Evaluation of different methods of performing cell block preparation and their role in accurate cytological diagnosis and reporting of fine needle aspiration cytology of the thyroid nodules

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Abstract

Background: Cytology is used to interpret cells obtained from various lesions. It is based on different sampling techniques that include fine needle aspiration cytology (FNAC), brush cytology, body fluids collection and study and collection of exfoliated cells. The use of cell block (CB) as an adjunct to routine fine needle aspiration can increase sensitivity to a considerable extent**.**

Objectives: to perform different techniques of cell block preparation and assess their utility in increasing the sensitivity of cytological diagnosis of fine needle aspiration cytology.

Patients and Methods: This was a prospective study carried out at Qena University Hospital's Department of Pathology (in the period from March 2021 to December 2022). The study included 40 individuals who were presented to the Outpatient Clinic of the General Surgery Department with thyroid nodules.

Results: Concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40 % formaldehyde) method showed the highest diagnostic accuracy and the highest score. Out of 40 cases (FNAC of thyroid nodules), there were 28 cases given a score of 3, while 12 cases were scored 2, and none was scored 1.

Conclusion: The cell block approach enables the recovery and processing of minute amounts of cellular material, allowing for better tumor categorization and for immunohistochemistry stains. We found that concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40 % formaldehyde) based cell block showed the highest scores and the highest diagnostic accuracy in FNAC samples of thyroid nodules.

Keywords: Cell Block; Cytodiagnosis; Malignant; FNAC; Immunohistochemical.
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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 collection and study and collection of exfoliated cells (**Wyse et al., 2018**).

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 (**Wyse et al., 2018**).

Another limitation of the typical FNA smear is the scarcity of materials for adjuvant diagnostic studies like immunocytochemistry and cytogenetic analysis. (**Sharma et al., 2015**)

The cell block approach might be utilized to circumvent this constraint. It is created from sediment cells that have been compressed into a solid pellet. It can be a valuable adjunct to regular cytological procedures. The advantage of cell blocks over cytological smears is the capacity to identify similarities in architecture that cannot be noticed in cytological smears alone (**Sharma et al., 2015**).

Due to the absence of appropriate standards for cell block production, few research testing its diagnostic effectiveness and utility exist. The use of CB as an addition to standard cytology smears of FNAC can significantly improve sensitivity (**Ranade et al., 2018**).

The availability of immunohistochemical (IHC) stains in recent years has also elevate the desire for CB preparation in cytopathology for further immunocytochemical study which is a tremendous advantage in the era of targeted therapy and biobanking (**Sharma et al., 2015**).

The aim of the work is to perform different techniques of cell block preparation and assess their utility in increasing the sensitivity of cytodiagnosis of fine needle aspirates. Comparison of results obtained from cytological smears with the results of the best cell block technique and the final histopathological diagnosis to evaluate the role of the CB in raising the sensitivity of cytological diagnosis.

Patients and methods

This is a prospective study performed at the Qena University Hospital Department of Pathology (in the period from March 2021 to December 2022). Patients who visited the Outpatient Clinic of the Department of General Surgery at Qena University Hospital complaining of thyroid nodules were examined.

All participants supplied informed permission before involvement in the study, and the study was approved by Qena Faculty of Medicine institutional ethical committee.

Ethical approval code: SVU-MED-PAT005-2-21-3-164.

Inclusion criteria: Patient who presented clinically with detectable thyroid nodules that is amenable for FNAC after performing required investigations.

Exclusion criteria: Any patient who has the previous inclusion criteria but

she or he refused to participate in this study and children less than 3 years**.**

Research strategy: All participants carried out the following procedures: Detailed history to address: Age, sex, duration of swelling, menstrual cycle, loss of weight and appetite, irritability, tachycardia, accompanying by pain or not, dyspnoea, taking any medicines, and family history.

Recording findings of thyroid examination: Palpable nodules, solitary or multiple nodules, site, size, well defined or not, consistency and moving with swallowing or not

Methodology: The patients with thyroid nodules were subjected to:

Patient position: The patient should be given a thorough explanation of the operation, and any of his or her questions should be answered thoroughly. We warn our patients that no local anesthetic will be administered, that the aspiration will last several minutes that three to six aspirations will be collected, that we do not expect any major complications, but that there will be discomfort equivalent to a venipuncture (blood draw for laboratory tests). The aspiration is carried out on a hospital bed-bound patient. Always have a clinical assistant ready to assist with the process. The patient with thyroid nodules is positioned supine with the neck hyperextended in order to expose the thyroid; a pillow is placed under the shoulders for support. The patient must refrain from swallowing, speaking, and moving during the operation. It is optimal to communicate with the patient and keep him or her updated on the progress of the aspiration. After the aspiration is finished, the site of the aspiration is maintained under firm pressure. Patients should ideally be observed for a few minutes, as infrequently they may experience dizziness or pain. If there are no issues, they are permitted to leave (**Shahzad and Rahat, 2023).**

Aspiration technique: After properly placing the patient, we locate and pinpoint the mass utilizing suitable illumination during the aspiration. The cytologist should be positioned at the side of the patient, ideally on the opposite side of the lesion. The nodule to be aspirated is identified, and alcohol is used to clear the skin. It is not essential to employ povidone-iodine (Betadine) or sterile method (**Muhammada et al., 2020**).

