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Ultrasound-guided fine needle aspiration versus non-aspiration techniques in the evaluation of solid thyroid nodules

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Abstract

Background: Comparison between the Fine Needle Non-Aspiration Cytology (FNNAC) and Fine Needle Aspiration Cytology (FNAC) in acquiring ultrasound-guided fine needle cytology (FNC) samples from solid thyroid nodules to determine the better technique, hence improving the sample quality aiming to decrease the number of unnecessary thyroidectomy operations.

Results: Prospective study showed statistically significant increase in cellular yield, preservation of cellular architecture and decrease in cellular trauma along with statistically significant increase in total sample quality with FNNAC (*P* value = 0.32, 0.004, 0.011, 0.21 respectively), and statistically insignificant difference in background blood in the sample (*P* value = 0.8).

Regarding sample convenience, FNNAC was found to be more convenient for both the patient (syringe is not seen by the patient reducing discomfort) and for the operator (better handling of the needle and fewer maneuvres).

Conclusions: FNC is an approved sensitive and cost-effective method to evaluate thyroid nodules, FNNAC technique was found to be more convenient for both the patient and the operator with statistically significant improvement in total sample quality compared to FNAC.

Keywords: Thyroid, Nodules, FNAC, FNNAC, Fine needle, Cytology, Ultrasound

Background

Thyroid nodules are relatively common diseases of the endocrine system, with estimated prevalence of about 34.2% by high-resolution ultrasound among randomly selected individuals with a female predominance [8].

Among detected thyroid nodules whether by palpation or ultrasound, the estimated risk of malignant nodules is about 5–15% [4].

Fine–needle aspiration cytology (FNAC) for tumours was first described by Martin and Ellis in the year 1930 in the United States [6].

Ultrasound-guided fine needle cytology biopsy appeared to be a relatively accurate method to evaluate thyroid nodules even those larger than 3 cm, with false-negative rates of about 2%. However much larger series would be required for evaluating its accuracy in nodules > 3 cm (Yoon et al. 2011).

Fine needle cytology (FNC) is usually done for the indeterminate thyroid nodules being safe, accurate and cost-effective method of evaluating thyroid nodules, ultrasound guidance allows direct visualization of the nodule with real-time guidance for the needle to the desired location within the nodules, with good accuracy (80%) (Parsa and Gharib 2019).

Unsatisfactory specimens, especially mixed with blood, poses an obstacle to proper cytological interpretation.

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To overcome this problem, an alternative method of fine needle non-aspiration cytology (FNNAC) technique was developed which relies on the capillary pressure only to suck the cells inside the needle bore [6].

FNNAC is also known as cytopuncture was developed in France in 1982 by Brifford et al., originally was developed for vascular organs to avoid haemorrhage and to obtain less haemorrhagic cytology samples from breast lesions [6].

FNNAC depends on insertion of a fine needle into the lesion without attaching a syringe, depending on the capillary principle of the needles that states that a fluid or semi-fluid material will ascend spontaneously in a tube (in inverse proportion to the tube diameter), without active suction or aspiration leading to less trauma to samples [2].

Nondiagnostic results and sensitivity are still the major challenges of FNC procedure. Despite high experiences, new aspiration and evaluation techniques, onsite cytopathology analysis and ultrasound guidance, up to 20% of initial aspirations may be nondiagnostic [1].

Many non-invasive tools for evaluating the thyroid nodules were used, the most convenient and practical till now is the American thyroid association revised guidelines for management of thyroid nodules these guidelines were first described in 1996, then revised in 2009 and again in 2015 [3].

These guidelines for active surveillance and management of thyroid nodules and differentiated thyroid cancer added a lot to the patients, as it led to more objective evaluation for the thyroid nodules, and decreased unnecessary fine needle cytology, and more importantly, it decreased unnecessary thyroidectomy operations that were done and advised instead to stick to US criteria and use FNC as sensitive and accurate tool for evaluating suspicious nodules.

Our study will question the better technique for acquiring FNC from thyroid nodules; whether aspiration (FNAC) or non-aspiration technique (FNNAC).

