Diagnostic accuracy of fine needle aspiration cytology in patients undergoing thyroidectomy in Uganda: tertiary hospital experience.

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Abstract

Background: Thyroid disease affects about 5% of the World’s population. Fine Needle Aspiration Cytology (FNAC) helps in planning extent of surgery. In some studies, FNAC has been found to have a low accuracy for malignancy.

Objective: To estimate the sensitivity and specificity of FNAC in detecting malignancy for thyroid disease using histopathology as the gold standard.

Methods: Patients who underwent clinical and laboratory evaluation and thyroidectomy at Mulago National Referral hospital and the Pathology department of Makerere University College of Health Sciences were consecutively recruited over a four months period. Analysis using STATA version 10 focused on sensitivity, specificity and accuracy of FNAC in detecting malignancy.

Results: In total, 99 patients were recruited, the F:M ratio was 15.5:1 and median age was 42 years (IQR 34-50). The median duration of symptoms was 364 weeks (IQR 104-986). The proportion of patients with malignancy was 13.3% with papillary thyroid carcinoma being the most predominant type and colloid goiter was the most predominant benign thyroid disease. The sensitivity was 61.5% and specificity 89.5%.

Conclusion: This study revealed high specificity and low sensitivity of Fine Needle Aspiration Cytology (FNAC) at detecting malignancy in thyroid nodules

Keywords: Diagnostic accuracy, fine needle aspiration, cytology, thyroidectomy, tertiary hospital.

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Introduction

Thyroid disease affects about 5% of the general population Worldwide ranging from hypothyroidism, hyperthyroidism, thyroiditis, cancer of the thyroid and nodules. Nodular thyroid disease is a common clinical problem with a prevalence of 4%-7% and annual incidence of 0.1% in some adult populations1-2. Nodules are more frequent in females and are mostly benign. Fine Needle Aspiration Cytology (FNAC) has been in use since the 1950s and is a safe, cost effective method of diagnosing thyroid nodules3.

Despite several studies showing a high accuracy with FNAC, emerging studies especially in tropical Africa and other developing countries with a high prevalence of nodular thyroid disease, have shown the accuracy of FNAC to be lower than previously reported4-5 and its diagnostic performance has been shown to vary across different studies6.

Knowledge on the burden of thyroid disease in Uganda is still limited. The Mulago hospital endocrine surgical outpatient clinic attends to about 25-30 patients with thyroid disease every week and an annual increase of about 350-370 new patients with thyroid disease has been reported over the last five years (Mulago hospital records).
In this hospital, nodular goiter was found to constitute about 82\%\textsuperscript{7}.

The diagnostic performance of FNAC in Mulago hospital is not known. The purpose of this study was to estimate the sensitivity and specificity of FNAC in detecting malignancy for thyroid disease using histopathology of excised specimen as a gold standard and to describe histopathological diagnoses of thyroid disease in patients following thyroidectomy.

Materials and methods

Study design
This was a cross sectional study with both prospective and retrospective arms carried out from January 2014 to April 2014. The retrospective arm involved retrieving patients’ records (cytological and histopathological reports) from the pathology department of Makerere University College of Health Sciences (MakCHS) that had undergone thyroidectomy from January 2008 to December 2013 and the other arm involved prospective recruitment of patients from January to April 2014. Consecutive sampling was used.

Sample size was estimated using the formula for single proportions\textsuperscript{8}. Using sensitivity and specificity proportions of 90\% initially found by Nyawawa et al\textsuperscript{9} in Tanzania and a significance level of 0.05, we estimated the sample size to be 175 participants (75 prospective and 100 retrospective arm). This division is because we expected to perform 20 thyroid surgeries per month for the study period of 4 months in the prospective arm.

Study setting
In the FNAC technique, equipment involved glass slides, cover slips, antiseptics, disposable gloves, fixative (absolute ethyl alcohol), swabs, 23 French (Fr) gauge hypodermic needles (23Fr, 24Fr, 25 Fr is recommended) and 10 ml syringes.

The patient was made to lie supine on an examination couch with slight neck extension and a sand bag underneath the shoulders.

Gloving of hands was done and thereafter the skin was prepped with 70\% ethyl alcohol in a swab. The thyroid nodule was immobilised and stabilised between the index finger and the thumb of the left hand.

