Fine Needle Aspiration (FNA) Cytology of the Thyroid: A Cyto-Histopathological Study of 361 Cases in Hospital Universiti Kebangsaan Malaysia

Nurismah MI, Sharifah NA, Usama AE, Rohaizak M, Naqiyah I, Jasmi A

1 Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur
2 Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur

ABSTRACT

Thyroid nodules are common but thyroid malignancies are not. Fine needle aspiration (FNA) cytology is a diagnostic tool used to screen patients with thyroid nodules who require surgery. We study the diagnostic accuracy of FNA as the initial diagnostic modality in the clinical assessment of thyroid nodules. Between January 1995 until December 2000, 2131 FNA of thyroid nodules were performed. Four hundred and forty-one (20.7%) of these were unsatisfactory and 1690 (79.3%) cases were satisfactory for cytological evaluation. Histopathological diagnosis were available for 361 cases. Cytohistopathological correlation was carried out for these cases. Our results showed a diagnostic accuracy of 96.2% with sensitivity and specificity rates of 87.7% and 98.4% res-

Address for correspondence and reprint requests: Professor Dr. Sharifah Noor Akmal Syed Husain, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia. Email: sharifah@mail.hukm.ukm.my
pectively. Our positive predictive value is 93.4% and our negative predictive value is 96.8%. From this study, we conclude that fine needle aspiration is an important initial screening diagnostic tool for the investigation of thyroid nodules.

Key Words: Fine needle aspiration cytology (FNAC), thyroid nodules, cytohistopathological correlation, sensitivity, specificity

INTRODUCTION

The incidence of thyroid nodules among adult population is between 4-7%, however, less than 5% of these nodules are malignant (Sclabas et al 2003). Few people die from thyroid carcinoma, on the other hand, thyroid surgery is associated with a number of risks like recurrent laryngeal nerve injury, hypoparathyroidism etc. Therefore, there is a need for an accurate test to screen the nodules that are likely to harbour malignancy and thus require operation while avoiding surgery of benign nodules.

Fine needle aspiration cytology was first described in the 1930s by Martin and Ellis, however it became widely accepted in the United States only in the late 1970s (Leonard et al 1997, Matesa et al 2002). Currently, fine needle aspiration (FNA) has emerged as one of the first-line diagnostic techniques in the evaluation of thyroid nodules. It is a simple, cost-effective and safe procedure with few complications. The main purpose of thyroid FNA is to select nodules that require surgery from those that do not. It can also be diagnostic for certain lesions such as classic nodular goiter, Hashimoto's thyroiditis, papillary carcinoma, medullary carcinoma, anaplastic carcinoma and metastatic carcinoma (Matesa et al 2002).

The aim of this study is to determine the diagnostic accuracy of FNA as the initial diagnostic modality in the evaluation of a thyroid nodule.

MATERIALS AND METHOD

A total of 2131 FNA thyroid were performed from January 1995 till December 2000 in Hospital Universiti Kebangsaan Malaysia (HUKM), a teaching hospital in Kuala Lumpur. Out of these, 441 FNAs were unsatisfactory for cytological evaluation. Of the remaining 1690 satisfactory samples, histopathological diagnoses were available for 361 cases.

Fine needle aspirations of palpable masses were performed by Pathology Registrars and Cytopathologists. Deep-seated lesions were performed by radiologists using ultrasound-guidance. The aspiration technique was the standard one described in the literature (Orell et al 2002). The thyroid mass was examined clinically to note its mobility, consistency and size. Cervical lymph nodes were palpated to detect enlargement. The skin was sterilized with alcohol. No local anaesthesia was used. Aspiration was performed under negative pressure using a disposable 23-gauge needle attached to a 20 ml syringe that was fitted to a Cameco syringe holder. Direct smears were prepared from the aspirated material. The procedure was repeated two to three times at different areas of the thyroid mass. In cases of cystic lesions, the cyst content was evacuated and smears were prepared from the cyst fluid. Reaspiration was performed if there was a remaining solid mass palpable.

