

## Comparing Aspiration and Non-aspiration Fine Needle Techniques in Cytodiagnosis of Thyroid Nodules

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**Background:** Nodular goitre remains a problem of enormous magnitude with an estimated prevalence of 19 to 35% worldwide. Of all thyroid nodules 5-10% are cancerous and require surgery. By identifying the benign ones unnecessary surgery, the associated morbidity and associated costs could be avoided. Fine needle cytology is recommended as the initial evaluation of thyroid nodules. Its main limitations are inadequate cellular harvest and indeterminate results. Aspiration (FNA) and non-aspiration (FNNA) techniques were evaluated in this study for purposes of judging which technique is better for cellular harvest. In providing the standard amount of follicular cells for cytodiagnosis (SAFC).

**Methods:** In a cross-sectional comparative descriptive study, 100 thyroid nodules were categorized by their widest diameter into <1cm, 1-4 cm and >4cm. Both FNA and FNNA were performed on each nodule (randomly assigned). After Papanicolaou and Diff-Quik staining, the follicular cells harvested were quantitatively analyzed for the SAFC by a cytopathologist blinded to the biopsy technique used and compared using Wilcoxon signed Ranks test and McNamara's test statistic. Ethical approval was secured.

**Results:** The patients' age range was 19 to 70 yrs (mean 43yrs) and 95.5% were females. Regarding the provision of the SAFC: FNNA had a higher mean cell count than FNA (108 Vs 63),  $p= 0.001$ ; FNNA was superior to FNA,  $OR= 6.5$ ,  $p<0.01$ ; and there was no significant difference between the nodules of diameter >4 cm and 1-4 cm, using FNNA or FNA.

**Conclusion:** The findings suggest that with regard to the provision of the standard amount of follicular cells for cytodiagnosis of thyroid nodules, FNNA is superior to FNA technique. There is no statistically significant association with the diameter of the thyroid nodule biopsied. FNNA technique is less technically challenging and does not require a syringe holder.

### Introduction

Nodular goitre remains a problem of enormous magnitude with an estimated prevalence ranging from 19% to 35% worldwide.<sup>1</sup> Thyroid nodules are evaluated to identify those that are cancerous, and account for approximately 5-10% of nodules. These require surgery. By identifying benign nodules unnecessary surgery is avoided.<sup>1, 2, 3.</sup>

In Uganda, at Mulago hospital, nodular goitre accounts for about 82% of goitres.<sup>4</sup> Kobusingye<sup>5</sup> found a high incidence of cancer of 19.6% of nodules from histopathology reports at Mulago. Fine needle biopsy (FNB) is the most accurate, cost effective and simplest screening test for the rapid diagnosis of the cause of thyroid nodules and it has become widely acceptable as an initial test.<sup>2, 6, 7, 8-14</sup>. The main limitation of FNB is inadequate specimen and indeterminate results<sup>3, 8</sup>. The techniques of FNB used are aspiration (FNA), and non-aspiration fine needle techniques (FNNA). Various studies comparing these two techniques have yielded conflicting results regarding adequacy of specimen. Some have shown FNNA to be simple and to significantly produce superior

quality material with less pain and discomfort to the patient compared to FNA.<sup>15-21</sup> At Mulago hospital, FNA, the commonly used technique, had about 50% inadequate results.<sup>22</sup> The effectiveness of FNNA is unknown. If FNNA is better, it will reduce the rate of both inadequate specimen and repeat biopsies as well as the cost incurred thereof. There is a need to determine the usefulness of FNNA compared to the conventional FNA in our environment in providing the SAFC of thyroid nodules and ensuring safe, reliable and effective screening of thyroid nodules.