A 23Fr gauge needle attached to a 10 ml syringe was inserted into the nodule. The plunger was retracted to create a vacuum in the needle for suction (in FNA) or without suction (FNNA). Backward and forward movements were used under constant suction with the needle moved at different depths and angles within the confines of the nodule. 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.

The needle was removed from the nodule and syringe detached. The syringe was reattached after withdrawing the plunger and air was used with the needle tip close to the glass slide, the sample was expressed on the slide. At least two passes were made in two different quadrants of the thyroid swelling/nodule. With a sterile swab, pressure was applied over the biopsy site for about five minutes.

The aspirated material was smeared on a slide labelled with the patient’s laboratory number and another labelled slide was placed on the smear to evenly and thinly spread it between the two slides on pulling them apart, this made two smears per pass and therefore four smears per patient.

Two slides were air dried where as the other two were fixed immediately by immersion into absolute ethyl alcohol.

The air-dried smears were stained with modified wright stain (Diff-Quick) and the smears fixed with absolute ethyl alcohol were stained with Papanicolaou stain. The attending cytopathologist examined the smears for standard adequate amount of follicular cells for cytodiagnosis.

Cytology results were categorised into 6 groups according to the Bethesda system for reporting thyroid cytopathology as Non-diagnostic, benign, follicular lesion of undetermined significance, follicular neoplasm, suspicious and malignant.

Histopathology
During histoprocessing of biopsy specimens after thyroidectomy, fixation was done with 10\% formalin immediately after surgery whose main objective was to preserve protoplasm with minimal alteration from the living state of the cell.

The specimen was embedded in paraffin wax. This provided rigid support to tissue blocks so that it was easy to cut them into thin sections.
The paraffin wax embedded tissue specimens were sliced into very thin sections of 3-10 microns thick. The section was then put on a clean glass microscope slide and warmed to let the specimen settle on the slide.

Staining was done with Eosin and Haematoxylin. The slide was then placed in a solution of paraffin solvent (xylol or toluol) to remove the paraffin. Excess dye was washed away with water. The section was dehydrated by increasing concentration of alcohol. A drop of mounting agent (canadian Balsam) which had the same refractive index similar to that of glass was placed on the section and the preparation covered with a cover slip and allowed to dry. The slide was read and interpreted by the histopathologist as benign or malignant and report of the results written.

Data collection, quality assurance and quality control

Interviews were conducted with all fully consenting patients using a standard pretested questionnaire aimed at capturing patients’ history, examination and investigation findings.

For the retrospective arm, enough information was extracted as much as possible from the cytology and histopathology reports.

Standard Operating Procedures (SOPs) for both FNAC and histopathology and Standards for Reporting of Diagnostic accuracy studies (STARD) were followed till this study was completed.

Quality control was assured by maintaining that FNB procurement, processing and reporting was done by experienced cytopathologists (using the Bethesda system for reporting thyroid cytopathology), the histopathologist examining excised thyroid specimens was blinded to pre-operative diagnosis with FNAC and the cytopathologists and histopathologists for FNAC and histopathology of excised specimens respectively were generally the same for both the retrospective and prospective arms of the study thus minimizing inter-observer bias.

Data analysis

Patients’ data was entered into EPIDATA 3.1 and exported to STATA Version 10 for analysis. Using the NCI (Bethesda) FNAC reporting system, only individuals with a “benign” report were considered benign cases on FNAC, where cases with “follicular neoplasm”, “suspicious for malignancy” or “malignancy” reports constituted malignant cases on FNAC. All patients enrolled in the study were included in the analysis to determine the sensitivity and specificity of FNAC for malignant conditions using a 2 X 2 table.

Ethical consideration

Before the start of this study, ethical clearance was obtained from the Institutional Review Board of Makerere University College of Health Sciences. Written informed consent was obtained from patients both for the surgery and for inclusion in the study. Waiver of consent was obtained for the retrospective arm of the study.

Results

A total of 99 patients were enrolled (45 prospective and 54 retrospective).