At least two smears were fixed with alcohol and two air-dried. Alcohol-fixed smears were stained by the Papanicolaou method while the air-dried smears were stained by the May-Grunwald-Giemsa technique. All the smears were screened and reported by the Registrars undergoing cytopathology training and reviewed by the
Cyto-pathologist on-call. The cytologic
smears were interpreted according to the
criteria described by Orell (Orell et al
2002), Ramzy (Ramzy 2001) and Cibas
(Cibas and Ducatman 2003).
Smears which contained no diagnostic
acellular material or were heavily blood-
stained are considered unsatisfactory for
cytological evaluation. A repeat FNA was
recommended in these cases.
The cytologic diagnosis was classified
as benign (goitre, Graves disease, colloid
cyst, and Hashimoto’s thyroiditis), incon-
clusive (follicular neoplasms and Hurthle
cell tumours) and malignant (papillary
carcinoma, medullary carcinoma, anap-
lastic carcinoma, squamous cell carcino-
ma and non-Hodgkin lymphoma).
Subsequent to FNA, the histopatho-
logical reports of the cases that were
operated on were traced from the histo-
pathology files. The final histopathological
diagnosis was then compared with the
 correponding cytological diagnosis and
cyto-histopathological correlations were
established in 361 cases.

Statistical analysis
Cases with cytological diagnosis of
follicular neoplasms and Hurthle cell
tumours were regarded as inconclusive
because it is not possible to differentiate
between follicular adenoma (benign) from
follicular carcinoma (malignant) based on
cytological assessment. Hence, these
cases were not included in the calculation
for sensitivity, specificity, positive and
negative predictive values and diagnostic
accuracy. Unsatisfactory cases were also
excluded from calculation.
True positives (TP) were defined as
cases diagnosed as malignant on cytology
which were histologically confirmed. False
positives (FP) were those diagnosed as
malignant on cytology that were benign on
histology. True negatives (TN) were benign
on both cytology and histology. False
negatives (FN) were negative on cytology
but positive for malignancy on histology.
The diagnostic accuracy was calculated as:
\[
\frac{(TP + TN)}{(TP + FP + TN + FN)}
\]

RESULTS
There were 2131 FNA thyroid performed in
HUKM from January 1995 till December
2000. Out of these, 441 (20.7%) were
reported as unsatisfactory, thus leaving
1690 (79.3%) satisfactory cases for cyto-
logical evaluation. Histopathological diag-
noses were available for 361 cases. Correlation between cytological and histo-
pathological diagnosis was done on these
cases.
From the 361 cases, 253 (70.1%) were
classified as benign, 61 (16.9%) malignant
and 47 (13.0%) were inconclusive on
cytology. These results are shown in Table
1.
The detail of cytological and the
corresponding histopathological diagnosis

<table>
<thead>
<tr>
<th>No.</th>
<th>FNA Classification</th>
<th>FNA diagnosis</th>
<th>No of cases (%)</th>
<th>Total no of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Positive for malignancy</td>
<td>Papillary carcinoma</td>
<td>47 (13.0%)</td>
<td>61 (16.9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anaplastic carcinoma</td>
<td>11 (5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Hodgkin lymphoma</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medullary carcinoma</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squamous cell carcinoma</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Negative for malignancy (Benign)</td>
<td>Goitre</td>
<td>233 (64.5%)</td>
<td>253 (70.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyst</td>
<td>18 (5.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Graves disease</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hashimoto’s thyroiditis</td>
<td>1 (0.3%)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Inconclusive (follicular lesion)</td>
<td>Follicular neoplasm</td>
<td>41 (11.3%)</td>
<td>47 (13.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hurthle cell tumour</td>
<td>6 (1.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>361 (100%)</td>
<td>361</td>
</tr>
</tbody>
</table>
is shown in Table 2. Histopathological examination confirmed the benign lesions in 245 (96.8%) of the 253 cytologically benign cases, which represent true negative cases. In these cases, FNA cytology correctly diagnosed 216 (92.7%) of the 233 goitres. There were 18 cases diagnosed as cyst on cytology. Out of these, subsequent histology revealed eight cysts, five goitres, two Hashimoto’s thyroiditis, one Hurthle cell tumour, one follicular adenoma and one papillary carcinoma.

There were eight (3.2%) false negative cases which included six papillary carcinoma cytologically diagnosed as goitre, one papillary carcinoma diagnosed as cyst and one follicular carcinoma which was interpreted as goitre on cytology.

Of the 61 patients diagnosed as malignant on FNA cytology, 57 (93.4%) were confirmed malignant on histopathological examination, thus were true positives (41 papillary carcinoma, one follicular carcinoma, one medullary carcinoma, 11 anaplastic carcinoma, one squamous cell carcinoma and one non-Hodgkin lymphoma). There were four (6.6%) false positive cases. These cases were cytologically diagnosed as papillary carcinoma from which three were histologically diagnosed as goitre and one as follicular adenoma. Table 2 depicts the detail of cytological and the corresponding histopathological diagnosis. Table 3 summarises the cytological and corresponding histopathological diagnosis.

