Comparison between Sonographic Features and Fine Needle Aspiration Cytology with Histopathology in the Diagnosis of Solitary Thyroid Nodule

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Abstract

Background: High resolution ultrasonography (USG) is the first-line investigation in evaluation of euthyroid nodules. Thyroid imaging reporting and data system (TIRADS) is an USG-based risk stratification system for classifying thyroid nodules. Subjects with high-risk category of TIRADS undergo fine needle aspiration cytology (FNAC) and FNAC findings are reported according to Bethesda classification. Bethesda categories are used for determining risk of malignancy. Data regarding sonographic classification of thyroid nodule and its cytological association with respect to final histopathological diagnosis remains scarcely available in India. Aims and Objective: The study evaluated euthyroid nodules for risk of malignancy and compared sonographic features and FNAC (Bethesda classification) findings with histopathology of excised samples. Material and Methods: This was a single-center observational study on 137 consecutive subjects of solitary euthyroid nodule. All subjects underwent USG according to TIRADS and FNAC where applicable. Surgical biopsy report was used as a gold standard. Results: The sensitivity, specificity, accuracy, positive predictive and negative predictive value of FNAC were 80%, 90%, 85%, 86%, and 86.6% and TIRADS were 80%, 47.2% 61%, 51.3%, and 77.3%, respectively. FNAC classification was equally sensitive and more specific than TIRADS. Among individual USG parameters, micro-calcification was most sensitive (80%) and specific (86%). Irregular margin and taller-than-wider shape had a specificity of 89% and 92%, respectively. 3 patients (14.28%) with benign cytology and suspicious USG features (specifically TIRADS 4 & 5) undergoing surgery had malignancy in final HPE. Conclusions: USG and FNAC are equally sensitive in diagnosing malignant thyroid nodule but FNA is more specific (90%). It’s a minimally invasive method which can be used to distinguish malignant from benign lesions with a high degree of accuracy (85%). In patient having high risk feature on USG, a benign cytology needs to be repeat FNAC and they should undergo surgical biopsy for confirmation.

Keywords: Fine needle aspiration cytology, histopathology, thyroid swelling, TIRADS

INTRODUCTION

Thyroid nodule is defined as a focal well-defined area of altered echogenicity within thyroid gland that is radiologically distinct from surrounding normal thyroid parenchyma.[1] Worldwide, thyroid nodule occurs with relatively high frequency in general population with an estimated prevalence of 4–8% by palpation alone and 19–67% by ultrasound examination. In India, thyroid nodules are seen in about 8.5% of the population.[2] An increase in the incidence of thyroid carcinoma has been noted in the recent decades due to markedly improved USG surveillance and USG-guided FNAC of thyroid nodules.

Majority of thyroid nodules are benign, but malignancy is found in approximately 5–15% of cases, high risk features like age, sex, radiation exposure history, family history, and

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other factors warrants further evaluation.\cite{3-5} Epidemiological studies have shown that thyroid nodules are more common in women. Despite their low prevalence in men, nodules are more aggressive with higher risk of malignancy.\cite{6}

Evaluation of a patient with thyroid nodule requires detailed history and imaging. High resolution ultrasonography (USG) is the first line investigations in clinically detected thyroid nodules who are biochemically euthyroid. Thyroid imaging recording and data system (TIRADS) is a risk stratification system for classifying thyroid nodules similar to BIRADS scoring for breast lesions. It was first proposed by Horvath \textit{et al.}\cite{8} in the year of 2009 with modified recommendation from Kwak JY \textit{et al.}\cite{9} Recently, thyroid nodules have been classified into 5 TIRADS categories based on 5 descriptors (composition, echogenicity, shape, margin, echogenic foci/calcification). Each descriptor gives a point, adding all points of all descriptors a numerical value is calculated which gives the TIRADS score. Sonographic findings suggestive of malignancy are solid nodules, hypoechogenicity, irregular margins, microcalcifications, and a shape taller than wide on a transverse view.