### Patients and Methods

This cross sectional descriptive study was carried out at Mulago Teaching and National Referral Hospital in Kampala, Uganda over a 4months period. Patients were recruited from the surgical and medical Endocrine units as well as Breast unit. The study population included all patients who presented with thyroid nodules, fulfilled the inclusion criteria and gave an informed consent. They underwent the routine clinical evaluation by the attending physician. Those with clinically palpable thyroid nodules were enrolled into the study. Eligible patients were consecutively recruited, and then each patient had both FNA and FNNA on their thyroid nodule

The thyroid nodules, identified were randomly assigned and measured their widest diameters in centimeters using Vernier calipers and aspirated. The nodules chosen for biopsy were those which were:

1. Solitary nodule.
2. Nodule in multinodular goitre that had grown steadily and had become distinctly dominant or changed in consistency. However, because the finding on the largest or firmest nodule need not be representative of those of the other nodules, all accessible nodules underwent both FNA and FNNA. 100 nodules were included in this study. Thyroid cysts identified at needle biopsy were not analyzed because of poor cellularity.<sup>3</sup>

Equipment used included glass slides, cover slips, antiseptic, disposable gloves, fixative (absolute ethyl-alcohol) in a Coplin jar, swabs, French gauge 23(23 FG) hypodermic needles, and 10 milliliter syringes.<sup>14</sup> Using a diamond pencil, one end of each slide was labeled with the patients' laboratory number. Slide labels with the initials A for FNNA specimen, B for FNA specimen was also used. The biopsies were carried out on patients lying supine on a couch supported by a pillow behind the shoulders. They were instructed not to talk or swallow as the biopsy was taken.

After gloving, the skin overlaying the thyroid nodule was cleansed with antiseptic-ethyl alcohol in a swab. The nodule was immobilized between the index finger and the thumb. In performing FNA, a 23 FG needle, attached to a 10ml syringe, was inserted into the nodule. The plunger was retracted to create a vacuum in the needle for suction. Using forward and backward movements under constant suction the needle was moved at different depths and angles within the confines of the nodule thus sampling multiple areas. The biopsy manoeuvre was terminated when fluid appeared in the hub of the needle. The plunger was released to prevent aspiration of the material into the syringe before recovering the needle from the nodule. The needle was then removed from the nodule, and the syringe detached. The syringe was filled with air and then re-attached to

the needle. Using the air, with the needle tip close to the slide, the sample was expressed onto the slide. With a swab, the patient applied firm pressure over the biopsied area.

***FNNA Technique:*** For this technique, a 23 FG hypodermic needle, held directly between the thumb and index finger of one hand, was inserted into the nodule. The needle was repetitively moved back and forth and twirling it within the nodule. The biopsy manoeuvre was terminated when fluid appeared in the hub of the needle. The needle was withdrawn then using an air-filled syringe the needle contents were expelled onto a labeled slide for smear preparation as in FNA above. Using a swab the patient applied firm pressure over the biopsied area for at least 5 minutes to reduce the chances of haematoma formation. For each nodule, FNA and FNNA were performed as far apart as possible

***Smear preparation:*** The aspirated material was smeared on a slide labeled with the patient's laboratory number. Another labeled slide was placed on the smear to thinly and evenly spread the smear between the two slides on pulling them apart. One slide was immediately immersed into absolute ethyl-alcohol fixative while the other was air-dried.

***Staining and smear evaluation:*** The air-dried smears were stained with a modified Wright stain (Diff Quik) The slides were examined for the standard adequate amount of follicular cells for cytodiagnosis and the cytodiagnosis made. The cytopathologist and laboratory technician covered the slides with cover slips for preservation. The PI re-labeled each slide according to the technique used as A for FNNA and B for FNA. The cytopathologist was blinded to the biopsy technique used. The slides were then presented to the cytopathologist to be examined for the number of groups of follicular cells and for the cytologic diagnosis. All the smears were evaluated by the same cytopathologist. Cytology results were categorized into the four groups suggested by the Papanicolaou Society of Cytology as non-diagnostic, benign, indeterminate, and malignant.<sup>33</sup> Smears with insufficient number of follicular cells were considered non-diagnostic. Results were recorded in another register. Ethical approval was sought prior to commencement of the study.

Analysis Data was corrected and entered into SPSS version 12. At univariate analysis data was analyzed for frequencies and proportions. Bivariate analysis was done using odds ratios and p-values. Where the data was not normally distributed, the Wilcoxon Signed Ranks test was used to compare paired medians of the same sample. The two techniques were analyzed as matched pairs for each nodule. For the paired samples in which one technique gave the SAFC and the other did not, analysis was done using McNemar's test statistic. Odds ratios were calculated for discordant pairs.

