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Original Research Article

To Determine the Sample Adequacy of FNAC Performed with 21 Gauge Needle in Thyroid Nodules

Article History:

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Abstract: ***Objective***: To determine the sample adequacy of fine needle aspiration cytology (FNAC) performed with a 21-gauge needle in thyroid nodules. ***Study Design and Setting***: This cross-sectional study was conducted at Liaquat National Hospital, Karachi, ***Methodology:*** A total of 184 patients, aged 20 to 60 years, with single or multiple thyroid nodules identified on ultrasound, were included after informed consent over six months from December 1st , 2021, to May 31st , 2022, . Patients with impaired coagulation profiles or refusal to participate were excluded. Ultrasound-guided FNAC was performed using a 21-gauge needle under a standardized technique. Six smears were prepared for each case and evaluated by a consultant pathologist for adequacy based on defined cytological criteria. Quantitative variables such as age and nodule size were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Post-stratification chi-square test was applied to assess associations, with a p-value ≤0.05 considered significant. ***Results:*** Out of 184 patients, the mean age was 47.14±6.49 years. The mean size of thyroid nodules was 3.41±1.24 cm, and the mean number of nodules was 2.85±1.56. Adequate FNAC samples were obtained in 160 patients (87%), while 24 patients (13%) yielded inadequate samples. There were no significant associations observed among sample adequacy and gender, duration of symptoms, size of nodule or number of nodules. ***Conclusion:*** Using a 21-gauge needle for FNAC generates a high rate of sample adequacy, and FNAC remains an effective, first line diagnostic test in evaluating thyroid nodules.

Keywords: Biopsy, Fine-Needle; Cytology; Thyroid Neoplasms; Thyroid Nodule.

INTRODUCTION

Thyroid nodules are among the most prevalent endocrine conditions seen in clinical practice and are rising in incidence worldwide.[1] In epidemiological studies, thyroid nodules may be palpated in 4-7% of adults, while ultrasound studies have reported visualization of thyroid nodules in 20-70% of adults, especially in females and older adults.[2] Clinically, high-resolution ultrasonography contributes substantially to the escalating recognition of both palpated and non-palpated thyroid nodules. Most thyroid nodules are benign, but 5-15% may be at risk for malignancy thus rendering evaluation and diagnosis important for managing thyroid nodules appropriately and avoiding unnecessary procedures.[3]

Fine-needle aspiration cytology (FNAC) has emerged as a key component in the initial assessment of thyroid nodules. FNAC is a cost-effective, reliable, and minimally invasive diagnostic procedure that is performed in an outpatient setting and typically involves little discomfort to the patient.[4,5] Numerous studies have shown that FNAC significantly increases diagnostic accuracy, allowing clinicians to accurately determine malignant vs. benign lesions with high sensitivity and specificity.[6] FNAC has resulted in an extraordinary decrease in unnecessary surgical intervention for thyroid nodules; now, greater than 50% of the nodules removed through surgery are malignant, unlike the past where many patients underwent surgery with benign disease.[7]

Both the American Thyroid Association (ATA) and the National Comprehensive Cancer Network

(NCCN) have strongly endorsed FNAC as the initial diagnostic modality for the evaluation of thyroid nodules due to its superior diagnostic performance, affordability, and safety. FNAC minimizes morbidity, avoids overtreatment, and facilitates standardization of pathological results thereby permitting timely recognition of surgically managed malignant nodules.[8]

Historically, larger core biopsy needles were employed in sampling thyroid nodules, but these resulted in higher complication risks (e.g., pain, damage to anatomic structures, bleeding, and discomfort). Thus, FNAC needles typically range from 21 to 27 gauge (G) today.[9] The gauge of a needle affects the volume and integrity of the cellular aspirate, and ultimately whether it achieves an adequate sample or not for cytological interpretation. Larger gauge needles theoretically aspirate higher numbers of cells but will also have a higher risk of blood in the specimen and patient discomfort. Conversely, extremely small needles (e.g. 25 - 27G) can, in some cases, yield insufficient cellularity for diagnosis.[10]