Methods

Study design and participants

48 subjects were enrolled in this prospective study in the period from August 2019 to April 2021, all subjects were presenting with solid thyroid nodules and referred to the radiology department for ultrasound-guided fine needle cytology (FNC) from these nodules. The inclusion criteria were as follows: (1) patient presenting with multiple or solitary solid thyroid nodules; (2) no age or sex predilection; (3) any thyroid profile were included whether euthyroid, hypothyroid or hyperthyroidism; (4) any nodule size. The exclusion criteria were: (1) Bleeding tendency defined by INR > 1.7; (2) other coagulopathies (either idiopathic or acquired).

Full history taking was obtained with emphasis on the clinical data, history of anticoagulants and coagulopathies. All patients presented INR not older than 3 weeks prior to the procedure. FNC was done for patients with solid thyroid nodules referred from and requested by either the surgery or the oncology departments. The whole study was done with proper infection control measures, ensuring patient safety, convenience & confidentiality.

Methods

23 G conventional needle was used in acquiring all the samples. All sampling procedures were performed by single operator to overcome operator bias. The sampling steps were done exactly as follows: (1) Sterilization of the neck skin and proper antiseptic rubbing. (2) Obtaining proper ultrasound view for the most suspicious solid thyroid nodule -ACR-TIRADS score—in the centre of the field of view [13]. (3) Evaluation of each nodule was done regarding; Size, Vascularity, Echogenicity, & Calcifications, and this data was recorded and archived. (4) Local anaesthesia was not routinely used unless requested by the patient, instead, cold packs for 5 min prior to procedure were used to relieve pain. (5) The needle was advanced through the skin aiming at the periphery of the target nodule directly with shortest and safest pathway ensuring single entry, uni-directional pathway to decrease needle contamination from other tissues. (6) 2 passes for each Non-aspiration (FNNAC) and Aspiration (FNAC) technique were done, where each pass consists of:i.Applying about 10 movements (To & Fro, Circumferential) within the nodule under complete ultrasound guidance for about 30 seconds or until the needle hub is filled with material.

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ii.During aspiration technique, 2–3 cm suction was applied upon the attached plastic syringe, then suction was stopped during withdrawal of the needle from the nodule.

iii.Withdrawal of the needle from the skin and compression.

(7) Immediate smearing of the obtained material from the needle only was done by attaching clean syringe filled with 5 cm air to force the material out of the needle and repeated as required. (8) Fixation by 70% Alcohol spraying over the glass slides with the smeared material. (9) Slides were put on rack in vertical position until drying and labelled randomly according to the technique used as

either (A) or (B) with registration of the technique used for each sample in a separate sheet, and then transferred to the pathology laboratory. (10) Alcohol-fixed slides are then processed and stained using Papanicolaou stains.

Sample quality assessment

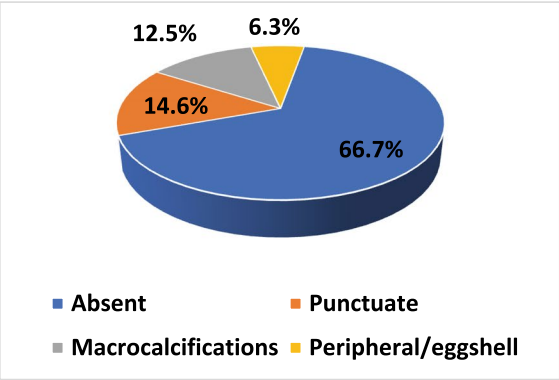
For every case, an expert pathologist compares the cytology results from FNNAC & FNAC with regards to the following: (1) Background blood; (2) Cellular yield; (3) Preservation of cellular architecture; (4) Degree of cellular trauma; (5) Quality of sample. Where quality of the sample is determined by the total score for the previous 4 criteria summed together score of each criterion is determined as illustrated in Table 1.

