block (86.67%) in effusions. Cytology showed the least accuracy (80%),

Conclusion: We advocated using of cell block technique with cytological cases, because of its great benefit in raising the accuracy of diagnosis for different cytological samples. we proved that Agar-based cell block showed the highest scores and the highest diagnostic accuracy in effusion samples. It shows better results for texture, intensity, and number of cells when we use mixture of 5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde as a fixative solution in this method. And Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) based cell block came in the second place in its scores and diagnostic accuracy. But the egg albumin-based cell block and the plasma thrombin based cell block have the least score and the least diagnostic accuracy in effusion samples.

Keywords: Cell Block; Cytological diagnosis; Malignant; Body effusions; immunohistochemical.

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1. INTRODUCTION

Cytology is used to interpret cells obtained from various lesions. It is based on different sampling techniques that include fine needle aspiration cytology, brush cytology, body fluids and study and collection of exfoliated cells. (1)

It is a less invasive simple procedure, inexpensive, accurate and has the advantages of faster reporting. At the same time, cytological sample evaluation particularly in the presence of onside evaluation can provide provisional diagnostic pathway for further management. (1)

Cytology is different from histology. As cytology involves looking at single cell types. The cell block approach could be utilized to overcome these limitations. It is created from sediment cells that have been compressed into a solid pellet. It can be a useful adjunct to regular cytological procedures. The advantage of cell blocks over cytological smears is the capacity to observe parallels in architecture that cannot be observed in cytological smears alone. (2)

In the era of targeted therapy and biobanking, the

availability of immunohistochemistry (IHC) stains has raised the demand for cell block preparation in cytopathology for further immunocytochemical analysis, which is a huge advantage. As cell-block is an outstanding archival material that may be utilized at any moment in the future. (3)
cell block can be prepared by different methods, the main principle in each technique is the concentration of cells and their processing into paraffin blocks for current or future use. (2) In our study we modified a new technique using agar gel which gives good results and scores in effusion samples.
Aim of the work to apply different cell block techniques on effusion samples (pleural and peritoneum) and correlate them to reach the best cell block techniques which give the highest diagnostic scores and accuracy.

2. PATIENTS AND METHODS

This is a prospective analytic comparative study conducted at the Department of Pathology, Qena University Hospital (in the period from March 2021 to December 2022).
Study carried out on patients who visited the Outpatient Clinic of Chest Department and Internal Medicine Department, at Qena University Hospital complaining of pleural effusion or peritoneal effusion. Before inclusion in the research, all participants gave an informed consent, and the study protocol was approved by the Local Ethics Committee.

- **Inclusion criteria:**

Patient who presented by pathologically detectable pleural effusion and peritoneal effusion.

- **Exclusion criteria:**

Any patient who has the previous inclusion criteria but she or he refused to participate in this study, pregnant women, children less than 3 years and patients with bleeding diathesis.

Research strategy:

The following steps were performed for all participants: Detailed history to address: Age, sex, duration of swelling, loss of weight and appetite, accompanying by pain or not, dyspnoea, taking any medicines, having any bleeding problems, pregnant or planning to get pregnant and family history. Recording findings of pleural or peritoneal effusion examination: Pleural effusion is unilateral or bilateral, peripheral oedema, transudate or exudate, if the condition is acute or chronic and if the patient has associated symptoms of other illness or tumours or not.

METHODOLOGY:

The patients with pleural or peritoneal effusion:

The physician or radiologist should have carefully explained the process to the patient and all of the patient's queries should have been answered in full. If the surgery is scheduled and not an emergency, we instruct patients not to eat or drink for 12 hours prior and to empty their bladder right before the procedure.
Paracentesis is done in a lateral decubitus or supine position. The level of ascites fluid is percussed, and a needle is inserted in the midline or lateral lower quadrant (lateral to rectus abdominis muscle, 2 cm to 4 cm superomedial to anterior superior iliac spine).
The patient is put in a left lateral posture during pleural effusion aspiration. The ultrasonic probe was positioned in the right mid-axillary region of the fifth intercostal space, and the enhancement of the pleural effusion was observed. (4)

The aspiration technique:

In a sterile manner, prepare and drape the patient. Apply an antiseptic solution to the skin to cleanse it. Give local anesthetic to the skin and subcutaneous tissue at the targeted site of needle or catheter placement. Put the standard needle or IV catheter attached to a syringe or the prefabricated catheter straight perpendicular to the skin or utilizing the z-track approach, which is believed to reduce the likelihood of fluid leakage following the surgery. (4)

Slides preparation:

The fluid specimen either from pleural or peritoneum effusion is put in the centrifuge for about 10 minutes at 3000 rpm. Then the smears are prepared by adding One drop of substance in the corner of a properly cleaned and labeled slide. The direct smears were then created by spreading the material on the slide using a spreader slide to form a monolayer of cells.
The slides for wet fixation are placed immediately in 95% alcohol for a minimum of 15 minutes for staining with the Papanicolaou stain and haematoxylin & eosin stain.