Non-diagnostic results from a fine needle aspiration cytology (FNAC) procedures can be a notable clinical challenge, as they occur in as many as 20% of ultrasound-guided FNAC procedures. There are numerous contributing factors of inadequate sampling, such as, operator inexperience, deeply situated or cystic nodules, unnecessarily excessive blood contamination, and issues related to specimen preparation or interpretation by the pathologist. Non-diagnostic samples are costly in that they not only delay clinical decision-making and cause patient anxiety, but studies show that 1-4% of initially non-diagnostic nodules may harbor malignancy. This underlines the necessity for optimal sampling techniques to ensure proper diagnosis.[11, 12]

In many clinical situations, including our own practice, a 21-gauge needle is used for FNAC of thyroid nodules. The question remains whether, a 21 gauge needle would always be the best choice to attain adequate cellular yield with minimal complications. It is important to determine whether, despite the outcome of the biopsy, the needle gauge used is generally adequate to obtain material for cytological evaluation. If adequate sampling frequency is insufficient, then repeat FNACs or higher risk intervention to obtain tissue, such as core needle biopsy, tru-cut biopsy, or empirically, with surgical excision of the nodule to rule out malignancy would be warranted.

Though the alternate methods are sometimes required, they also are more expensive, more uncomfortable for the patient, and suspicious for bleeding events and longer recovery times. Hence, it is important to study the sample adequacy rate of FNAC with a 21-gauge needle in relation to diagnostic efficiency in the assessment of thyroid nodules. The demonstration of sample adequacy using a 21-gauge needle will help promote timely and accurate diagnosis while lessening the burden on patients, reducing unnecessary invasive procedures, and ultimately better clinical outcomes. There is a need to study if FNAC with a 21-gauge needle is providing adequate cellular material for determining FNAC adequacy in our clinical setting. If it is determined to be adequate, then it bolsters current practice with regards to getting a diagnosis in a timely manner. However, if a high frequency of inadequate samples is observed, this may cause changes in either needle gauge used or technique when performing FNAC to optimize diagnostic yield and improve patient care. The objective of this study was to determine sample adequacy of fine-needle aspiration cytology (FNAC) with a 21-gauge needle in patients with thyroid nodules.

METHODOLOGY

This cross-sectional study was conducted in the Department of Radiology at Liaquat National Hospital, Karachi, over a period of six months from December 1st , 2021, to May 31st , 2022, after approval of the study from Ethical Review Committee (ERC) of Ethical Review Committee Liaquat National Hospital & Medical College Karachi, approval no: 0704-2021-LNH-ERC, Dated November 10, 2021. The sample size was calculated to be 184 patients through open Epi software, based on a prevalence of 78%, a confidence interval of 95%, and a precision level of 6%.[13]

A non-probability consecutive sampling technique was used for patient recruitment. Individuals aged 20 to 60 years, of either gender, presenting with single or multiple thyroid nodules on ultrasound and who agreed to participate were included in the study. Patients who had impaired coagulation profiles or declined consent were excluded. Patients referred to the Radiology Department who met the inclusion criteria were enrolled after obtaining informed consent.

All fine needle aspiration (FNAC) procedures were carried out utilizing the same ultrasound machine (TOSHIBA Xario 100) with a 7.5MHz multifrequency linear-array transducer. The echogenicity of the thyroid nodules were classified as hypoechoic, isoechoic, hyperechoic, or heterogeneous in relation to surrounding thyroid tissue. In cases where patients had multiple nodules, we aspirated the largest or most concerning nodule classified by a hypoechoic density, microcalcifications, or regular margins. The overlying skin was cleaned using povidone-iodine

solution and local anesthesia was administered with 2% Xylocaine.