Two fingers of the free (left) hand clasp the nodule firmly, while the other hand holds a 23-gauge needle attached to a 5-milliliter syringe. The needle is then immediately injected into the nodule through the skin. Once the needle tip is positioned within the nodule, gentle suction is given as the needle is pushed vertically within the nodule. Cell debris may be more easily dislodged and sucked into the needle with this motion. During this 5-10 second period, suction is maintained, and as soon as fluid or aspirate collects in the hub of the needle, suction is released and the needle is withdrawn (**Muhammada et al., 2020**).

After the aspiration has been completed, a 4x4-inch gauze pad is used to provide firm pressure to the site of aspiration. As soon as the bleeding has ceased, an adhesive bandage is applied to the puncture site(s) and the patient is watched for a

few minutes. If there are no issues, the patient is permitted to depart. The presence of fluid signals that the nodule is cystic; maintain suction and aspirate all fluid. Before removing the needle, it is essential to release the syringe plunger and relieve the vacuum; this prevents the aspirate from being sucked into the syringe. After detaching the needle from the syringe, 5 mL of air is sucked into the syringe (**Fig.1**) (**Muhammada et al., 2020**).

Slide preparation: Reattaching the needle to the syringe, one drop of aspirated material is pushed onto each of numerous glass slides. The slides for wet fixation should be placed immediately in 95% alcohol for a minimum of 15 minutes for staining with the Papanicolaou stain and haematoxylin & eosin stain. And other slides were stained by Diff-Quik (**Shahzad and Rahat, 2023**).

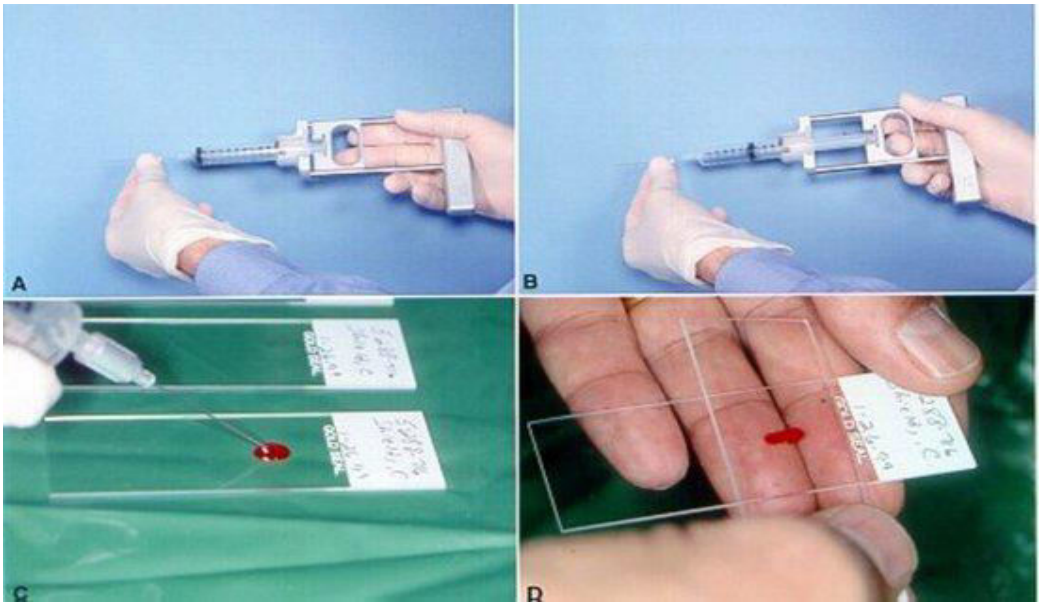

Fig.1. A, Quickly, the syringe's needle is pulled out. **B**, After reattaching the needle, you pull five milliliters of air into the syringe. **C**, One drop of the aspirated material is delivered onto each of several glass slides with the needle bevel pointing downward. In preparation for aspiration, slides are located and laid out on the table. **D**, a second slide is used in the same way as a blood smear would be made. Wet-fixing the slides in a bottle of liquor produces quick results (**Muhammada et al., 2020**).

Cell block preparations

Other passes of FNA from thyroid were randomly used for 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), formalin/ ethanol fixative (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde) and concentrated Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde).

Agar embedding method: We modified a new agar method technique. First, we mix the aspirated material with a mixture of (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde) at the same amount of aspirated material, for about one hour then specimen with fixative was centrifuged for ten minutes at

3000 rpm. Then mix the mixture with 10 percent formaldehyde at half of the amount of the mixture for 2 hours and centrifuge it again for 10 minutes at 3000 rpm. The cell button was carefully removed after removal the supernatant, then we take small amount of agar gel about 1x1 cm. and mix it with our mixture by using warm water bath 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, Sections obtained by microtome were stained with Hematoxylin and Eosin for morphological examination, and the material was treated and embedded in the same way as standard histopathological specimens (**Miceli et al., 2017**).

Plasma thrombin method: The aspirated material is just rinsed in 10 percent formaldehyde for few seconds. The mixture is centrifuged at 2500 rpm for ten minutes. After removing the supernatant, the sample was immediately agitated with the addition of 0.5 mL of plasma and 2 drops of thrombin (5000 units in 10 ml distilled water). Within 30-60 seconds, a clot had developed. The clot was then treated and embedded in paraffin wax in the same way as standard histopathology specimens are. Hematoxylin and eosin staining was used to examine cellular structure in tissue sections (**Nair and Manjula, 2015**).

Egg albumin method: Egg albumin powder (10 grams) and distilled water (100 milliliters) should be mixed into a smooth paste without any clumps. Egg albumin (at same amount of the aspirated material) was combined with the aspirated material, and the resulting specimen was centrifuged at 3000 rpm for 10 minutes to remove excess fixative. The cell pellet has been properly packaged in some Cotton cloth, and the supernatant fluid has been discarded. Hematoxylin and eosin were used to analyze the morphology of the specimen after it had been treated and embedded in paraffin wax, as is standard procedure for histopathological specimens (**Cretu et al., 2021**).