Cell block preparations:

For each case, we use the aspirated material in preparing cell blocks by six different techniques, these techniques are: Agar embedding method, plasma thrombin method, Egg albumin method, formalin/ ethanol fixative (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde) method, formalin/ ethanol fixative (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde)method and concentrated Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) method.

Agar embedding method:

We modified a new method using agar gel. First, we mix the aspirated material with mixture of 5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde for about one hour then specimen with fixative was centrifuged for 10 minutes at 3000 rpm. Then mix the mixture with 10 percent formaldehyde for 2 hours and we centrifuge it again for 10 minutes at 3000 rpm. Cell button was carefully removed after

discarding the supernatant, then we take small amount of agar gel about 1x1 cm. and mix it with our mixture by using warm water path until the mixture becomes homogenous. Then the mixture was put in freezer until it turns from liquid to solid. The cell pellet is carefully wrapped in a piece of Joann fabric, The specimen was then processed and embedded in the same way as that of routine histopathological specimens, Sections were stained with Hematoxylin and Eosin for morphological evaluation.

Plasma thrombin method:
The aspirated material is rinsed in 10 percent formaldehyde. The mixture is centrifuged at 2500 rpm for 10 minutes. The supernatant fluid was removed, 0.5 mL of plasma and 2 drops of thrombin (5000 units in 10 ml of distilled water) were added, and the sample was agitated rapidly. Within 30-60 seconds, a clot formed. The clot was then placed in a tissue cassette lined with filter paper, after which it was treated and embedded in the same manner as standard histopathological specimens. Hematoxylin and Eosin were used to stain sections for morphological analysis. (5)

Egg albumin method:
Avoid the creation of clumps as you combine 10 grams of egg albumin powder with 100 cc of distilled water to make a smooth paste. After combining the aspirated material with egg albumin, the specimen was centrifuged at 3000 rpm for 10 minutes. The supernatant fluid has been removed, and the cell pellet has been gently wrapped with Cotton cloth.
Sections were stained with Hematoxylin and Eosin to evaluate the morphology of the specimen.

Formalin/ ethanol fixative (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde):
The aspirated material was first placed in container or plastic syringe, containing the fixative solution which is a mixture of (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde). The mixture was centrifuged for 10 minutes at 3000 rpm. Then, a piece of fabric from Joann was used to gently wrap the cell pellet. Sections were stained with Hematoxylin and Eosin for morphological examination, after the specimen had been prepared and embedded in the same way as standard morphologic specimens.

Formalin/ ethanol fixative (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde):
The aspirated material was first placed in container or plastic syringe, containing the fixative solution which is a mixture (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde). The mixture was centrifuged for 10 minutes at 3000 rpm. And the cell pellet was carefully wrapped in a piece of Joann fabric. The specimen was then processed and embedded in the same way as that of routine histopathological specimens, Sections were stained with Hematoxylin and Eosin for morphological evaluation.

Concentrated Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde):
The aspirated material was first placed in container or plastic syringe, containing the fixative solution which is a mixture (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde). The specimen with fixative solution was left for one night, then this mixture was centrifuge for 10 minutes at 3000 rpm. The cell pellet was carefully wrapped in a piece of Joann fabric. Sections were stained with Hematoxylin and Eosin for morphological examination, and the specimen was treated and embedded in the same manner as standard histopathological specimens.