All FNACs were performed by the same radiologist to maintain consistency in the regimented technique utilizing a multiple pass approach with a 30mL syringe using a 21 gauge needle. The aspirated material was dispensed onto six glass slides and immediately fixed via a cytology fixative spray (M-FixTM, Germany). There was no cytology technician on-site during the performance of the procedures, and thus, the adequacy of the samples was not checked in person. All slides made were assessed later by the designated pathologist (AVS) for cytological interpretation and assessment of adequacy of samples.

The ultrasound of thyroid nodules classification was based on the British Thyroid Association (BTA) 2014 guidelines on the management of thyroid cancer. The classification puts nodules into five groups, U1 to U5, according to their sonographic features. U1 represents a normal thyroid gland with no detectable nodules. U2 nodules are considered benign and are typically hyperechoic or isoechoic with a peripheral halo; they may also show cystic areas with a ring-down artifact, a spongiform or microcystic appearance, peripheral "egg-shell" calcification, or predominantly peripheral vascularity. U3 nodules are classified as indeterminate and may appear as solid homogeneous, markedly hyperechoic nodules with a halo, or hypoechoic nodules with equivocal echogenic foci or partial cystic change. These may show mixed or central vascularity. U4 nodules are labeled suspicious and are usually solid and hypoechoic compared to normal thyroid tissue, or markedly hypoechoic when compared to surrounding strap muscles.

They may also demonstrate disrupted peripheral calcification or lobulated margins. U5 nodules are considered malignant and often present as solid hypoechoic lesions with irregular or lobulated margins and microcalcifications, features suggestive of papillary carcinoma. Some malignant nodules, such as medullary carcinoma, may show globular calcification. Additional features, such as intranodular vascularity, a "taller-than-wide" shape (anteroposterior diameter greater than transverse diameter), and associated suspicious lymphadenopathy, further support a malignant classification. According to these guidelines, U1 and U2 nodules typically do not require FNAC unless clinical suspicion exists, whereas U3 to U5 nodules warrant FNAC and further evaluation based on cytological and clinical correlation.

Sample adequacy for FNAC was assessed based on standard cytological criteria. A sample was considered adequate if it met at least one of several established features: the presence of at least six groups of well-visualized follicular cells with each group containing a minimum of ten cells; the presence of follicular cells exhibiting significant cytological atypia, regardless of cell count; the presence of numerous inflammatory cells, in which case the number of follicular cells was not required; or the presence of abundant thick colloid, allowing diagnosis even in the absence of sufficient follicular cells. On the other hand, an insufficient sample was one that exhibited ill-prepared, ill-staining, or obscured follicular cells; one that had aspirate that consisted of more than cyst fluid with less than six groups of ten benign follicular cells; or one which contained predominantly red blood cells with few inflammatory cells. In this study, any one fulfillment of adequacy criteria served to show an adequate sample, and any one of inadequacy criteria, served to show the inadequacy of the sample.

Analysis of the data was conducted by using SPSS version 21. Quantitative variables (age, nodule size, and number of nodules) were given mean and standard deviation. Categorical variables that were computed by use of frequencies and percentages include gender, duration of symptoms, nodule characteristics, sample adequacy, and FNAC findings. To control the possible effect modifiers they stratified by age, gender, duration of symptoms and nodule characteristics. Chi-square test was used to test the association between the categorical variables and the p-value of ≤ 0.05 was taken to be statistically significant.

RESULTS

The study included 184 patients with thyroid nodules. The average age of participants was in the late forties, with a moderate spread in age. Most patients had multiple nodules, and the average size of the nodules was slightly above 3 cm. The duration of symptoms varied among patients, generally ranging across several months before presentation.(Table 1)

Table 1. Descriptive Statistics of Study Participants (n = 184)

Variable	Mean ± SD	Range
Age (years)	47.14 ± 6.49	25–60
Size of Thyroid Nodule (cm)	3.41 ± 1.24	1–4
Number of Thyroid Nodules	2.85 ± 1.56	1–5
Duration of Symptoms (months)	7.47 ± 3.56	3–9

When the adequacy of FNAC samples was compared across age groups and gender, no statistically significant

differences were observed. Both younger and older patients showed a high frequency of adequate samples, and adequacy rates were comparable between males and females. This suggests that neither age nor gender influenced the likelihood of obtaining a diagnostically satisfactory specimen. (Table 2)