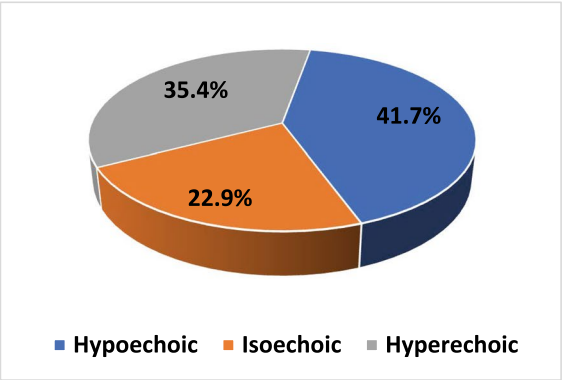
Table 1 Sample quality score

Background blood	Large 1	Moderate 2	Minimal 3
Cellular Yield	Minimal	Sufficient	Abundant
	1	2	3
Cellular architecture	Lost	Moderately preserved	Preserved
	1	2	3
Cellular trauma	Marked	Moderate	Minimal
	1	2	3
Quality of sample	Non-diagnostic	Adequate	Superior
	1–4	5–8	9–12

Table 2 Ultrasound appearance of the nodules

	Category	Number	Percentage
Echo-pattern	**Hyperechoic**	**17**	**35.42%**
	Isoechoic	**11**	**22.92%**
	Hypoechoic	**20**	**41.67%**
Vascularity	**Unnoticeable**	**9**	**18.75%**
	Mild	**23**	**47.92%**
	Moderate	**13**	**27.08%**
	Marked	**3**	**6.25%**
Calcification	**Absent/Comet tail**	**32**	**66.67%**
	Punctuate	**7**	**14.58%**
	Macrocalcifications	**6**	**12.50%**
	Peripheral/eggshell	**3**	**6.25%**





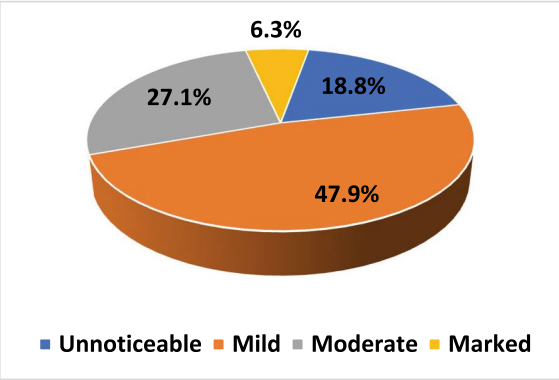


Table 3 Mean scores for sample quality criteria

	FNAC		FNNAC		P-value
	Mean	± S.D.	Mean	± S.D.	
Background blood	1.979	0.79	1.938	0.78	0.755
Cellular yield	1.604	0.76	1.9375	0.70	0.012
Cellular trauma	2.271	0.84	2.58	0.61	0.038
Cellular architecture	1.85	0.82	2.33	0.72	0.003
Total score	7.708	2.24	8.792	1.74	0.011

Pathologist opinion in each sample -although was recorded and considered the most important- but was not used in this study being subjective criterion thus would have implied bias within the study.

Statistical analysis

Data entry, processing and statistical analysis were carried out using MedCalc ver. 20 (MedCalc, Ostend, Belgium). Tests of significance (paired *t*-test, Wilcoxon's, McNemar's, logistic regression analysis, and ROC Curve analysis and Kappa statistics) were used. Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. *P* values less than 0.05 (5%) were considered statistically significant.

Results

Forty-eight patients (41 females, 7 males, mean age in women were 47.1, mean age in males was 50.6, age range 18–70, age range in females 18–70, age range in males 33–62) with solid thyroid nodules were included in the study, mean INR was 1.1, mean nodule size was 2.57 cm, ultrasound appearance of the nodules is detailed in Table 2. All 48 patients had fine needle cytology by both techniques aspiration (FNAC) and non-aspiration (FNNAC), all results were presented to histopathology for evaluation by an expert pathologist blinded to the technique used in each sample and asked to give score for each the mentioned 4 criteria and then calculate the total sample quality score.

All scores were collected and assigned to its respective sample, descriptive & inferential analytical tests were done for all collected data, results showed better performance with statistically significant difference between the non-aspiration technique versus the aspiration technique in obtaining more cellular yield with less cellular trauma and more preservation of the cellular architecture with better total sample quality score. On the other side, there's was a statistically insignificant decrease in background blood in samples obtained by aspiration technique, Table 3 summarized the means and standard deviations for each criterion with its respective p-value.