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) at the same amount of the aspirated material. The mixture was centrifuged for 10 minutes at 3000 rpm. And the cell pellet was carefully wrapped in a piece of Joann fabric. Sections were stained with Hematoxylin and Eosin for morphological examination, and the material was treated and embedded in the same way as standard histopathological specimens (**Shidham, 2019).**

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) at the same amount of the aspirated material. The mixture was centrifuged for ten 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 (**Ireka et al., 2019**).

Concentrated Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde):

we modified this method, and it is the first time to use concentrated alcohol with concentrated formalin. 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 concentrated 40% formaldehyde) at the same amount of the aspirated material. 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. Material was treated and embedded in the same way as standard histopathological specimens. Sections were stained with Hematoxylin and Eosin for morphological examination, (**Fig. 2**).

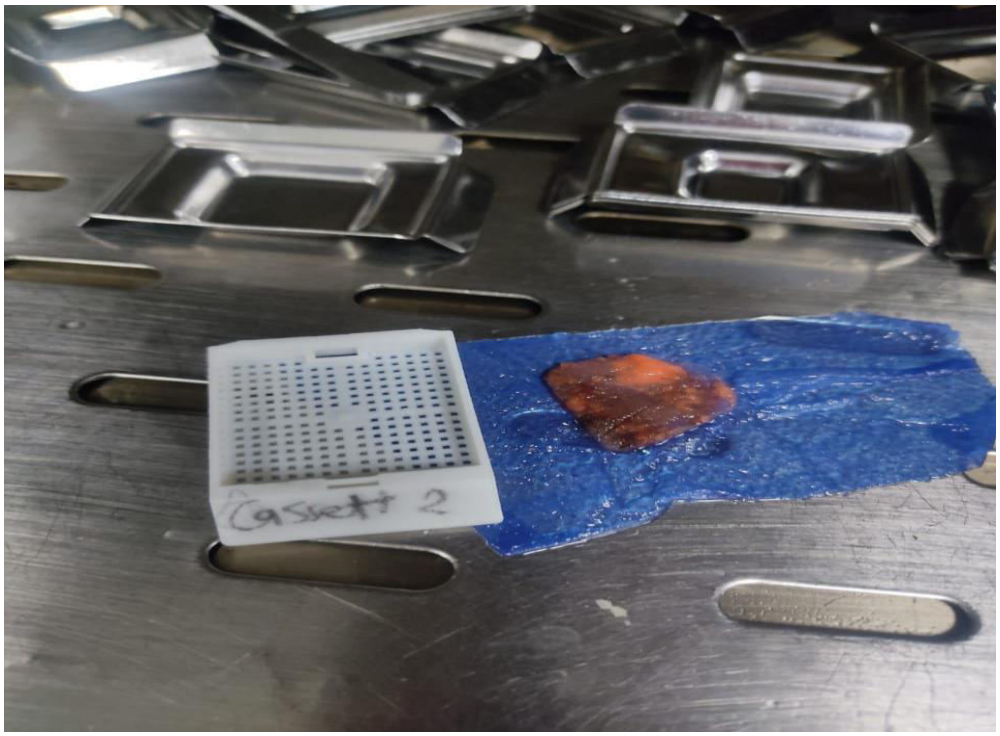

Fig.2. The cell pellet on a piece of Joann fabric, done by concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) method.

Cytological evaluation

A) FNAC of thyroid nodules

Cellular adequacy: It is necessary to collect a sufficient sample size. 5-6 groups of well-preserved follicular epithelial cells with 10 cells per group are proposed as criteria for the adequacy of thyroid cytology. Additionally, the smear should be technically well-prepared, the aspirate should be properly smeared to prevent clotting, and smears should be read in a clinical context. As 5-15% of these cases have been associated with malignancy, unsatisfactory aspirations ought to be repeated (**Lee et al., 2015**).

Diagnostic categories: the cytological findings were classified into five classifications as benign (negative), Atypia of undetermined significance, follicular lesions, suspicious for malignancy and malignant. Colloid goiter, colloid or adenomatous nodule, Hashimoto's thyroiditis, subacute thyroiditis, and thyroglossal duct cyst are all examples of benign aspirates. Follicular adenoma, cellular adenomatoid nodules, and hurthle cell proliferations were all seen in smears of follicular lesions. Malignant lesions, such as papillary carcinomas, were found in

smears that looked suspicious. Due to inadequate or delayed fixation, aspirates with insufficient cellularity or low-quality smear were signed as unacceptable (**Pandey et al., 2022**).

B) Cell block evaluation: After all the CB samples were processed by histology and H & E sections were cut, six slides taken from each different method of cell block were reviewed for each case and compared with each other. Slide scoring was performed with predetermined criteria established at the outset of the study. The slides were all scored on a scale of 1 (worst) to 3 (best) for the following criteria: cellularity, architecture, morphology, use of ancillary test and recovery of cell cluster and fragments, in addition, the overall best slide was also recorded As shown in **(Table .1)** (**Kasichhwa et al., 2019**).

Table 1. Scoring Criteria for evaluation of cell block.