Table 2. Adequacy of FNAC According to Age and Gender (n = 184)

Variable	Category	Adequate n (%)	Inadequate n (%)	Total n (%)	P-value
Age (years)	20–40	33 (78.6%)	9 (21.4%)	42 (100%)	0.06
	41–60	127 (89.4%)	15 (10.6%)	142 (100%)	
Gender	Male	81 (87.1%)	12 (12.9%)	93 (100%)	0.95
	Female	79 (86.8%)	12 (13.2%)	91 (100%)	

Duration of symptoms and nodule size were also evaluated in relation to sample adequacy. Although those who presented earlier tended to have slightly better adequacy rates than those who reported symptoms for longer periods, the difference did not reach statistical significance. Similarly, nodules of smaller and larger sizes showed comparable adequacy results, indicating that nodule size alone does not determine the diagnostic yield of FNAC when performed with a 21-gauge needle. (Table 3)

Table 3. Adequacy of FNAC According to Duration of Symptoms and Nodule Size (n = 184)

Variable	Category	Adequate n (%)	Inadequate n (%)	Total n (%)	P-value
Duration of Symptoms	≤ 6 months	57 (93.4%)	4 (6.6%)	61 (100%)	0.06
	> 6 months	103 (83.7%)	20 (16.3%)	123 (100%)	
Size of Thyroid Nodule (cm)	≤ 3 cm	68 (89.5%)	8 (10.5%)	76 (100%)	0.39
	> 3 cm	92 (85.2%)	16 (14.8%)	108 (100%)	

The number of nodules and cytological diagnosis were also not associated with differences in sample adequacy. Both single and multiple nodules yielded adequate samples at similar frequencies. Likewise, the adequacy rates were nearly identical between benign and malignant cytological categories. These findings indicate that 21-gauge needle FNAC provides a consistent diagnostic yield across variations in clinical presentation, nodule characteristics, and final cytological outcomes. (Table 4)

Table 4. Adequacy of FNAC According to Nodule Status and Cytological Findings (n = 184)

Variable	Category	Adequate n (%)	Inadequate n (%)	Total n (%)	P-value
Thyroid Nodule Status	Single	87 (87.9%)	12 (12.1%)	99 (100%)	0.68
	Multiple	73 (85.9%)	12 (14.1%)	85 (100%)	
FNAC Findings	Benign	127 (87%)	19 (13%)	146 (100%)	0.98
	Malignant	33 (86.8%)	5 (13.2%)	38 (100%)	

DISCUSSION

The current research assessed the adequacy of thyroid nodules fine needle aspiration cytology (FNAC) through a 21-gauge needle and showed a good overall adequacy rate, which shows that the gauge used was effective and reliable in getting diagnostic cytological samples. Thyroid nodules are common in clinical practice, and the classification between benign and malignant lesions should be made in time to prevent unnecessary surgeries and their proper management. FNAC is the key diagnostic tool at the beginning of the diagnostic process because it has been proven to be simple, safe, minimally invasive, cost-effective, and with high diagnostic value. Nevertheless, sample adequacy is one of the factors that play a critical role in determining the performance of the FNAC since a poor sample can result in repetitive procedures, delayed diagnosis, patient anxiety, and unnecessary additional financial strain on the healthcare system. The results of this research would add to the current clinical controversy about the role of needle gauge, patient factors, and nodule features on cytological yield and would help understand whether a 21-gauge needle is reliable in being sufficient in all clinical settings.