Cytological evaluation:
Aspiration of pleural and peritoneal effusion: Cellular adequacy: As effusions are not standard samples and might exhibit a wide range of cellularity, protein, blood, and clot content, several different methods for processing fluids have been developed. Cells are often harvested after the fluid has been concentrated. (6). Samples must include adequate cellular constituents, for example, and research suggests processing a minimum of 50–75 mL of fluid to minimize false negatives and maximize test sensitivity. (7)

Diagnostic categories:
The cytological results were categorized into benign (negative), suspicious for malignancy and malignant. Benign aspirates are those that meet the sample adequacy criteria and show no signs of mesothelial or non-mesothelial malignancy in the cells. Suspicious aspirates had the cytological characteristics often seen in malignant tumors and met adequacy requirements, but there was not enough of the sample to make a definitive diagnosis of malignancy. Malignant aspirates have cytomorphological characteristics that are symptomatic of a primary or secondary malignancy, either on their own or in conjunction with other tests. (7)

Cell block evaluation:
Histology was used to prepare all of the CB samples, and H & E sections were then cut. Six slides from each cell block technique were inspected and compared for each instance. Slides were graded based on criteria decided upon at the study's inception. Each slide was given a score between one and three stars based on its cellularity, architecture, morphology, using an ancillary test, and recovery of cell clusters and fragments; a record of the best slide was also kept. (8)

Diagnostic categories of cell block slides:
After we recorded the best two slides which were representative of the best two methods of cell block preparation, their diagnostic results were categorized

into different diagnostic groups: benign (negative), suspicious for malignancy and malignant. Results of cytological smears, cell block slides and histopathological sections were later correlated to evaluate the efficiency of the cell block. Then, the diagnostic accuracy of cell block results was calculated.

STATISTICAL ANALYSIS:

Data will be entered into SPSS (Statistical Program for the Social Sciences) 26.0, Microsoft Excel 2016 and MedCalC (19.1) for tabulation and statistical analysis.

For numerical parametric data, descriptive statistics were calculated as mean SD (standard deviation), minimum, and maximum; for numerical non-parametric data, descriptive statistics were calculated as median and first and third inter-quartile range; and for categorical data, descriptive statistics were calculated as number and percentage.

Analytic statistics:

The Chi-square test is utilized to examine the relationship between two qualitative variables. Using Kappa statistics to compute the degree of concordance between two investigative techniques.

Diagnostic validity test:

It contains: Diagnostic specificity: It is the proportion of truly diagnosed disease cases (TP) relative to the overall number of disease cases (TP+FN). The diagnostic specificity: It is the proportion of truly non-diseased patients removed by the test (TN) relative to the overall number of non-diseased cases (TN+FP). The effectiveness or diagnostic precision of the test: It is the proportion of diseased and non-diseased cases within the entire amount of cases.

3. RESULTS

This prospective research was undertaken on 40 cases that are confirmed indicated for aspiration cytology, they were included in this research of which cytology smears, cell block and biopsy specimens were available. 26 were from lung aspirates pleural effusion (12 males & 14 females) and 14 were from peritoneum effusion (7 males &7 females) as shown in **Table (1).**

Table (1): Distribution of the studied cases as regards organ and gender

Parameters		Gender		Studied cases (N=40)	
		Male	Female	N	%
	Effusion	19	21	40	100%
	pleural effusion	12	14	26	65%
	peritoneum effusion)	7	7	14	35%

In effusion samples, agar embedding methods showed the highest score. As out of 40 cases, there were 35 cases were given score 3, while 5 cases were score 2 and no cases were score 1. As illustrated in **Table 2** and **Figures 1:4.**

Table (2): Distribution of the effusion samples cases as regards scores of different methods performed

Parameters		Effusion samples (N=40)	
Type of cell block	**score**	**N**	**%**
Alcohol/formalin (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde)	1	13	32%
	2	19	47.5%
	3	8	2%
Alcohol/ formalin (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde)	1	19	47.5%
	2	18	45%
	3	3	7.5%
Alcohol/ formalin (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde)	1	4	10%
	2	32	80%
	3	4	10%
Agar embedding	1	0	0%
	2	5	12.5%
	3	35	87.5%
Egg albumin	1	31	77.5%
	2	9	22.5%
Plasma thrombin	1	33	82.5%
	2	7	17.5%