Fine needle aspiration cytology (FNAC) is considered as an essential tool in providing a rational approach to the clinical management of thyroid nodules and determines the correct surgical procedure when surgery is needed. Similar to other clinical tests in medicine, it is expected that thyroid FNA should demonstrate high degree of sensitivity and specificity. Therefore, it is prudent that thyroid FNA reporting should be close to uniform among pathologists to give the path for rational management strategies and avoid confusion among clinicians.\cite{9,10} According to current standards of thyroid cytopathology, Bethesda classification is used for determining proper localization of nodule. Thyroid is a superficial structure and thus easily accessible to invasive and noninvasive procedures. USG-guided FNA has been done routinely for proper localization of nodule.

Studies have been done worldwide regarding stratification of risks of malignancy in subjects with thyroid nodule by ultrasound and cyto pathological examination. Kwak \textit{et al.}\cite{9} have proposed a TIRADS score by retrospective analysis of thyroid nodules in ultrasound and FNA, using five ultrasound criteria that can be used during thyroid evaluation. This article describes that a malignancy risk of\cite{11} 0% is expected for TIRADS 2, 1.7% for TIRADS 3, a risk of 3.3–72.4% for TIRADS 4, and of 87.5% for TIRADS 5. Srinivas \textit{et al.}\cite{12} also concluded that the risk of malignancy for TIRADS categories 1, 2, 3, 4A, 4B, 4C, and 5 was 0, 0, 0.64, 4.76, 66.67, 83.33, and 100%, respectively. But still data regarding sonographic classification of thyroid nodule and its cytological correlation remains scarcely available in India.

In this background, the aim of this study was to compare high resolution USG by TIRADS scoring and cytological diagnosis by Bethesda scoring with histopathological diagnosis in subjects with solitary thyroid nodule. We evaluated all consecutive subjects of solitary thyroid nodules by TIRADS Scoring, and cytopathology (if done) by Bethesda classification and compared the TIRADS and Bethesda classification with final diagnosis as reported by HPE (gold standard) in those who had excisional biopsy done. The sensitivity, specificity, PPV, and NPV of TIRADS and Bethesda scoring in detecting malignancy (confirmed by HPE) were also evaluated.

**Materials and Methods**

This was a single center observational study in which 150 patients of was recruited of which 137 subjects with thyroid nodules giving informed consent for this study were selected. Demographic and other clinical data were collected from the subjects according to specified protocol and all subjects with clinically detected nodule underwent thyroid function tests.

Serum TSH and free T4 were estimated by chemiluminescence technique (CLIA) using commercially available kits from Siemens Diagnostics (Germany) with Immulite-1000 analyzer. The analytical sensitivity and total precision values (as given by the providers) for TSH were 0.01 µIU/ml and 2.2%, respectively, and for free T4 assays were 0.35 ng/dl and 2.7%, respectively. The laboratory reference ranges for TSH was 0.4–4 µIU/ml, for free T4 was 0.8–1.9 ng/dl and the inter-assay coefficients of variation (CV) for the assays were 8.9% and 5.5%, respectively (as determined locally).

Those who had normal TSH and free T4 levels were included in the study and they subsequently underwent high resolution USG and USG-guided FNAC. All USG were performed by one of two dedicated persons who were trained in the subject. Ultrasound report was prepared according to the TIRADS Score. TIRADS 1: Benign (No FNA), TIRADS 2: Not Suspicious (No FNA), TIRADS 3: Mildly Suspicious (FNA if ≥2.5 cm and follow-up if ≥1.5 cm), TIRADS 4: Moderately Suspicious (FNA if ≥1.5 cm and follow-up if ≥1 cm), and TIRADS 5: Highly Suspicious (FNA if ≥1 cm and follow-up if ≥0.5 cm). Then USG-guided FNAC done from thyroid nodules if indicated.

Cytology reports were prepared according to Bethesda classification as Bethesda 1 (non-diagnostic), Bethesda 2 (Benign), Bethesda 3 (AUS/FLUS), Bethesda 4 (folicular neoplasm), Bethesda 5 (suspicious for malignancy), and Bethesda 6 (malignant). All FNA slides were examined by a single cytopathologist oriented about the protocol and nature of the study.

Of the total 137 subjects with thyroid nodules, 61 underwent surgical biopsy, and hence histopathological examination. Evaluation of all histopathology slides was done by a single pathologist. Out of 61 biopsy proved subjects, 21 with “Bethesda 2” cytology and thus having a benign etiology were also operated due to increasing size, compressive symptoms as described by patient or due to cosmetic
De, et al.: TIRADS versus Bethesda staging in histopathology proved thyroid nodule

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Written informed consent was taken from all subjects. The study was approved by Institutional Ethics Committee of Institute of Post Graduate Medical Education and Research. Kolkata, West Bengal, India.