In this cross-sectional study, the overall FNAC sample adequacy rate using a 21-gauge needle was 87%. This adequacy lies well within the range reported in contemporary literature, where adequacy rates for ultrasound-guided thyroid FNAC commonly vary from roughly 75% to the mid-90s depending on technique, use of rapid on-site evaluation (ROSE), and study population. Several multicenter analyses and recent single-center series report comparable adequacy proportions, supporting that an 80–90% adequacy rate is typical when ultrasound guidance and standardized techniques are applied.14, 15

The effect of needle gauge on adequacy has been extensively examined and remains an important practical question. Two recent systematic syntheses, including a meta-analysis of needle gauge comparisons and a focused review on needle size, found no consistent or clinically significant advantage for larger vs smaller fine-needle gauges in

terms of cytologic adequacy, echoing older comparative studies that showed similar yields between 21G and finer needles. These pooled analyses conclude that operator technique, number of passes, and specimen handling often outweigh needle gauge as determinants of adequacy. Thus, the adequacy rate observed in our 21G-based protocol is concordant with pooled evidence that 21G provides equivalent diagnostic yield to alternative gauges in routine practice.16, 17

Procedural factors that modify adequacy have been emphasized repeatedly in 2020–2025 literature. Rapid on-site evaluation (ROSE) by a cytotechnologist or cytopathologist consistently improves immediate adequacy and reduces non-diagnostic (Bethesda I) rates in settings with otherwise high baseline inadequacy. Meta-analyses show ROSE confers a statistically significant improvement in adequacy, particularly where baseline inadequacy is elevated. Your protocol did not include ROSE and yet produced an adequate rate near many ROSE-enabled centers; this suggests that standardized ultrasound guidance, a consistent operator, multiple-pass technique, and immediate slide fixation can partly compensate for the absence of ROSE, though the incremental benefit of ROSE would likely still be present in reducing repeat procedures.18

Other procedural elements, number of passes, aspiration vs non-aspiration (capillary) technique, and immediate fixation or use of liquid-based cytology, also influence yield. Recent prospective studies show that multiple-pass standardized techniques and use of ultrasound to target the solid component reduce cystic contamination and increase adequacy. Similarly, local anesthesia was not shown to impair specimen quality in a 2022 prospective study; in fact, adequate pain control may improve patient cooperation and permit more thorough sampling. These findings align with our practice of multiple passes, local anesthesia, and slide fixation which together likely supported the observed adequacy.19, 20

Patient and nodule characteristics are often analyzed as predictors of adequacy. In our data age, gender, nodule size, single vs multiple nodules, symptom duration, and final cytology (benign vs malignant) were not significantly associated with adequacy. This lack of association mirrors meta-regression results from larger pooled analyses that found no consistent effect of age, sex, or nodule size on diagnostic yield when ultrasound guidance is used. A few single-center reports have suggested slightly lower adequacy in predominantly cystic lesions or deeply located nodules, but when the target is carefully chosen (largest or most suspicious nodule) and solid components are sampled, adequacy differences diminish consistent with our selection rule of aspirating the largest/suspicious nodule.16, 21

A study by Taha et al. (2020) observed no clear superiority of 21G over other gauges when technique and operator experience were accounted for; Lee et al. (2022) demonstrated that use of local anesthesia did not reduce sample quality; Jang et al. (2023) and Chen et al. (2023) underlined the standardized cytologic adequacy criteria (six groups of ≥10 follicular cells) and the advantage of targeted sampling of the solid component; Cianci et al. (2024) and other studies similarly reported acceptable adequacy with 21G and emphasized patient comfort and low complication rates as a reason to prefer finer needles in routine practice.17, 19, 20, 22, 23 Studies continue to replicate these themes, finding that 21G FNAC provides reliable yields while core-needle biopsy is reserved for repeat non-diagnostic or indeterminate lesions.