Criteria	**Score** 1	**Score** 2	**Score 3**
Cellularity	Paucicellular (rare cells)	Moderately cellular (Numerous cells)	Abundantly cellular (Numerous cells and clusters)
Morphology Clarity	Poor	Fair	Good
Nuclear feature	Nuclear contours indistinct Chromatin not preserved. Nucleoli not apparent Mitotic figures not identified	Some nuclear contours visible Chromatin partially preserved Some nucleoli visible Few identifiable mitotic figures	Distinct nuclear contours Distinct chromatin pattern Prominent nucleoli Mitotic figures readily identified
Cytoplasm	No vacuoles/granules No cell boundaries	Some vacuoles/granules Some cell boundaries	Cytoplasmic vacuoles/granules evident/Distinct membrane boundaries
Use of ancillary tests	Not suitable for use	Not done	Done
Recovery of cell clusters and	Poor recovery, worse than conventional smears	Fair recovery, some cell clusters, and fragments, comparable to conventional smears	Good recovery, distinct patterns, and architectural assessment possible

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: The diagnostic results of cell block from thyroid nodules were categorized into: five categories as benign (negative), Atypia of undetermined significance, follicular lesions, suspicious for malignancy and malignant. Slides which were classified as benign included colloid goiter, colloid or adenomatous nodule, Hashimoto's thyroiditis, sub-acute thyroiditis, thyroglossal duct cyst. Follicular adenoma, cellular adenomatoid nodules, and hurthle cell proliferations

were all seen in smears of follicular lesions. The effectiveness of the CB was determined by correlating the results of cytological smears, cell block slides, and histopathological sections when were available (**Pandey et al., 2022**).

In our study the histopathological sections were available in 30 cases. 12 cases underwent hemithyroidectomy operation and 18 cases underwent total thyroidectomy. accuracy of CB data as a diagnostic tool was then determined.

Immunohistochemical staining: was performed for selected cell blocks with the highest score. Using the autostainer Ventana XT. For protein detection, we used the I-VIEW DAB Universal Kit from Ventana. The intensity and distribution of staining patterns were examined. A case was classified to be positive if greater than 5% of tumor cells were found; otherwise, the case was classified as negative. In terms of specific staining patterns, Cytoplasmic staining was classified as positive for CK19. Nuclear staining was classified as positive for TTF-1 (**Joshi et al., 2020**).

Statistical analysis

The acquired data was tabulated and statistically evaluated using the SPSS (Statistical Program for Social Sciences) program software version 26.0, Microsoft Excel 2016,

Results

This prospective research was carried out on 40 cases that are confirmed indicated for FNAC, they were included in this study of which

The concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) method showed the highest score. As out of 40 cases of thyroid nodules there were 28 cases given score 3, while 12 cases were score 2, and no case was score 1. The and the MedCalC program software version 19.1. Descriptive statistics were computed for numerical parametric data as mean ±SD (standard deviation) and minimum and maximum of the range, for numerical nonparametric data as median and first and third interquartile ranges, and for categorical data as number and percentage Chi-square test; used to investigate the relationship between two qualitative variables. Kappa statistics were used to calculate the degree of agreement between two investigative procedures.

Analytic statistics: The Chi-square test is used to investigate the relationship between two qualitative variables. Kappa statistics were used to determine the level of agreement between two investigative procedures.

Diagnostic validity test: It contains: Diagnostic specificity: It is the proportion of actually diagnosed illness cases (TP) relative to the overall number of disease cases (TP+FN). The diagnostic particularity: It is the proportion of really 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 total number of instances.

FNAC smears, cell block and biopsy specimens were available. Out of 40 specimens from the thyroid nodules, there were 5 males and 35 females.

score of Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) and agar embedding method showed significant difference between different methods in thyroid (<0.001) As illustrated in **(Table .2)**.

Table 2. Distribution of thyroid nodules aspirates cases as regards scores of different methods performed

Parameters		(N=40)			
Type of cell block	**score**	**N**	**%**	**Mean± SD**	**P- value**
Alcohol/formalin (5 parts of 95 percent ethanol/ 5 parts of 10 percent formaldehyde)	**1**	13	32.5%	1.95±.78	.230
	2	16	40%		
	3	11	27.5%		
Alcohol/ formalin (8 parts of 95 percent ethanol/ 2 parts of 10 percent formaldehyde)	**1**	18	45%	1.70±0.72	.008
	2	16	40%		
	3	6	15%		
Alcohol/ formalin (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde)	**1**	0	0%	2.70±0.46	<.001
	2	12	30%		
	3	28	70%		
Agar embedding	**1**	4	10%	2.03±0.48	<.001
	2	31	77.5%		
	3	5	12.5%		
Egg albumin	**1**	28	70%	1.30±0.46	.135
	2	12	30%		
Plasma thrombin	**1**	33	82.5%	1.18±0.38	.359
	2	7	17.5%		

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant, SD= standard deviation.

Cases in (**Fig 3, 4 and 5**) were diagnosed papillary thyroid carcinoma, their cell blocks gave score 3 and were done by Concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde) method. The cell blocks show high cellularity, good architecture, and morphology. In addition to the possibility of good immunohistochemical staining due to the high antigenicity (**Fig.3**).

An example of cell block done by agar embedding method is illustrated in (**Fig.6**). The cell block gave score 2 as it showed moderate cellularity and moderate immunohistochemical staining.

(Table .3) illustrates the frequency and percentage of each diagnostic category in FNAC, cell block as well as histopathological diagnosis in thyroid cases. Most cases were benign on FNAC (56.7%), cell block (56.7% on agar based and 53.3% on formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde)) as well as final diagnosis (66.7%). Malignant lesions were diagnosed by FNAC in (20%), cell block (26.7% on agar based and 26.7% on formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) while final diagnosis revealed malignant diagnosis in (33.3%) concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) based cell block had fewer equivocal cases.