The average total (sample quality) score was (7.708 ± 2.24 and 8.792 ± 1.74) with FNAC & FNNAC respectively, P value $= 0.011$.

Table 4 summarizes the diagnostic categories representing the diagnostic quality of the sample, more diagnostically superior samples were acquired by the FNNAC than by FNAC (60.4% and 37.5% respectively), while FNAC acquired more diagnostically adequate samples than FNNAC (50% and 39.6% respectively), non-diagnostic samples were only 12.5% with FNAC and 0% with FNNAC, (P value $= 0.024$).

Discussion

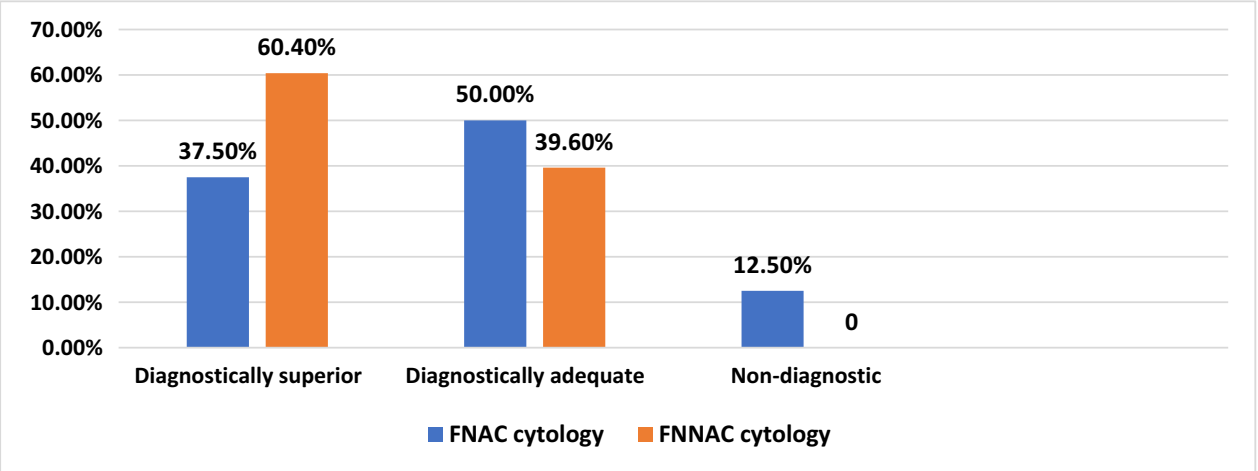
Fine needle cytology (FNC) is a well-recognized modality for evaluation of thyroid nodules, very safe and highly sensitive, however, the major problem facing FNC is bad quality samples impairing proper diagnosis which leads to redo the procedures to obtain satisfactory samples, our study aimed to improve the quality of samples by hypothesis that non-aspiration technique would yield better results and quality of samples.

The non-aspiration technique was first developed in France to overcome this situation in 1982 by Brifford et al., and was referred to as cytopuncture, since then non-aspiration technique was not well implemented in all fine needle cytology procedures, and few studies compared its effectiveness in acquiring quality samples to the conventional aspiration technique that is more often used in sampling.

The non-aspiration technique entangles insertion of the fine needle directly into the nodule with minimal manipulation in its track to avoid contamination without attaching syringe for aspiration, instead, it depends on the capillary property in narrow channels (the fine needle itself), where according to the capillarity principle "fluid or semi fluid substance will ascend spontaneously in the narrow channel with the degree of ascend is inversely proportional to the diameter of the channel.

Table 4 diagnostic quality categories percentage with each technique

Technique used / Diagnostic quality	FNAC		FNNAC	
	Number	Percentage	Number	Percentage
Non-diagnostic	6	12.5	0	0.0
Diagnostically adequate	24	50.0	19	39.6
Diagnostically superior	18	37.5	29	60.4



This prospective study was carried out to compare the diagnostic quality of FNC sample obtained by aspiration versus non-aspiration techniques, aiming to improve the overall quality of FNC and hence better evaluation of thyroid nodules avoiding repeating FNC and unnecessary thyroidectomies.