Overall, median age (IQR) was 42 years (34-50), 92 females and 7 males with a female to male ratio of 15.5:1 [Table 1].
Table 1. Social demographic characteristics of patients who underwent thyroidectomy at Mulago Hospital during the study period (prospective arm).

| Characteristics                  | Prospective arm (N=45) |
|----------------------------------|------------------------|
| **Gender, n (%)**                |                        |
| Female                           | 41 (91.1)              |
| **Tribe, n (%)**                 |                        |
| Ganda                            | 11 (24.4)              |
| Nkole/Kiiga                      | 6 (13.3)               |
| Soga                             | 3 (6.7)                |
| Others*                          | 25 (55.6)              |
| **Marital status, n (%)**        |                        |
| Married                          | 28 (62.2)              |
| Not married                      | 17 (37.8)              |
| **Address, n (%)**               |                        |
| Central                          | 17 (37.8)              |
| Western                          | 7 (15.6)               |
| Eastern                          | 16 (35.6)              |
| Northern                         | 5 (11.1)               |
| **Education, n (%)**             |                        |
| Formal education                 | 42 (93.3%)             |
| No formal education              | 3 (6.7%)               |
| **Occupation, n (%)**            |                        |
| Peasant                          | 19 (42.2)              |
| Salaried                         | 14 (31.1)              |
| Self-employed/business           | 9 (20.0)               |
| Others#                          | 3 (6.7)                |

# refers to students, unemployed.
* refers to Banyoro, Iteso, Batoro, Bagisu, Karamojong

Of the five solitary nodules, two (40%) were malignant and of the 40 multinodular goiter, two (5%) were malignant (p value=0.055) [Table 2 and Table 3]
Table 2. Baseline clinical characteristics of patients who underwent thyroidectomy at Mulago Hospital during the study period (prospective arm).

| Characteristic                                               | Prospective arm (N=45) |
|-------------------------------------------------------------|------------------------|
| Anterior Neck Swelling, n (%)                               | 45 (100)               |
| Duration of symptoms in weeks, Median (IQR)                 | 364 (104 – 986)        |
| Difficulty in breathing, n (%)                              | 33 (73.3)              |
| Change in Voice, n (%)                                      | 16 (35.6)              |
| Family history of thyroid cancer, n (%)                     | 3 (6.7)                |
| Goitre grade, n (%)                                         |                        |
| Grade 1                                                     | 0 (0.0)                |
| Grade 2                                                     | 0 (0.0)                |
| Grade 3                                                     | 12 (26.7)              |
| Grade 4                                                     | 33 (73.3)              |
| Biochemical diagnosis, n (%)                                |                        |
| Euthyroid                                                   | 37 (84.1)              |
| Hyperthyroid                                                | 5 (11.4)               |
| Hypothyroid                                                 | 2 (4.6)                |
| Number of nodules, n (%)                                    |                        |
| Solitary                                                    | 5 (11.1)               |
| multinodular                                                | 40 (88.8)              |
| Size of largest nodule, mm (IQR)                            | 39 (29-54)             |
| Site of nodules, n (%)                                      |                        |
| Left                                                        | 10 (22.2)              |
| Right                                                       | 3 (6.7)                |
| Bilateral                                                   | 32 (71.1)              |

Table 3. Distribution of histopathological diagnoses among patients who underwent thyroidectomy in Mulago Hospital by method of participant enrollment

| Diagnosis                    | Overall, N=99, n (%) | Prospective, N=45, n (%) | Retrospective, N=54, n (%) |
|------------------------------|----------------------|--------------------------|----------------------------|
| Colloid goitre               | 66 (66.7)            | 35 (77.8)                | 31 (57.4)                  |
| Nodular goitre               | 16 (16.2)            | 4 (8.9)                  | 12 (22.2)                  |
| Papillary carcinoma          | 11 (11.1)            | 3 (6.7)                  | 8 (14.7)                   |
| Follicular carcinoma         | 2 (2.0)              | 1 (2.2)                  | 1 (1.9)                    |
| Chronic thyroiditis          | 2 (2.0)              | 1 (2.2)                  | 1 (1.9)                    |
| Follicular adenoma           | 1 (1.0)              | 0 (0.0)                  | 1 (1.9)                    |
| Inflammatory cyst            | 1 (1.0)              | 1 (2.2)                  | 0 (0.0)                    |
Malignant nodules tended to be bigger with median size of 50 mm (IQR 39.5-76.5) compared to benign nodules with median size of 37 mm (IQR 27-51) but this was not statistically significant (p value of 0.055). Sensitivity and specificity were 61.5% (CI 35.5 – 69.0) and 89.5% (CI 81.3 – 94.4) respectively. [Table 4].