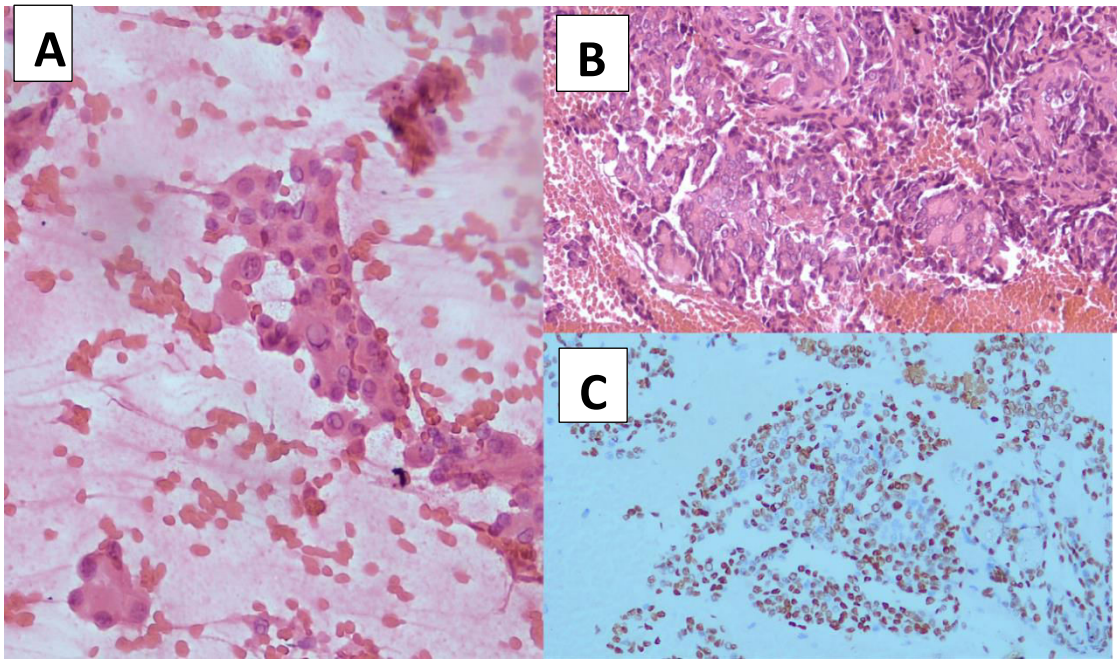

Fig.3. Papillary thyroid carcinoma. (A, x200 power, H&E stain. FNAC, showing anisonucleosis, nuclear pseudoinclusion and irregular nuclear membrane). (B, x200 power, H&E stain. cellblock by concentrated formalin/ alcohol method. Score 3. Showing papillary structures lined by overlapping follicular cells with nuclear clearing and pseudoinclusion). (C, x200 power immunostaining with TTF1 showing positive nuclear immunoreactivity).

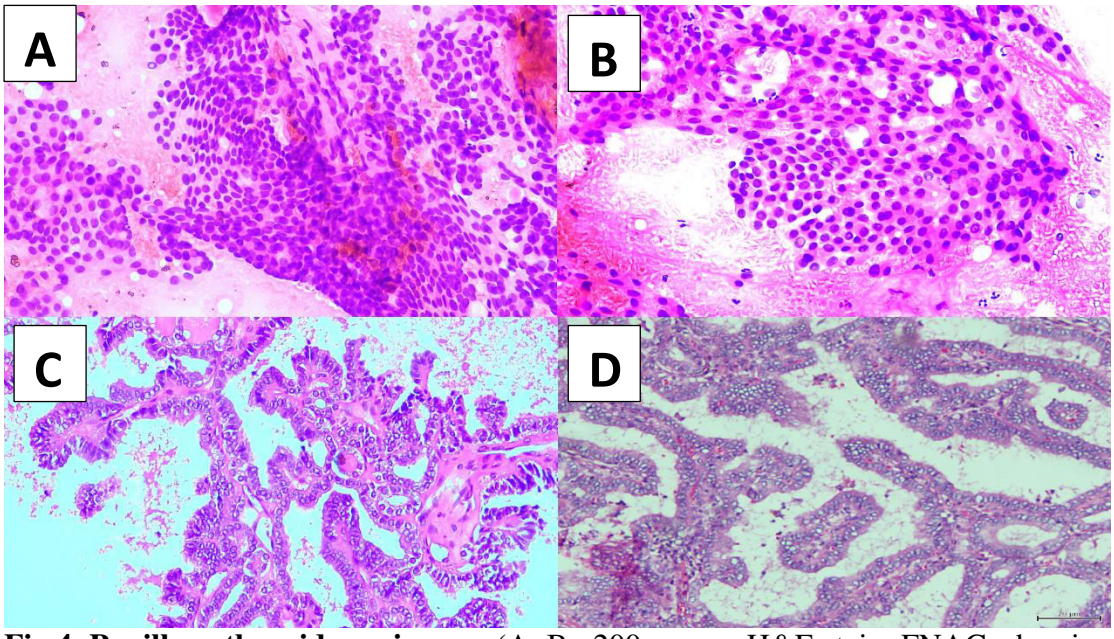

Fig.4. Papillary thyroid carcinoma. (A, B x200 power, H&E stain, FNAC, showing overlapping, anisonucleosis, nuclear pseudoinclusion and irregular nuclear membrane). (C, x200, H&E stain, cellblock by concentrated formalin/ alcohol method. Score 3 Showing papillary structures lined by overlapping follicular cells with nuclear clearing and pseudoinclusion). (D, x200, H&E stain, histopathological section showing papillary configurations lined by follicular cells showing papillary carcinoma nuclear features)