We classified 47 cases as inconclusive (41 follicular neoplasms and six Hurthle cell tumours) because it is not possible to differentiate between follicular adenoma and follicular carcinoma based on cyto-

<table>
<thead>
<tr>
<th>Histology Cytology</th>
<th>N</th>
<th>Goitre</th>
<th>Cyst</th>
<th>Graves</th>
<th>HT</th>
<th>FA</th>
<th>HCT</th>
<th>FC</th>
<th>PTC</th>
<th>MC</th>
<th>AC</th>
<th>SCC</th>
<th>NHL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goitre</td>
<td>2</td>
<td>216</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>233</td>
</tr>
<tr>
<td>Cyst</td>
<td>-</td>
<td>5</td>
<td>8</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>Graves</td>
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<td>-</td>
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<td>1</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>-</td>
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<td>-</td>
<td>1</td>
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<td>-</td>
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<td>-</td>
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<td>1</td>
</tr>
<tr>
<td>Follicular neoplasm</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31</td>
<td>5</td>
<td>1</td>
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<td>-</td>
<td>41</td>
</tr>
<tr>
<td>Hurthle cell tumour</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>4</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>41</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>47</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
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<td>1</td>
<td>-</td>
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<td>-</td>
<td>1</td>
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<tr>
<td>Anaplastic carcinoma</td>
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<td>11</td>
<td>11</td>
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<tr>
<td>Squamous cell carcinoma</td>
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<td>-</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2</td>
<td>228</td>
<td>9</td>
<td>1</td>
<td>8</td>
<td>37</td>
<td>5</td>
<td>8</td>
<td>49</td>
<td>1</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>361</td>
</tr>
</tbody>
</table>

N: Normal
HT: Hashimoto’s thyroiditis
FA: Follicular adenoma
HCT: Hurthle cell tumour
FC: Follicular carcinoma
MC: Medullary carcinoma
AC: Anaplastic carcinoma
SCC: Squamous cell carcinoma
NHL: Non-Hodgkin lymphoma

Table 2. Results of FNA cytology with subsequent histopathological diagnosis.
Table 3. Summary of cyto-histopathological correlation of all 361 cases

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Histology</th>
<th>Benign</th>
<th>Malignant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td></td>
<td>245 (96.8%)</td>
<td>8 (3.2%)</td>
<td>253</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td>4 (6.5%)</td>
<td>57 (93.4%)</td>
<td>61</td>
</tr>
<tr>
<td>Inconclusive</td>
<td></td>
<td>41 (87.2%)</td>
<td>6 (12.8%)</td>
<td>47</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>290 (80.3%)</td>
<td>71 (19.7%)</td>
<td>361</td>
</tr>
</tbody>
</table>

TN: True negative, FN: False negative
TP: True positive, FP: False positive

Table 4. Calculations of diagnostic accuracy, sensitivity, specificity, negative and positive predictive values

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Formula</th>
</tr>
</thead>
</table>
| Accuracy                     | \[
|                             | \frac{TP + TN}{TP + TN + FP + FN} \times 100     |
|                             | \frac{57 + 245}{57 + 245 + 4 + 8} \times 100     |
|                             | = 96.2%                                          |
| Sensitivity                  | \[
|                             | \frac{TP}{TP + FN}                             |
|                             | \frac{57}{57 + 8} \times 100                    |
|                             | = 87.7%                                          |
| Specificity                  | \[
|                             | \frac{TN}{TN + FP}                             |
|                             | \frac{245}{245 + 4} \times 100                  |
|                             | = 98.4%                                          |
| Positive Predictive Value    | \[
|                             | \frac{TP}{TP + FP}                             |
|                             | \frac{57}{57 + 4} \times 100                    |
|                             | = 93.4%                                          |
| Negative Predictive Value    | \[
|                             | \frac{TN}{TN + FN}                             |
|                             | \frac{245}{245 + 8} \times 100                  |
|                             | = 96.8%                                          |

DISCUSSION

For thyroid FNA cytology to be clinically useful, a satisfactory sample must be obtained. Smears from thyroid aspirates are considered satisfactory when the material is representative of the lesion, adequate in quantity and the cytological preparation is excellent. Our unsatisfactory rate is 20.7% which is on the high side compared with other previous studies which reported unsatisfactory rates between 0 to 25% (Ramachandra et al 1995). This could be attributed to the aspirations being performed by Trainee Registrars, who had limited experience during the initial part of their training. The quality of the smears usually improved as each student completed his or her three months cytology training. At our centre, a repeat FNA will be done for cases classified as inadequate. According to the Papanicolaou Society of Cytopathology Task Force, an acceptable rate of inadequate smears should be kept less than 15% (The Papanicolaou Society of Cytopathology Task Force 1996).