### Table 4. Summary of results from the 2 x 2 table

|                      |               |
|----------------------|---------------|
| Sensitivity, % (CI)  | 61.5 (35.5-69.0) |
| Specificity, % (CI)  | 89.5 (81.3-94.4) |
| Positive predictive value (PPV), % (CI) | 47.1 (26.2-69.0) |
| Negative predictive value (NPV), % (CI) | 93.9 (86.5-97.4) |
| False positive rate (FPR), % (CI)        | 10.5 (5.6-18.7) |
| False negative rate (FNR), % (CI)        | 38.5 (17.7-64.5) |
| Accuracy, % (CI)       | 85.8 (77.7-91.4) |

### Discussion
The aim of this study was to estimate the sensitivity and specificity of FNAC in detecting malignancy for thyroid disease using histopathology as the gold standard. We found a sensitivity of 61.5% and specificity of 89.5%. [Table 4].

The median age of patients was 42 years, the youngest was 16 years and the oldest was 78 years. In a related study in Uganda the median age was 43.2 years, in Tanzania it was 42.7 years, in Egypt it was 44 years. The female to male (F:M) ratio was 15.5:1 meaning that this is predominantly a disease of women. This was observed in related studies within the East African region.

In all patients the main presenting complaint was an anterior neck swelling with median time interval to presentation of 364 weeks (7 years) [Table 2]. The long time interval to presentation could probably explain the fact that all patients had either grade 3 or grade 4 goitre [Table 2].

On Ultrasound scan, majority of patients (88.9%) had multinodular goiter and 71.1% were bilateral. Most goiters in resource poor settings are multinodular and this emphasizes the fact that iodine deficiency still remains top most cause of thyroid disorders on the African continent.

The sensitivity and specificity for detecting malignancy on FNAC was 61.5% and 89.5% respectively and the accuracy was 85.9%. Just like other studies with a high prevalence of multinodular goiter, the sensitivity for detecting malignancy is relatively low where as specificity is high. For most studies, the diagnostic accuracy of more than 90% is seen in malignant disease and about 77.8% in benign disease.

For a rare disease (thyroid cancer is relatively uncommon), specificity is the most desirable so that when the test result is negative then chances are very high that the individual has no disease. The negative predictive value in this study was 93.9% meaning that when FNAC classifies a patient as having benign disease, then chances of having thyroid cancer are very low.

Majority of the goiters were benign (86.9%). The proportion of patients with malignancy in this study was 13.1%. Kobusingye found malignancy rate of 19.6% in Uganda whereas in Tanzania it was 18.6%. This contrasts with what is reported in Europe and North America where incidence of malignancy in thyroid nodules is about 5%. The difference could probably be attributed to the fact that a significant proportion of long standing multinodular goiter in areas endemic for iodine deficiency tend to undergo malignant transformation.

Of the malignant histopathology, majority(84.6%) were papillary thyroid carcinoma (PTC) and the rest were follicular thyroid carcinoma (FTC). In a related study in Uganda, FTC predominated at 59.1% and in Tanzania, FTC predominated at 72.2%. It is now noted that for differentiated thyroid carcinoma (DTC), there is a changing trend towards the frequent occurrence of PTC compared to FTC and this may be attributable to wide spread iodization programs.
Study limitations
For the retrospective arm, blinding of the histopathologist to the corresponding cytological diagnosis with FNAC may not have been observed. The many pathologists involved in reporting histopathology could have over-estimated or under-estimated malignancy rate (random error).

We were unable to achieve the estimated sample size within the study period. The prospective arm was dependent on availability of theatre space whereas the retrospective arm was limited by poor and to a large extent non computerized record keeping.

The findings of this study may not be generalizable because of the relatively small numbers used in analysis compared to the relatively big number of patients with nodular thyroid disease.

Conclusion
This study reveals high specificity and a low sensitivity for FNAC at detecting malignancy in thyroid nodules. These findings give good preliminary data on performance of FNAC in thyroid disease in our setting.

There appears to be a changing trend towards the frequent occurrence of papillary thyroid carcinoma (PTC) compared to follicular thyroid carcinoma (FTC).

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Author contributions
RM developed the concept, participated in data collection and manuscript writing, PKO participated in data collection, analysis, manuscript writing, JOF and DW reviewed the concept, data analysis and manuscript. All the authors contributed equally and agreed to the final manuscript.

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Conflict of interests
The authors declare no conflict of interests

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