48 patients with solid thyroid nodules were included in this study, our results showed statistically significant improvement in overall quality of the samples obtained by the non-aspiration (FNNAC) technique with less cellular trauma, more cellular yield, better preservation of cellular architecture, however, there was no significant decrease in background blood.

As a side observation, the non-aspiration technique was more convenient for the patient due to non-visualization of the syringe which is very near to the patient's face in case of acquiring sample from thyroid.

Furthermore, the non-aspiration technique resulting in better handling of the needle instead of handling the larger syringe allowing better control over the needle and more convenience for the operator, also was less traumatic if the patient accidentally swallowed while the needle was inside the thyroid gland.

In the currently available literature, few studies were conducted to evaluation of the difference between sample quality acquired by aspiration and non-aspiration techniques, in 2010, Maurya et al. conducted a study over 50 thyroid lesions to compare the samples obtained by both techniques, the study results were matching our study where Maurya et al. found that samples acquired by non-aspiration technique were more diagnostically superior with better overall sample quality but the difference was not statistically significant, on the other hand, the non-aspiration technique yielded more inadequate sample than those yielded by the aspiration technique, detailed results of the study is discussed in Tables 5 and 6.

Another larger study conducted over 289 patients with thyroid nodules from 2006 to 2008, showed that aspiration technique yielded more number of diagnostically

Table 5 Sample quality [7]

Category	FNAC	FNNAC
Diagnostically superior	40% ($n=20$)	46% ($n=23$)
Diagnostically adequate	24% ($n=12$)	18% ($n=18$)
Unsuitable for diagnosis	34% ($n=17$)	38% ($n=38$)

Table 6 Samples scores [7]

Criteria	FNAC	FNNAC	*P* value
Background blood	1.16	1.24	> 0.05
Amount of cellular material	1.35	1.42	> 0.05
Degree of cellular degeneration	1.18	1.32	> 0.05
Degree of cellular trauma	1.27	1.29	> 0.05
Retention of appropriate architecture	1.13	1.28	> 0.05

Table 7 Samples scores [5]

Criteria	FNAC	FNNAC	*P* value
Background blood	1.82 ± 0.42	1.87 ± 0.36	0.01
Amount of cellular material	1.28 ± 0.65	1.19 ± 0.65	0.04
Degree of cellular degeneration	0.99 ± 0.57	0.93 ± 0.62	0.1
Degree of cellular trauma	0.98 ± 0.58	0.93 ± 0.62	0.1
Retention of appropriate architecture	0.97 ± 1.03	0.83 ± 0.61	0.02
Total score	6.00 ± 2.17	5.76 ± 2.3	0.08

Table 8 Comparison between different studies regarding background blood using FNAC vs. FNNAC (Song etal.,[10])

Background blood	Technique			
	FNAC		FNNAC	
	Mean	± S.D	Mean	± S.D
Pinki et al., [11]	0.56	0.499	1.74	0.485
Ghosh et al., [15]	0.714	0.468	1.28	0.468
Mahajan et al., [14]	1.04	0.7348	1.32	0.8021
Kashi et al., [5]	1.82	0.42	1.87	0.36
de Carvalho et al., [12]	1.39	0.71	1.39	0.72

superior samples (122) than non-aspiration technique (98), moreover non-aspiration technique lead to more unsatisfactory samples (40) versus only (31) in aspiration technique, however, there was slight difference between the two techniques regarding the background blood, amount of cellular material, degree of cellular degeneration, and retention of cellular architecture, the study results are detailed in Table 7.

The main limitation of this study was that not all samples were acquired under ultrasound guidance whereas the ultrasound guidance was only reserved for very small nodules which may lead to biased specimens. However, the conclusion of this study was very interesting as it showed that the best samples were achieved when using both techniques for each sample which is logic being easy and cost-effective [5].