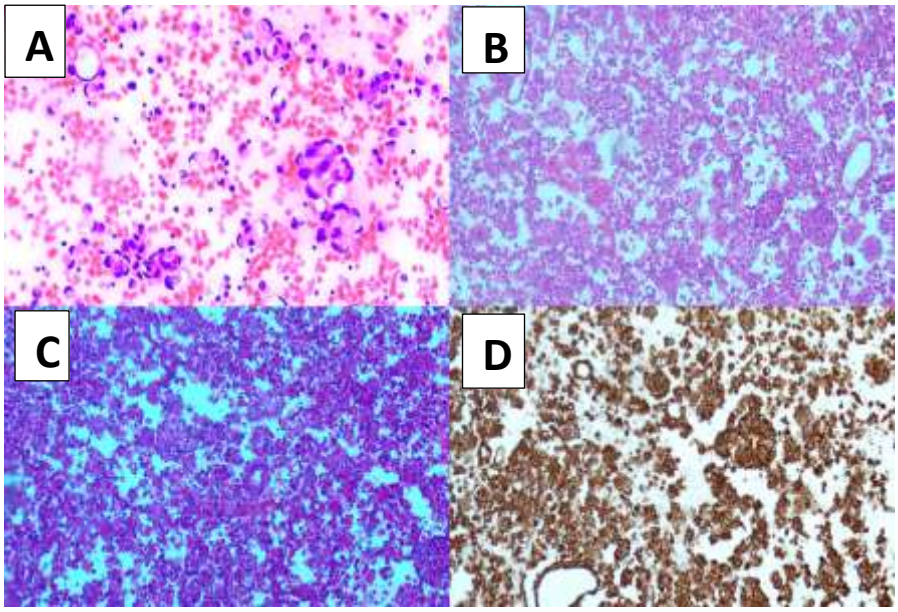

Fig (1): Pleural effusion showing adenocarcinoma metastasis.

(A, x200 power, H&E stain, FNAC showing cohesive groups of carcinomatous cells with eccentric nuclei that touch the periphery of the cells admixed with Some cells are less cohesive seen as solitary carcinoma cells). (B, C x100 power, H&E stain, cell block made by agar embedding method. Score 3. Showing numerous groups of malignant epithelial cells with pleomorphic nuclei, irregular nuclear contours, prominent nucleoli, and moderate to abundant foamy cytoplasm.). (D, x100 power, immunostaining with Cytokeratin (CK) showing positive cytoplasmic and membranous immunoreactivity).

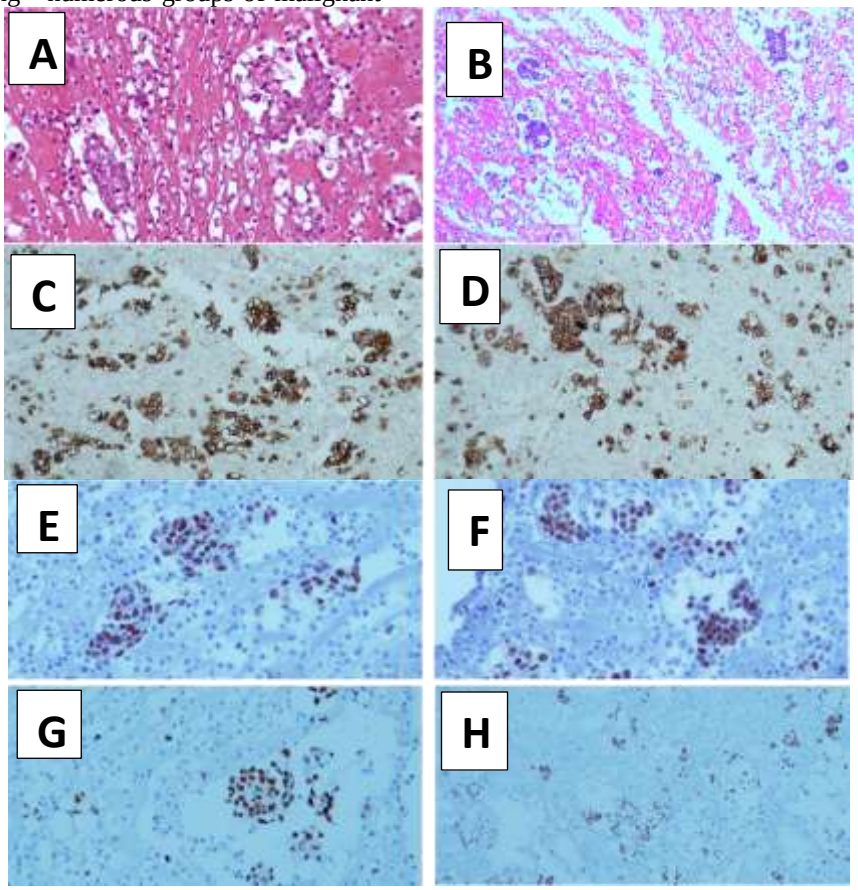

Fig (2): Pleural effusion showing adenocarcinoma metastasis.