## Results

The study involved 88 patients, in whom 100 nodules were biopsied. The patients' age range was 19 to 70 yrs (mean 43yrs) and 95.5% were females. Table 1 shows the age and sex distribution. Table 2 shows the pattern of clinical characteristics of thyroid nodules. Table 3 shows the bivariate analysis of nodularity and nodule size. Follicular cell counts for eighty-five nodules were available for statistical analysis. In this study, FNNA technique provided a greater mean cell count than did FNA. (108.9 vs. 63,  $p=0.01$ ). (Figures 1 and 2). The proportion of nodules with smears having SAFC using FNNA was

76/85 (0.894) compared with 65/85 (0.765) using FNA (Tables 4). The difference between the proportions was therefore 0.129, 95% C.I, 0.053 – 0.206. This means that the rate of providing the SAFC was between 5.3% and 20.6% higher if FNNA was used than if FNA was used.

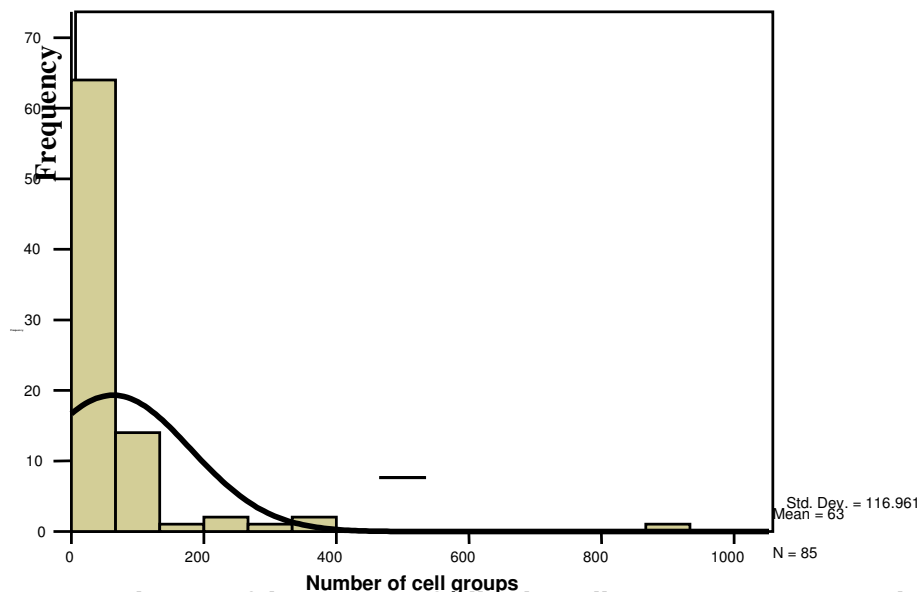
FNNA was significantly superior ( $p < 0.01$ ) to FNA in providing the SAFC. In two nodules (2.3%), FNA yielded the SAFC while FNNA did not. Both of the nodules measured 1-4 cm in their widest diameter. One nodule was a solitary thyroid nodule while the other was in a multinodular goitre. Previous studies by Ciatto et al, and Mariyan et al among others similarly showed superiority of FNNA,  $p < 0.01$ .<sup>17, 19, 20, 26, 27, 32</sup>. However, Suen found FNA to provide more cells than FNNA in some cases and vice versa in others<sup>25</sup>. On the other hand, Ghosh et al found that FNA was superior to FNNA. The difference is statistically significant<sup>18</sup>.

**Table 1.** Age and Sex distribution (n = 88)

Characteristic	Number	(%)
<b>Age-group</b>		
< 30 yrs	17	19.3
30-59 yrs	57	64.8
60 and above yrs	14	15.9
<b>Sex</b>		
Male	4	4.5
Females	84	95.5

**Table 2:** Pattern of Clinical Characteristics of Thyroid Nodules.

Clinical Features	Frequency (n)	%
<b>Nodularity</b>		
Solitary	53	60.2
Multinodular	35	39.8
<b>Nodular size (cm)</b>		
1-4	60	60
> 4	40	40