A meta-analysis study by Song and colleague was published in 2015 comparing the different results of other studies comparing the aspiration and non-aspiration sampling techniques for fine needle cytology, the study showed the following:

i- Regarding background blood, less background blood was associated with non-aspiration technique in 3 studies while 2 studies showed non-significant difference between the 2 techniques as shown in Table 8.
ii- 2 studies showed less cellular trauma with non-aspiration technique while 1 study showed less trauma with the aspiration technique, and 2 studies showed no significant difference between the 2 techniques as shown in Table 9.
iii- Regarding the cellular yield, there was no significant difference between the 2 techniques in all reviewed studies as shown in Table 10.
iv- The aspiration technique led to less cellular degeneration according to 2 studies while non-aspiration was better in another 2 studies and 1 study showed no significant difference as shown in Table 11.
v- 3 studies showed better retention of cellular architecture with the non-aspiration technique, while 2 stud-

Table 9 Comparison between different studies regarding degree of cellular trauma using FNAC vs FNNAC [10]

Degree of cellular trauma	Technique			
	FNAC		FNNAC	
	Mean	± S.D	Mean	± S.D
de Carvalho et al., [12]	1.7	0.61	1.74	0.55
Ghosh et al., [15]	1.07	0.474	1.357	0.497
Kashi et al., [5]	0.98	0.58	0.93	0.62
Mahajan et al., [14]	1.48	0.7141	1.52	0.7703
Pinki et al., [11]	1.32	0.584	0.85	0.479

Table 10 comparison between different studies regarding amount of cellular yield using FNAC vs FNNAC [10]

Amount of cellular yield	Technique			
	FNAC		FNNAC	
	Mean	± S.D	Mean	± S.D
de Carvalho et al., [12]	1.54	0.68	1.51	0.69
Ghosh et al., [15]	1.28	0.611	1.375	0.497
Kashi et al., [5]	1.28	0.65	1.19	0.65
Mahajan et al., [14]	1.28	0.7371	1.36	0.8103
Pinki et al., [11]	1.25	0.557	1.76	0.474

Table 11 Comparison between different studies regarding degree of cellular degeneration using FNAC vs FNNAC [10]

Degree of cellular degeneration	Technique			
	FNAC		FNNAC	
	Mean	± S.D	Mean	± S.D
de Carvalho et al., [12]	1.71	0.58	1.72	0.58
Ghosh et al., [15]	1.07	0.474	1.42	0.513
Kashi et al., [5]	0.99	0.57	0.93	0.62
Mahajan et al., [14]	1.48	0.7141	1.52	0.7703
Pinki et al., [11]	1.33	0.711	0.92	0.662

Table 12 Comparison between different studies regarding the retention of the appropriate cellular architecture using FNAC vs FNNAC [10]

Retention of appropriate cellular architecture	Technique			
	FNAC		FNNAC	
	Mean	±S.D	Mean	±S.D
de Carvalho et al., [12]	1.63	0.67	1.58	0.69
Ghosh et al., [15]	0.92	0.257	1.357	0.497
Kashi et al., [5]	0.97	1.03	0.83	0.61
Mahajan et al., [14]	0.88	0.7257	1.12	0.7257
Pinki et al., [11]	0.96	0.53	1.83	0.428

Table 13 Comparison between different studies regarding the total sample quality score using FNAC vs FNNAC [10]

Total sample quality score	Technique			
	FNAC		FNNAC	
	Mean	±S.D	Mean	±S.D
de Carvalho et al., [12]	7.94	2.84	7.96	2.81
Kashi et al., [5]	6	2.17	5.76	2.3
Mahajan et al., [14]	6.16	2.8531	6.84	3.3749
Pinki et al., [11]	5.42	2.113	7.1	1.761
Torabizadeh et al., 2008	5.82	2.3	5.7	2.4

ies showed better retention of cellular architecture with the aspiration technique as shown in Table 12.

Only 1 study showed better total performance of the non-aspiration technique while the other four studies showed no significant difference between both techniques as shown in Table 13.

The main limitation of this meta-analysis study is the great difference in number of patients, and needle gauge and other factors leading to great variations and inconsistencies between the results.

In our study, however, the needle gauge was fixed, and the operator was the same in all the samples, whether aspiration or the non-aspiration technique was done first was totally random to avoid any bias.