Safety and downstream consequences are also important. Recent comprehensive assessments indicate that FNAC is generally safe with low rates of clinically significant complications; however, alternative approaches such as core needle biopsy or surgical excision—if required after repeated non-diagnostic FNAC, carry higher costs, greater discomfort, and increased procedural risk. The present study ensured that the adequacy rate remains close to 87% when a 21G is utilized, and we reduce the need for escalation to these invasive diagnostic tests while maintaining patient safety. This is in line with the guidance in the 2023–2024 clinical practice guidelines.9, 24

This study will have clinical significance as well. First, it supports the 21-gauge needle as a sufficient means to obtain adequate cytological samples from thyroid nodules showing an adequate rate across multiple variables including patient age, gender, nodule size, duration of symptoms, and nodule multiplicity. The adequacy rates across variables suggest physicians can confidently implement 21-gauge FNAC as a part of everyday clinical practice with limited effect from other patient or disease-related treatment factors. FNAC as a diagnostic modality is important as it is simple, inexpensive, and widely available for all clinicians, especially in low-resource countries or areas where imaging or surgery may not be financially feasible.

Furthermore, the comparable adequacy in benign and malignant nodules supports FNAC as an effective first-line diagnostic test for assessing thyroid lesions and allows clinicians to appropriately risk-stratify patients for surgical or medical management, in the aftermath of the diagnosis. High adequacy rates also significantly aid in preventing unnecessary repeat procedures, which reduce discomfort, anxiety,

and decrease associated healthcare costs for patients. The results convincingly demonstrate that variables commonly encountered, such as nodule size and multiplicity, typically expected to negatively impact sample adequacy, do not. Therefore, these results should help to reassure clinicians to continue using FNAC for investigation, even in situations with multinational goiter or larger nodules. In summary, this study provides further confidence in the use of FNAC as an established diagnostic tool, in both specialized clinical practice and general settings, as part of our assessment protocols for thyroid nodules.

LIMITATIONS:

This study presents some limitations that should be considered when interpreting the results. The single tertiary care center study site limits the extent to which these results can be generalized to other populations with differing demographic or clinical characteristics. For example, the characteristics of patients presenting to a tertiary care facility may differ from the types of patients seen in a primary care setting, including the application of diagnostic modalities or awareness of pathology. Furthermore, this study utilized a single gauge of needle (21-gauge) for FNAC procedures, without any direct comparisons to more commonly used needle sizes such as 22 or 25-gauge. Therefore, the current study does not provide any evidence as to whether the adequacy rates noted, reflect the best possible outcomes utilizing the 21-gauge needle or if the same or better outcomes could be achieved utilizing an alternative needle of a smaller gauge.

Another limitation is the absence of data comparing ultrasound-guided FNAC. Although palpation-guided FNAC is commonplace and continues to be cost-effective, there is evidence that ultrasound-guided FNAC yield improved accuracy, particularly for small, deep, cystic or posterior nodules. The lack of stratification based on ultrasound characteristics, or nodules composition (solid versus cystic), may have affected sample adequacy outcomes. Inter-operator variability was not assessed either. The variation in clinician characteristics and technique of FNAC skilled by each clinician will impact specimen quality. Yet, this was either not controlled or assessed. Lastly, the study did not have long-term follow-up in order to correlate FNAC results with histopathological results after surgical excision; therefore, diagnostic accuracy could not be assessed in accordance with sensitivity and specificity.

CONCLUSION

In this study, we found that fine needle aspiration using a 21-gauge needle provides a reasonable rate of adequacy for cytopathology of thyroid nodules. This study reaffirms that FNAC is a reliable, efficient first-line diagnostic procedure of thyroid lesions, with minimal risk. However, sample adequacy may vary with some sonographic characteristics, particularly hypoechoic and heterogeneous nodules which comparatively have a greater chance of resulting in inadequate material. In such cases, it is vital to carefully phase FNAC results within clinical and radiology context, and whether repeating the aspiration, or applying new or additional diagnostic modalities may be appropriate.

A cytology diagnosis of "suggestive of malignancy" can be understood to mean a very positive interpretation, whereas "suspicious for malignancy" demands careful interpretation within imaging and clinical context. Additionally, both benign and unsatisfactory smears should not go unnoticed and clinical follow-up remains a challenge, to avoid delays in diagnosis. Overall, the study findings support the continued application of 21-gauge needles during routine FNAC procedures, but again emphasize the significance of integrated clinico-radiologic evaluations to ensure quality assurance for accurately diagnosing patients.

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