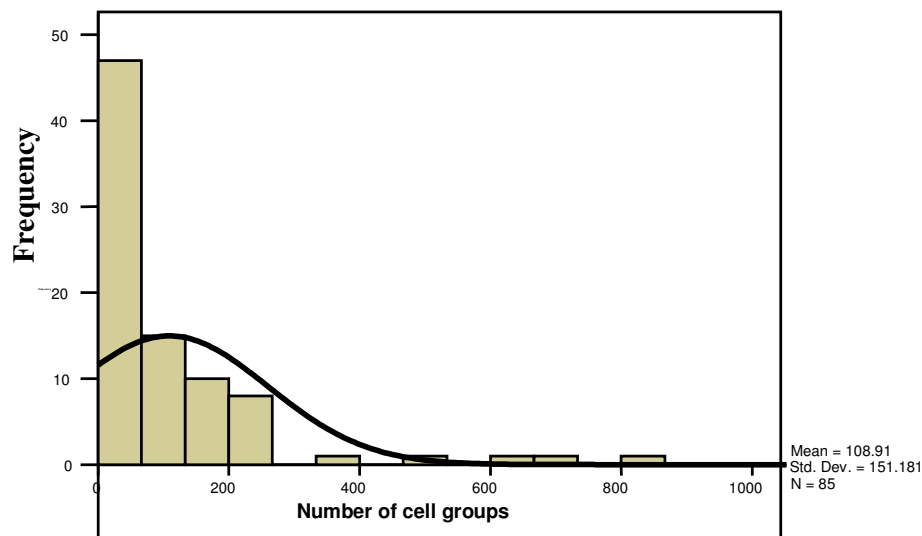
**Figure 1.** Distribution of the amount of follicular cell groups using FNA Technique

**Table 3.** Bivariate analysis of nodularity and nodule size

	Nodule Size (cm)		Totals	OR (95%CI)	X <sup>2</sup> (p-value)
	1-4	> 4			
STN	42	11	53	6.15 (2.33-16.58)	17.4 (0.0000302)
MNG	18	29	47		
Totals	60	40	100		

**Table 4.** Performance of FNA and FNNA techniques

Sample	Methods	
	FNA	FNNA
SAFC	65 (76.5%)	76 (89.4%)
No SAFC	20 (23.5%)	9 (10.6%)
Total	85 (100%)	85 (100%)

**Figure 2.** Distribution of amount of follicular cell groups using FNNA Technique

The FNNA technique draws up cells by capillary action with minimal dilution with blood. The FNA technique, on the other hand, is not infrequently complicated by aspiration of significant quantities of blood, which compromises cellular concentration, preservation, and interpretation. This is the likely reason for the provision of more cells by FNNA.<sup>2,6,23,24</sup>

## Discussion

Fine needle biopsy of the thyroid is widely used in the cytodagnosis of thyroid nodules since it is quick, safe, inexpensive and reliable. Inadequate cell harvest is a major limitation, while previous studies comparing FNA and FNNA techniques with regard to this limitation show conflicting results<sup>3,8,17,20,25</sup>. Over a four months period, eighty-eight

patients with thyroid nodules were recruited from the medical and surgical endocrine clinics.

In this study, FNNA technique provided a greater mean cell count than did FNA. (108.9 vs. 63,  $p=0.01$ ). The proportion of nodules with smears having SAFC using FNNA was 76/85 (0.894) compared with 65/85 (0.765) using FNA. The difference between the proportions was therefore 0.129, 95% C.I, 0.053 – 0.206. This means that the rate of providing the SAFC was between 5.3% and 20.6% higher if FNNA was used than if FNA was used. FNNA was significantly superior ( $p < 0.01$ ) to FNA in providing the SAFC. In two nodules (2.3%), FNA yielded the SAFC while FNNA did not. Both of the nodules measured 1-4 cm in their widest diameter. One nodule was a solitary thyroid nodule while the other was in a multinodular goitre. Previous studies by Ciatto et al, and Mariyan et al among others similarly showed superiority of FNNA,  $p < 0.01$ .<sup>17, 19, 20, 26, 27, 32</sup> However, Suen found FNA to provide more cells than FNNA in some cases and vice versa in others<sup>25</sup>. On the other hand, Ghosh et al found that FNA was superior to FNNA. The difference is statistically significant<sup>18</sup>.

The thyroid gland is very vascular. The FNNA technique employs capillary action, which draws up the cells into the biopsy needle while the FNA employs high suction pressures. The FNNA technique draws up cells by capillary action with minimal dilution with blood. The FNA technique, on the other hand, is not infrequently complicated by aspiration of significant quantities of blood, which compromises cellular concentration, preservation, and interpretation. This is the likely reason for the provision of more cells by FNNA<sup>2,6,23,24</sup>.