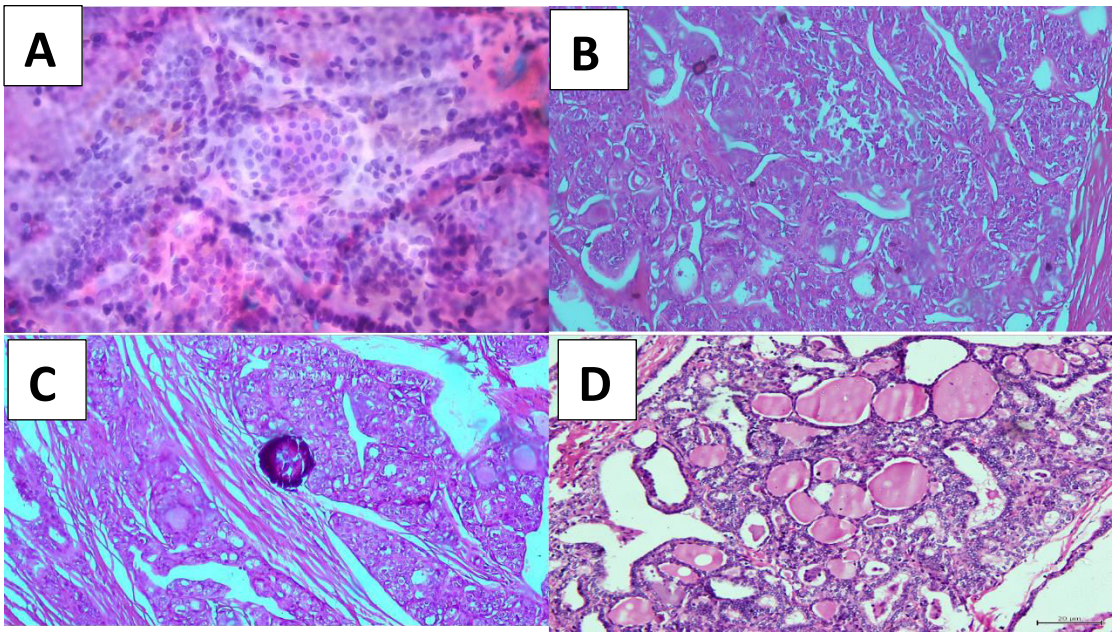

Fig.5. Papillary thyroid carcinoma. (A, x200 power, pap stain. FNAC, showing overlapping, anisonucleosis, nuclear pseudoinclusion and irregular nuclear membrane). (B, C x100 power, H&E stain. cellblock by concentrated formalin/ alcohol method. Score 3. Showing papillary structures lined by overlapping follicular cells with nuclear clearing, pseudoinclusion and psammoma body). (D, x100 power H&E stain. Histopathological section showing thyroid follicles lined by follicular cells with papillary carcinoma nuclear features).

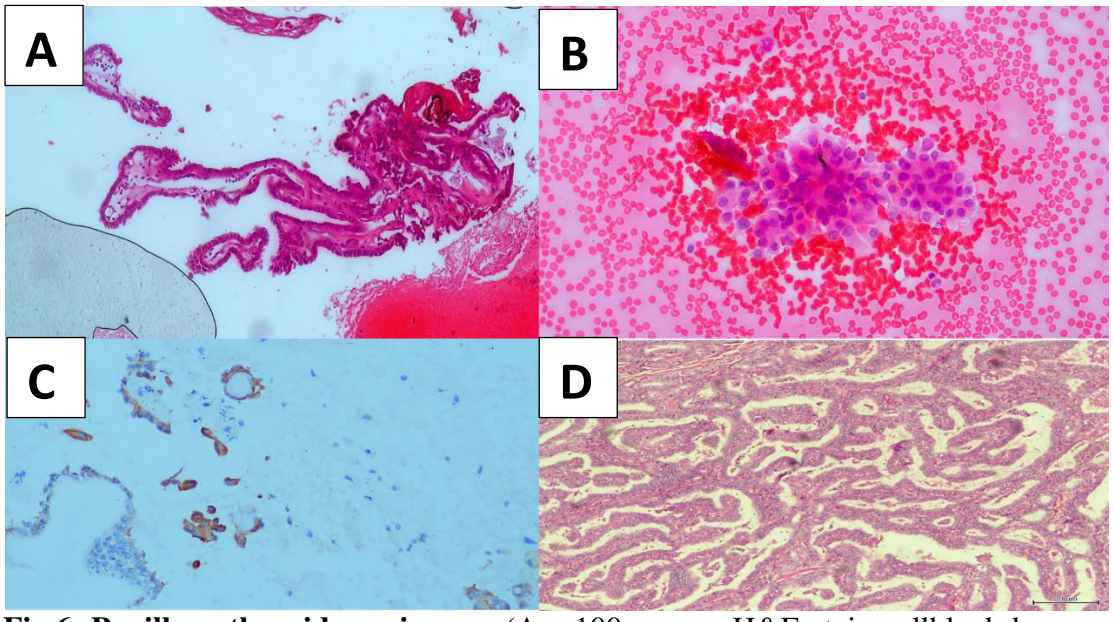

Fig.6. Papillary thyroid carcinoma. (A, x100 power, H&E stain cellblock by agar embedding method. Score 2. Showing papillary structures lined by overlapping follicular cells with nuclear clearing, pseudoinclusion). (B, x200 power, H&E stain. FNAC, showing overlapping, anisonucleosis, nuclear pseudoinclusion and irregular nuclear membrane). (C, x200 power, immunostaining with CK19 showing membranous and cytoplasmic moderate positive immunoreactivity). (D, x100 power, H&E stain, histopathological section showing papillary configurations lined by follicular cells with papillary carcinoma nuclear features)

Table 3. Distribution of the studied cases as regards results of cell block, smear of fine needle aspiration cytology and histopathological diagnosis of thyroid nodules.