Another study by Sasikumar et al. [9], published in 2018 that was conducted over 91 patient comparing the efficacy of FNAC versus FNNAC regarding the diagnostic superiority where samples are categorized into diagnostically superior, diagnostically adequate, and unsuitable for diagnosis according the pathologist opinion regarding cellular aggregates, amount of cellular material and amount of background blood as well as retention of cellular architecture, in this study FNNAC produced

Table 14 Comparison between FNAC and FNNAC regarding diagnostic quality [9]

	FNAC		FNNAC	
	No	%	No	%
Diagnostically superior	18	19.7	42	46.1
Diagnostically adequate	62	68.2	44	48.6
Unsuitable	11	12.08	5	5.49

statistically significant better quality samples as detailed in Table 14.

However, the main limitation of this study is that it relied on the pathologist opinion as a subjective method for deciding the category of the sample rather than having objective scoring system.

In our study, significant patient convenience was achieved by the non-aspiration technique as described by most of the patients however being subjective point, this point was not included in our study statistics although it carries a great importance for the study as patient convenience is an ultimate goal.

Moreover, the non-aspiration technique was more convenient for the operator while handling the needle instead of handling whole syringe and undergoing suction, another important factor was the less trauma when the patient accidentally swallows where the needle alone is more mobile with the thyroid gland than the needle with the syringe attached.

Also, our study showed very good agreement between the FNC and tissue histopathology in patients who underwent thyroidectomy, with sensitivity = 100% and specificity = 97.5%, however, this high sensitivity may be partially affected by lack of definite histopathological diagnosis for samples with negative malignancy by FNC although all these patients were subjected to 6 months follow up by ultrasound and none of them showed any suspicious changes in nodule size or appearance by ultrasonography according to the TIRADS.

Furthermore, many other studies have already proved the very high sensitivity and specificity of the FNC in cases of good sample quality that are beyond the scope of this study.

Limitations

The main limitation of our study includes small number of patients involved in this study, although this sample was well representative larger sample volume would have increased the strength of this study.

Another limitation in our study is the lack of tissue histopathology for patients with negative malignancy by FNC, and although it's already approved for patients with negative malignancy by FNC to have ultrasound

follow-up only, however, longer follow-up period will have more accurate results regarding the specificity and sensitivity of the fine needle cytology.

Conclusions

Both aspiration and non-aspiration techniques can be implemented and used for acquiring cytology samples, however, non-aspiration technique (FNNAC) was associated with overall better-quality samples with statistically significant better performance compared to FNAC. Non-aspiration technique is more convenient for both the patient that does not see the syringe while sampling and for the operator due to better handling of the needle alone rather than the needle with the syringe attached to it and applying suction simultaneously. In some instances, combining both aspiration and non-aspiration techniques may yield better results, especially in very hard nodules where non-aspiration technique alone could not acquire satisfactory samples.

Abbreviations

FNC: Fine needle cytology; FNNAC: Fine needle non-aspiration cytology; FNAC: Fine needle aspiration cytology; TIRADS: Thyroid Imaging Reporting & Data system; ACR: American College of Radiology; NPV: Negative predictive value; PPV: Positive predictive value; INR: International normalized ratio; US: Ultrasound.

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Author contributions

MAAH: reviewing the literature, data collection and analysis, perform statistical analysis, write, and revise the manuscript, and prepare figures and tables. MA: Reviewing the literature. MGA: manuscript editing. MF: Reviewing literature, data analysis, and manuscript editing. HEHE: histopathological evaluation of the samples, reviewing the literature regarding histopathology topics. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author when needed.

Declarations

Ethics approval and consent to participate

Institutional (Ain Shams University, Maadi Armed Forces hospital and Aswan Cancer hospital and Aswan cancer centre) ethical approval was taken before conducting this prospective study; Research ethics committee (REC) in Ain shams university approved the study protocol on 21/7/2019 from the ethical point of view (FWA: 000017585). Verbal consent & ethical permissions were obtained from all subjects after informing each of them about the nature & purpose of the study, ensuring confidentiality was mandatory.

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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