(A, x200 power, B x 100 power, H&E stain, cell block made by agar embedding method. Score 3. Showing numerous groups and glandular structures lined by malignant epithelial cells with

pleomorphic nuclei, irregular nuclear contours, prominent nucleoli, and moderate to abundant foamy cytoplasm.). (C, D, x100 power, immunostaining with Cytokeratin7 (CK7) showing positive cytoplasmic and membranous immunoreactivity). (E, x100 power. F, x200 power. Immunostaining with WT1 showing positive nuclear immunoreactivity). (G, x200 power. H, x 100 power. Immunostaining with estrogen (ER) showing positive nuclear immunoreactivity).

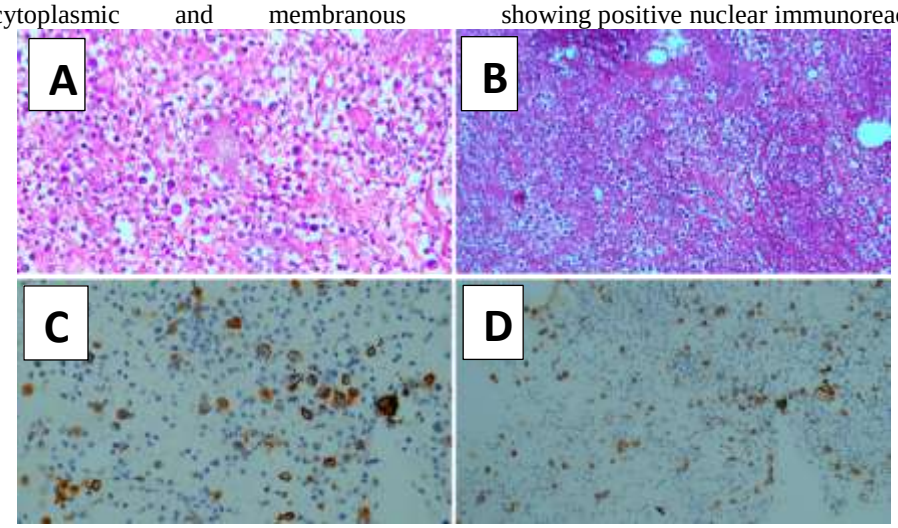

Fig (3): Pleural effusion showing metastatic undifferentiated carcinoma.

(A, x200 power, B x 100 power, H&E stain, cell block made by concentrated formalin/ alcohol method. Score 2. Showing borderline cellularity formed of atypical epithelial cells with pleomorphic nuclei, irregular nuclear contours, prominent nucleoli, and moderate to abundant foamy cytoplasm.). (C, x200 power. D x100 power. immunostaining with epithelial membrane antigen (EMA) showing positive apical luminal membrane immunoreactivity).

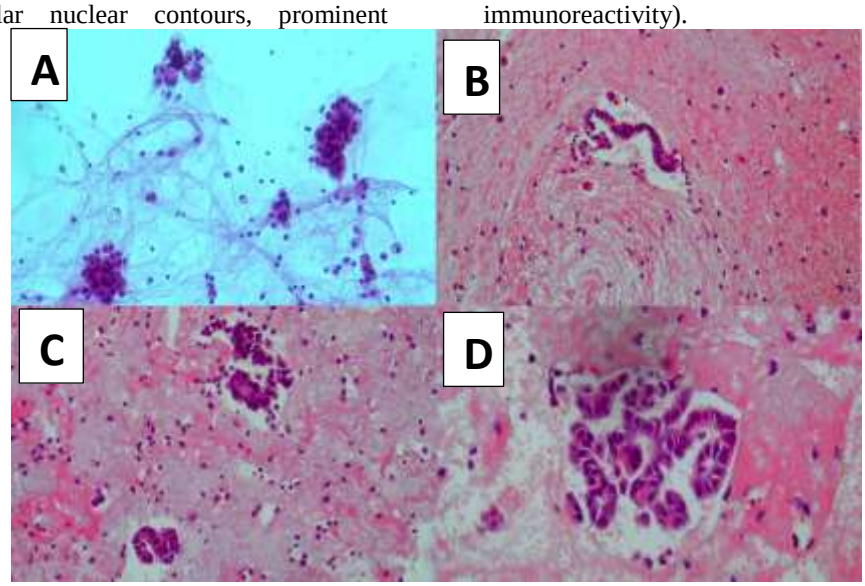

Fig (4): Peritoneum effusion with ovarian cystadenocarcinoma metastasis.