Parameters		Studied cases (N=30)	
		N	%
FNAC Diagnosis	Benign	17	56.7%
	Atypia of undetermined significance	1	3.3%
	Follicular neoplasm	6	20.0%
	Suspicious of malignancy	0	0.0%
	Malignant	6	20.0%
Agar based cell block diagnosis	Benign	17	56.7%
	Atypia of undetermined significance	0	0.0%
	Follicular neoplasm	5	16.7%
	Suspicious of malignancy	0	0.0%
	Malignant	8	26.7%
Formalin (30)/alcohol (70) based cell block diagnosis	Benign	16	53.3%
	Atypia of undetermined significance	0	0.0%
	Follicular neoplasm	6	20.0%
	Suspicious of malignancy	0	0.0%
	Malignant	8	26.7%
Histopathological diagnosis	Benign	20	66.7%
	Malignant	10	33.3%

In thyroid lesions, one case previously was diagnosed atypia of undetermined significance and three cases were diagnosed as follicular neoplasm by FNAC, were diagnosed malignant on final histopathological diagnosis. Only 3 cases previously diagnosed as benign on FNAC were malignant on final histopathological diagnosis. Kappa statistics revealed fair agreement between FNAC and histopathological diagnosis results (kappa =0.22) (**Table.4**).

Table 4. Inter-rater agreement of FNAC results with histopathological diagnosis results of thyroid nodules.

Variables		FNAC				Total	Agreement	
		Benign	Malignant	Atypia of undetermin-ed significance	Follicular neoplasm		Kappa	P-value
Histo-pathological diagnosis	Benign	14	3	0	3	20 (66.7%)	0.220	0.07
	Malignant	3	3	1	3	10 (33.3%)		
	Total	17(56.7%)	6 (20%)	1 (3.3%)	6 (20%)	30 (100%)		

Two cases previously diagnosed as follicular neoplasm by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of 100 percent formaldehyde), were diagnosed malignant on the final histopathological diagnosis. Only one case previously diagnosed as benign on formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts concentrated 40% formaldehyde) cell block, was malignant on the final histopathological diagnosis (**Table.5**).

Table 5. Inter-rater agreement of Formalin (30)/alcohol (70)-based cell block results with histopathological diagnosis results of thyroid nodules.

Variables		*Formalin (30)/alcohol (70) based cell block*			Total	Agreement	
		Benign	Malignant	Follicular neoplasm		Kappa	P-value
Histo-pathological diagnosis	Benign	15	1	4	20 (66.7%)	0.520	**<0.001**
	Malignant	1	7	2	10 (33.3%)		
	Total	16(53.3 %)	8 (26.7)	6 (20%)	30 (100%)		

Two cases previously were diagnosed as follicular neoplasm by agar-based cell block, were diagnosed malignant on final histopathological diagnosis. Only two cases were previously diagnosed as benign on agar-based cell block, were malignant on final histopathological diagnosis. Kappa statistics revealed moderate agreement between agar-based cell block and histopathological diagnosis results (kappa =0.437). (**Table.6**).

Table 6. Inter-rater agreement of agar-based cell block results with histopathological diagnosis results of thyroid nodules.

Variables		*Agar-based cell block*			Total	Agreement	
		Benign	Malignant	Follicular neoplasm		Kappa	P-value
Histo-pathological diagnosis	Benign	15	2	3	20 (66.7%)	0.437	**0.002**
	Malignant	2	6	2	10 (33.3%)		
	Total	17(56.7%)	8 (26.7%)	5 (16.7%)	30 (100%)		

Formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) cell block showed the highest accuracy (86.67%) followed by agar -based cell block (80%). FNAC showed the least accuracy (66.7%). as illustrated in (**Table.7**).

Table 7. Comparison between accuracy of different methods in relation to histopathological diagnosis of thyroid nodules.

Variables	FNAC	Agar -based cell block	Formalin -based cell block
Sensitivity	50.00%	75.00%	87.50%
Specificity	70.83%	81.82%	86.36%
PPV	30.00%	60.00%	70.00%

NPV	85.00%	90.00%	95.00%
Accuracy	66.67%	80.00%	86.67%

PPV: Positive predictive value, NPV: Negative predictive value

Discussion

In current work, we studied 40 cases demonstrating cytological samples from FNAC from thyroid nodules representing patients who visited the Outpatient Clinic of General Surgery Department, at Qena University Hospital complaining of thyroid nodules. Our study showed that the number of males was 5 (47.5%) cases that is less than females (35 (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 the studied groups.

Our study included patients with an 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.

In our study, we performed the scoring system on the cell blocks of the 40 cases which were done by six different 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)**.

Regarding agar embedding method there were 35 cell blocks (87.5%) gave score 3 and 5 cell blocks (12.5%) gave score 2. Compared to 4 cell blocks (10%) prepared by concentrated Formalin/ ethanol fixative method (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) gave score 3 and 32 cell blocks (80%) gave score 2.