In this study, we obtained a sensitivity rate of 87.7% and a specificity rate of 98.4%, which gives a diagnostic accuracy.
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of 96.2%. Our results are comparable with several other studies which reported sensitivity rates between 65% to 98%, specificity rates between 72% to 100% (Sanggali et al 2006) and diagnostic accuracy rates between 70% to 90% (Vojvodich et al. 1994). The sensitivity, specificity and diagnostic accuracy depend on the experience of the person performing the aspiration and the cytopathologist interpreting the smear, the statistical method employed and whether the follicular lesions were included or not; and if they were included whether they were classified as benign or malignant. In our analysis, we did not include the cases classified as inconclusive which represent follicular neoplasms and Hurthle cell tumours, because the objective of this study is to calculate the diagnostic accuracy of fine needle aspiration cytology in thyroid lesions at our centre rather than its impact on clinical management.

Our false negative rate is 3.2%. This figure is in agreement with the other studies which reported false negative rates between 1-11% (Sanggali et al 2006). False negative rate is a concern because a missed malignant lesion will result in dire consequences for the patient. Review of these cases showed that most of them were due to sampling errors especially in the case of a cystic lesion where papillary carcinoma was missed. One case of follicular carcinoma was cytologically diagnosed as goitre (Table 2). This was also considered as sampling error because the relative abundance of cellular material lacking in microfollicular pattern in a background of moderate amount of colloid as seen in this case would inevitably render a cytologic diagnosis of hyperplastic goitre. After all, one has to make diagnosis based on the available cytologic material and according to standard criteria. We feel that in these cases, radiology-guided fine needle aspiration may reduce this problem in some of these cases. Clinical correlation is strongly recommended in cases where suspicion of malignancy is high but cytologically reported as benign. For these cases, we recommend a repeat aspiration or radiologically-guided aspiration. Therefore, communication between the clinician and the cytopathologist is extremely important.

There were four false positive cases giving a rate of 6.6%. The reported false positive rates in the literature vary from 0.3% to 10% (Suresh et al 1995). This was not considered a major problem because these patients would have been operated based on other clinical parameters. Three goitres were cytologically diagnosed as papillary carcinoma of the thyroid. Review of these cases showed that these were due to interpretative errors. In these cases, diagnosis of papillary carcinoma of the thyroid was offered due to hypercellularity of the specimens together with scant amount of colloid and the occasional presence of longitudinal grooves accompanied by intranuclear pseudo-inclusion-like features. The other false positive case was a follicular adenoma misdiagnosed as papillary carcinoma of the thyroid. Because longitudinal grooves and intranuclear pseudo-inclusions are occasionally observed in other thyroid lesions, Cibas (Cibas et al 2003) stressed the significance of pale, powdery chromatin pattern in papillary carcinoma (Figure 1) as a helpful distinguishing feature from the coarse chromatin pattern seen in follicular lesions (Figure 2). Awareness by the cytopathologists of this problem should reduce this particular diagnostic error in future.

Finally, even though we excluded the inconclusive cases from our calculations, it was interesting to note that we correctly assigned 36 (87.8%) out of 41 cases diagnosed as follicular neoplasms. Histological diagnosis confirmed 31 cases of follicular adenoma and five cases of follicular carcinoma. Despite the extensive work done on separating follicular adenoma and carcinoma on cytological basis, only marginal success has been achieved. Molecular technique is another potential area which may help resolve this problem.
in future. Molecules like CD44v6 and Galectin 3 seem to be promising markers to detect neoplastic thyroid epithelial cells (Matesa et al 2002, Inohara et al 1994, Ermac et al 1995). However, more work and results are needed before they can be routinely used clinically. One of the follicular neoplasms turned out to be a follicular variant of papillary carcinoma of the thyroid. This mistake is not common due to the presence of overlapping follicular and papillary features. Careful assessment of the nuclear cytological features is important to avoid future mistakes in diagnosis of this variant.

In conclusion, results of our study showed that our sensitivity, specificity and diagnostic accuracy were high, thus confirming the important role of fine needle aspiration cytology as the initial diagnostic utility in the management of thyroid nodules. Sampling error was the major cause of our false negativity, which may be reduced by incorporating the other clinical parameters as index of suspicion of malignancy and utilizing radiology-guided aspiration.

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