(A, x200 power. H&E stain, FNAC showing cohesive groups of carcinomatous cells with eccentric nuclei, irregular nuclear contours and hyperchromatism admixed with reactive mesothelial cells). (B, x400 power. C, x200 power. D, x200 power. H&E stain, cell block made by concentrated formalin/ alcohol method. Score 2. Showing numerous groups and glandular structures of malignant epithelial cells with pleomorphic nuclei, irregular nuclear contours, prominent nucleoli, and moderate to abundant foamy cytoplasm).

Most cases were benign on cytology (53.3%), cell block (56.7% on agar-based and 53.3% on formalin (30)/alcohol (70) based) as well as final diagnosis (66.7%). Malignant lesions were diagnosed by cytology in (26.7%), cell block (33.3% on both agar and formalin (30)/alcohol (70) based) while final diagnosis revealed the malignant diagnosis in (33.3%). Agar-based cell block preparations had fewer equivocal cases. **(Table-3)**

Table (3): Distribution of the studied cases as regards results of cell block, cytology, and final diagnosis in effusion samples

Parameters		**Studied cases (N=30)**	
		N	**%**
Cytological Diagnosis	**Benign**	16	53.3%
	Suspicious of malignancy	6	20.0%
	Malignant	8	26.7%
Agar based cell block diagnosis	**Benign**	17	56.7%
	Suspicious of malignancy	3	10.0%
	Malignant	10	33.3%
Formalin (30)/alcohol (70) based cell block diagnosis	**Benign**	16	53.3%
	Suspicious of malignancy	4	13.3%
	Malignant	10	33.3%
Histopathological diagnosis	**Benign**	20	66.7%
	Malignant	10	33.3%

In effusions samples four cases previously diagnosed suspiciously malignant on cytology were diagnosed malignant on final histopathological diagnosis. Kappa statistics revealed moderate agreement between cytology and histopathological diagnosis results (kappa =0.520). **(Table-4).**

Table (4): Inter-rater agreement of cytology results with histopathological diagnosis results in effusion

		Cytology			**Total**	**Agreement**	
		Benign	**Malignant**	**Suspicious of malignancy**		**Kappa**	**P-value**
Histo-pathological diagnosis	**Benign**	16	2	2	20 (66.7%)	0.520	**<0.001**
	Malignant	0	6	4	10 (33.3%)		
	Total	16 (53.3%)	8 (26.7%)	6 (20%)	30 (100%)		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant.

One case from effusions samples previously diagnosed suspicious of malignancy on agar-based cell block was diagnosed malignant on final histopathological diagnosis. Kappa statistics revealed good agreement between agar-based cell block and histopathological diagnosis results (kappa =0.739). **(Table-5).**

Table (5): Inter-rater agreement of agar-based cell block results with histopathological diagnosis results in effusions

		Agar-based cell block			**Total**	**Agreement**	
		Benign	**Malignant**	**Suspicious of malignancy**		**Kappa**	**P-value**
Histo-pathological diagnosis (Biopsy)	**Benign**	17	1	2	20 (66.7%)	0.739	**<0.001**
	Malignant	0	9	1	10 (33.3%)		
	Total	17 (56.7%)	10 (33.3%)	3 (10%)	30 (100%)		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant.

Two cases previously diagnosed suspicious of malignancy on Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block, were diagnosed malignant on final histopathological diagnosis in effusions. Kappa statistics revealed good agreement between formalin -based cell block and histopathological diagnosis results (kappa =0.625). **(Table-6).**

Table (6): Inter-rater agreement of Formalin (30)/alcohol (70) based cell block results with histopathological diagnosis results in effusions:

		Formalin (30)/alcohol (70) based cell block			**Total**	**Agreement**	
		Benign	**Malignant**	**Suspicious of malignancy**		**Kappa**	**P-value**
Histopath-ological diagnosis	**Benign**	16	2	2	20 (66.7%)	0.625	**<0.001**
	Malignant	0	8	2	10 (33.3%)		
	Total	16 (53.3%)	10 (33.3%)	4 (13.3%)	30 (100%)		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant.

Agar-based cell block showed the highest accuracy (93.33%) followed by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block (86.67%) in effusions. Cytology showed the least accuracy (80%) as illustrated in table 7.