So, the highest score detected was from agar embedding method, followed by formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) method.

In this work we detected that using absolute formalin is perfect in lysis of red blood cells, so that increases the cellularity of the cell block and makes the details of the cells more obvious. Also, absolute formalin was very good in increasing the antigenicity and in raising the quality of immunohistochemical stains on the cell block. This was agreed with the study of **Ireka et al. (2019)**, that said that formalin-fixed tissues provide significantly better immunostaining results (84% good staining and exhibited 100% and 76% histoscore on E-cadherin and Ki-67 immunostaining respectively).

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.

Also, in the study of **Lindsey et al. (2016)** proved that using 10% neutral buffered formalin on cell block were judged to be superior in all cases as the cytoplasmic and nuclear details were good preserved. They also proved that using fixatives other than formalin can interfere with IHC results.

In addition to that, in our study we found that using absolute alcohol gives us better preservation of the cellularity and higher stability of fixed tissue than diluted alcohol .Plus, it raises the immunogenicity of the cell block that may be due to the ability of alcohol in precipitation of the proteins and in optimal preservation of DNA and RNA.

That goes in line with the study of **Rahman et al. (2022)**, where they proved that alcohol-based fixatives induced simultaneous dehydration, fixation, and coagulation of proteins rather than masking antigenic sites. They also proved that alcohol is faster in penetration of tissues and has a higher coefficient of infusibility.

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

So, in our study we used the combination of absolute alcohol and absolute formalin (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% 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.

They found that rinsing the FNAC needle directly into saline rinsing solution appeared to lyse most of the cells, and they advised to use 10 % neutral buffered formalin for rinsing the FNAC needle. In our study we tried this advice; we used 10 % neutral buffered formalin for rinsing. The formalin led to lysis of RBCS and made the background less hemorrhagic. However, we got less cellular and less preserved cell blocks also.

That also was in line with the study of **Nambirajan et al. (2018)**, where they found that plasma thrombin cell block had less cellularity, and it is a source of extraneous DNA that may interfere in molecular studies. 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.

The study of **Balassanian et al. (2016)** also proved that both cell preservation and cellularity were improved with the agar gel technique when compared with the plasma thrombin method. As in the plasma thrombin method, the clot formed was often sparsely cellular due to cell lysis. so, there were very few cells present in the H & E sections.

In contrast to our investigation, **Kanjilal et al. (2021)** reported good diagnostic accuracy in the detection of juvenile abdominal neoplasms using a cell block produced using the plasma thrombin approach. This procedure enabled the recovery of minute cellular material compared to previous cell-block techniques, and auxiliary techniques were successfully done in challenging situations since it did not affect antigenic preservation.

In Our study, we compare between the frequency and percentage of each diagnostic category in FNAC, cell block as well as histopathological diagnosis in thyroid cases. FNAC had overall sensitivity, specificity, and diagnostic accuracy of 50%, 70.83% and 66.67% respectively in detecting the malignant lesions in thyroid. Positive predictive value was 30%

while the negative predictive value was 85%.

Agar-based cell block had overall sensitivity, specificity, and diagnostic accuracy of 75%, 81.82% and 80% respectively in detecting the malignant lesions in thyroid cases. Positive p-value was 60% while the negative p-value was 90%.

Concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) cell block had overall sensitivity, specificity, and diagnostic accuracy of 75%, 81.82% and 80% respectively in detecting the malignant lesions in thyroid. Positive p-value was 60% while the negative p-value was 90%. This technique had fewer equivocal cases.

As only one case previously diagnosed as benign on formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) cell block, was malignant on the final histopathological diagnosis, but only two cases were previously diagnosed as benign on agar-based cell block, were malignant on final histopathological diagnosis.

The study of **Vance et al. (2019)** goes in line and proved that the nondiagnostic diagnosis rate of FNAC of thyroid lesions reduces by 7.1% when combined cytology and cell block slides are evaluated for adequacy vs when only cytology slides without cell block are examined (9.3% vs 16.4%; P = .0764).

We proved that in thyroid lesions concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) cell block showed the highest accuracy (86.67%) followed by agar -based cell block (80%). FNAC showed the least accuracy (66.7%).

That was agreed with the study of **Vance et al. (2019)** where they proved that using formalin-based cell block increases diagnostic yield of the specimen of FNAC from thyroid nodules. The cell block material proved beneficial for immunohistochemical staining of histiocytic and epithelial markers in challenging instances.

But in the study of the **Mitteldorf et al. (2018)** they found that using agar embedding method cell block in cases of FNAC of thyroid nodules showed rich cellular cell blocks in 52.8% of the cases. The cell blocks gave good morphological information and helped in applying an immunohistochemical panel predictive of malignancy, including cytokeratin 19, galectin-3 and HBME-1.

However, contrary to our findings, **Ireka et al. (2019)** demonstrated that agar-based cell block was compact and avoided considerable loss of diagnostic cells or tissue during cell block preparation from FNAC of thyroid nodules. The immunocytochemical characteristics were sufficient for the diagnosis of challenging cases.

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. In FNAC of thyroid gland, concentrated formalin/ ethanol fixative (7 parts of 100 percent ethanol/ 3 parts of concentrated 40% formaldehyde) based cell block is the best method as it showed the highest score and the best diagnostic accuracy. And we found that using absolute alcohol in the fixative solution preserves the cellular architecture and morphology. In addition to that, we found that absolute formalin has great benefits in improving the results of the immunohistochemical staining of the cell blocks.

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