Common technical errors leading to inadequate specimen include aspirating a mass without a syringe holder, aspirating a mass without moving the needle back and forth through the specimen and aspirating of air after the biopsy is completed and the needle is withdrawn, allowing the specimen to be lost in the syringe<sup>28</sup>. In this series, in 20/85 (23.5%) of nodules the specimen was inadequate. This may have partly been contributed to by not using a syringe holder and the loss of part of the specimen in the syringe. During biopsy, it was more cumbersome aspirating the smaller nodules while maintaining suction in the syringe when using the FNA technique. The FNNA technique afforded better control of both the needle and nodules during biopsy than did the FNA technique. This has been observed by other researchers as well<sup>29</sup>. It was difficult to control the syringe movement while maintaining suction with one hand when using the FNA technique. Where the syringe holder is not available for FNA, the biopsy material can easily be sucked up into the syringe. This makes it difficult to express onto slides. The reasons could have contributed to worse performance of FNA. In the present study, biopsies were performed by a single operator. This avoids bias introduced by differing skills and experience by different performers<sup>15, 27</sup>. The possibility of trauma caused by the first procedure affecting the outcome of the second was minimized by placing the punctures as far apart as possible.

In this study, nodule size in diameter was categorized into  $< 1\text{cm}$ ,  $1\text{-}4\text{cm}$ , and  $> 4\text{cm}$ . There were no nodules in the  $< 1\text{cm}$  category. Thyroid nodules with widest diameter  $1\text{-}4\text{ cm}$  were commoner in the solitary nodules than in multinodular goitres, while nodules  $> 4\text{cm}$  were commoner in the multinodular goitre-nodules. Ultrasound was not



used in the evaluation of all the thyroid nodules in this study. Results of ultrasound were available for 24/88 (27.2%) of the patients. The positive predictive value of clinical examination as compared to ultrasound was 86%.

Makoba<sup>4</sup> in his study found that regarding nodular thyroid disease, clinical diagnosis was made in 48.8% of the patients while with ultrasonography it was 82.2%. Other studies found that about 50% of solitary nodules on palpation, were multiple nodules on ultrasound evaluation<sup>30</sup>. This implies that the multinodular goiters in this study was an under estimation and that some nodules were probably missed. Similarly, clinical determination of nodule size using Vernia calipers is likely to have over-estimated nodule size. The widest diameter clinically might not be real because of inaccessibility. There were no nodules of diameter <1 cm. These could have been missed because of their position, being inaccessible. The use of ultrasound to determine nodule sizes would certainly give more accurate measurements as other researchers have found<sup>4</sup>.

For each of the techniques the difference in providing the SAFC from the different nodule sizes was not statistically significant. These findings suggest that no particular technique performed better with regard to nodule size. Brownridge et al<sup>15</sup> had similar findings.

It is likely that patients with smaller nodules in this case < 1cm widest diameter had sub-clinical nodules and therefore, were not recruited. The current study did not undertake to screen thyroid glands for sub-clinical nodules, neither were any biopsies done using ultrasound. The main indication for ultrasound-guided FNA/FNNA is following unsatisfactory biopsy by palpation<sup>32</sup>. Bivariate analysis demonstrated a tendency towards a larger cell provision in nodules 1-4 cm category, however possibly because of the small sample size, the statistical significance of this could not be demonstrated by the current study. Larger nodules tend to have centres undergoing degeneration and less numerous follicular cells as compared to small ones.<sup>3</sup> This probably is one of the reasons for a larger cell provision in the smaller 1-4cm nodules.

Even though the order of FNA or FNNA was randomly assigned and these were performed as far apart as possible, it is conceivable that some bias would be introduced especially to the technique that was performed second. However random assignment minimizes this limitation.

## Conclusion

In assessment of thyroid disease without Ultrasound scan, thyroid nodules could be missed. Thyroid nodules are better assessed with FNNA, which is less technically challenging and does not require a syringe holder. The association between the diameter of the thyroid nodule biopsied and the provision of adequate standard amount of follicular cells for cytodiagnosis of thyroid nodules using either FNA or FNNA at Mulago was not statistically significant.

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