Table (7): Comparison between accuracy of different methods in relation to histopathological diagnosis in effusions

	Cytology	Agar -based cell block	Formalin (30)/alcohol (70) based cell block
Sensitivity	75.00%	90.00%	80.00%
Specificity	81.82%	95.00%	90.00%
PPV	60.00%	90.00%	80.00%
NPV	90.00%	95.00%	90.00%
Accuracy	80.00%	93.33%	86.67%

PPV: Positive predictive value, NPV: Negative predictive value

4. DISCUSSION

The cell block (CB) is a regular cytopathology treatment that has acquired significance due to its central role in diagnosis and ancillary research. CBs can be derived from practically all cytological sample types. In the current era of personalized medicine, cytological specimens, particularly CBs, enhance the utility of cytological samples for assessing molecular changes as efficiently as surgical biopsies or resection specimens (8).

In present work, we studied 40 cases demonstrating cytological samples from effusion samples representing patients who visited the Outpatient Clinic of Chest Department and Internal Medicine Department, at Qena University Hospital complaining of pleural effusion and peritoneal effusion.

Our study showed that the number of males was 19 (47.5%) cases that is less than females (21 (52.5%)). This observation was in conjugation with studies performed by **Vance et al (2019)** that showed increase in number of female patients over than male patients. In contrast, the study done by **Melo et al (2020)** showed slight increase in male cases over female cases by 20%. This may be related to patient demographics circumstances among studied groups. (9, 10)

Our study included patients with age range between 21 and 71 years with mean ±SD was 53.18± 13.81 years and median was 42 years. This finding was similar to studies done by **Melo et al (2020).** Where mean age was 55.8 and 40.4 ± 19.5 years respectively. These findings may be related to the same age range of lesions in patients of studied groups. (10)

In our study, we performed the scoring system on the cell blocks of the 40 cases which were done by different 6 techniques. The scoring scale ranges from score 1 (worst) to score 3 (best) according to the following criteria: cellularity, architecture, morphology, use of ancillary test and recovery of cell cluster and fragments. According to the scoring system in the study of **Kasichhwa et al (2019).** (8)

Regarding agar embedding method there were 35 cell blocks (87.5%) gave score 3 and 5 cell blocks (12.5%) gave score 2. 4 cell blocks (10%) done by concentrated Formalin/ ethanol fixative method (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) gave score 3 and 32 cell blocks (80%) gave score 2.

Only 3 cell blocks (7.5%) done by Formalin/ ethanol fixative method (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde) gave score 3 and 8 cell blocks (20%) done by Formalin/ ethanol fixative method (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde) gave score 3. While there was no cell block done by the plasma thrombin method or egg albumin method gave a score 3.

So, the highest score detected was from agar embedding method, followed by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) method.

This was agreed with the study of **Miceli et al (2017)**, where the study proved that the agar embedded method shows better results for texture, intensity, and number of cells especially if we use 10% formalin or alcohol 50% as a fixative with the agar. (11)

But in our study, we obtained the best results when we use mixture of 5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde as a fixative.

In the study of **Nambirajan and jain (2018)** also proved that Pretreatment of cell pellet with 95% ethyl alcohol or CytoRich Red improves agar cell block cellularity due to clearing of mucoid substances. (12)

And in the study of **Lindsey et al (2016)** found that adding formalin as the fixative, decrease the diameter of the cell pellet (thereby increasing the thickness), and make the media that holds the cell pellet less likely to obscure the tissue. (13)

In the study of **Lindsey et al (2016)** said that the heating process in the agarose method may affect cell morphology and in the study of **Nambirajan and jain (2018)** also found that heat is induced artifacts in cells. In our study we overcome this obstacle by using warm water bath until the mixture becomes homogeneous and avoid using of oven or microwave

to warm the agar gel. We found that this technique helps in preservation of cells morphology and in absence of any artifacts. (12, 13)

In contrast, the study of **Balassanian et al (2016)** showed that the agar-based cell block technique did not capture all the material obtained by aspiration and the free-floating cells were often not well represented resulting in poorly concentrated and paucicellular CBs. This is most likely reflecting the dilution of the sample by the agar gel clotting agent. (14)

This differences between our study's findings and those of this study's may be due to using a combination of formalin and alcohol as a fixative in our study, but they used formalin alone as a fixative in their study. Moreover, we found that adding alcohol to the fixative reagent helps in concentration of the cells and increasing the cellularity of agar-based cell block.

The second highest score in our study was for concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) method after agar-based method. The score of this method was more than the scores of the rest methods where we use different concentration of diluted formalin and alcohol as a fixative reagent.

This was agreed with the study of **Ireka et al (2019),** that said that formalin-fixed tissues provides significantly better immunostaining results (84% good staining and exhibited 100% and 76% histoscore on E-cadherin and Ki-67 immunostaining respectively). (15)

But the study of **Layfield et al (2019)** suggested that 10% neutral buffered formalin was optimal for IHC and as their study shows that formalin fixation may deliver consistently more cells for morphologic analysis, immunohistochemical staining and DNA extraction. (16)

some studies have shown that using alcohol alone as a fixative was negatively affected the immunohistochemistry outcomes as in study of **Obiajulu et al (2020).** (17)

So, in our study we used the combination of absolute alcohol and absolute formalin (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde), and this combination gives us the best scoring in the cellularity, architecture, morphology, using of ancillary test and in the recovery of cell cluster and fragments.

Our study showed that the lowest scores were for the egg albumin method and the plasma thrombin method. That was shown also in the study of **Kasichhwa et al (2019)** where they found that the sections from plasma thrombin cell blocks had less cellularity and were less well preserved. (8)

That also was in line with the study of **Nambirajan and jain (2018)**, where they found that plasma thrombin cell block had less cellularity. Also, they found in their study that egg albumin method gave less cellular cell blocks and had a distracting dense pink background with partially obscuring folds. (12)

In contrast to our study, the study of Kanjilal et al (2021) said that they get high diagnostic accuracy in the diagnosis of pediatric abdominal neoplasms with cell block made by plasma thrombin method. This method had allowed them to recover minute cellular material when compared with other cell-block techniques and ancillary techniques were performed successfully in difficult cases as it did not compromise the antigenic preservation. (18)

In Our study, we compare between the frequency and percentage of each diagnostic category in cytology smears, cell block as well as histopathological diagnosis. In effusions samples we found that Cytology had overall sensitivity, specificity, and diagnostic accuracy of 75%, 81.82% and 80% respectively in detecting the malignant lesions in effusions samples. Positive predictive value was 60% while the negative predictive value was 90%.

Agar-based cell block had overall sensitivity, specificity, and diagnostic accuracy of 90%, 95% and 93.3% respectively in detecting the malignant lesions in effusions. Positive predictive value was 90% while the negative predictive value was 95%. This technique had fewer equivocal cases.

Concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block had overall sensitivity, specificity, and diagnostic accuracy of 80%, 90% and 86.67% respectively in detecting the malignant lesions in effusions. Positive predictive value was 80% while the negative predictive value was 90%.

As one case from effusions samples previously diagnosed malignant on agar-based cell block was diagnosed benign on final histopathological diagnosis. But two cases previously diagnosed malignant on concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block, were diagnosed benign on final histopathological diagnosis.

So, in effusion samples Agar-based cell block showed the highest accuracy (93.33%) followed by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) cell block (86.67%) in effusions. Cytology showed the least accuracy (80%).

That also was proved in the study of **Obiajulu et al (2020)**, the study showed that Equivocal cases constituted 16 (16%) cases of diagnosed on cytology alone compared to 3 (3%) on cell block. It was possible to detect stromal invasion in some malignant cases with cell block resulted in improved yield for malignancy. (17)

And the study of **Miceli et al (2017)** found that using agar embedding method in ascitic effusion showed better results for texture, intensity, and number of cells and Immunohistochemical staining of CA125

showed strong membrane positivity in about 70% of cells in cases of metastatic ovarian cancer. (11)
After all, in our study we proved that agar-based cell block has the highest score and the best diagnostic accuracy in effusion cases (pleural and peritoneal effusions). The cytology alone showed the least diagnostic accuracy.

5. CONCLUSION

We advocated using of cell block technique with cytological cases, because of its great benefit in raising the accuracy of diagnosis for different cytological samples. we proved that Agar-based cell block showed the highest scores and the highest diagnostic accuracy in effusion samples. It shows better results for texture, intensity, and number of cells when we use mixture of 5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde as a fixative solution in this method. Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) based cell block came in the second place in its scores and diagnostic accuracy. But the egg albumin-based cell block and the plasma thrombin based cell block have the least score and the least diagnostic accuracy in effusion samples.

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