Statistical analysis
All the data collected was compiled in MS Excel. Appropriate statistical methods like Fisher exact test and Chi-square test were used. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each of major ultrasound features that highly suggest malignancy (irregular margin, taller than wider shape, presence of microcalcifications and hypoechogenicity). Odds ratio (risk estimates) were calculated and presented using 95% confidence interval (CI) statistic. The risk of malignancy of each TIRADS category and Bethesda category was determined with respect to histopathological reports. All statistical analysis was performed using the software IBM SPSS 20.0 and Graph Pad Prism 8.0.

Results
In this study, 150 subjects of thyroid nodules were recruited of which 137 subjects fulfilled our inclusion criteria. The basic demographic profile of solitary thyroid nodule is shown the [Table 1]. Mean age of subjects is 40.18 ± 13.64 years. Distribution pattern of solitary thyroid nodule in various age groups was 3.64% (5 patients) in age group between 18 and 20 years, 54% cases (i.e., 74 patients) were in between 21 and 40 years group, 24 (17.51%) cases were recorded in age group 41–50, and least in age group 51–60 with 34 cases, that is, 24%. Our study population had a female predominance with 119 (86.6%) female and 18 (13.1%) male subjects.

Table 1 also shows the percentages of subjects in different TIRADS category and percentage of subjects with different FNA finding as per Bethesda classification. TIRADS 3 was the most common category. Subjects with TIRADS categories 3, 4, and 5 underwent USG-guided FNAC. Of total 137 subjects, 61 had undergone surgery with 36 subjects having benign pathology and 25 having thyroid malignancy on HPE.

Table 2 shows final histopathological diagnosis in different categories of USG findings as per TIRADS. The risk malignancy increased with the TIRADS categories 3–5. The incidence of malignancy in solitary thyroid nodule was more in females. Out of 25 malignant patients 8 were males (32%) and 17 were females (68%).

Preoperative investigation of TIRADS score with histopathological findings is shown in Table 3. Combining TIRADS 4 and 5 (Moderately and highly suspicious lesion) as probably malignant US findings, and TIRADS 3 as probably benign US findings and the sensitivity, specificity, PPV, and NPV were respectively 80%, 47.2%, 51.28%, and 77.27%. The overall accuracy of ultrasound was 61%. The risk of malignancy in our study for TIRADS 3, TIRADS 4, and TIRADS 5 were 22.7%, 29.16% and 86.66%, respectively.

From this data it is clear that USG is a good initial screening test but has poor specificity.

On analysis of this subgroup of subjects using HPE as the gold standard, the individual parameters of TIRADS like shape, echogenicity, and presence of microcalcifications were statistically significant, and serves to differentiate benign from malignant nodule. The nodule margin and consistency were not statistically significant. Major ultrasound features according to TIRADS Score are shown in Table 4. Sensitivity, specificity, PPV, NPV, OR, and likelihood ratio were calculated for each feature and tabulated in Table 5. Combining TIRADS 4 and 5 together, a sensitivity of 80% in diagnosing thyroid cancer was documented. Table 5 show the different statistical analysis of the major ultrasound features suggestive of malignancy with respect to histopathological reports, and their respective performance. Major ultrasound features like microcalcification is highly sensitive (80%) and specific (86.11%) parameter, taller than wider shape is highly specific (92%) but low sensitivity (36%) parameter, hypoechogenicity is also specific (78%) but not very sensitive (68%) parameter and irregular margin is highly specific (89%) but not sensitive (28%) in differentiation of malignant and benign thyroid nodule.

A total of 61 out of 137 cases (44.5%) underwent surgery after FNA procedures, which included 20 benign (Bethesda 2), 1 non-diagnostic (Bethesda 1), 14 AUS/FLUS(Bethesda 3), 12 FN/SFN (Bethesda 4), and 14 were suspicious for malignancy (Bethesda 5). In the “indeterminate” cytological categories of Bethesda 3, out of 14 cases, 10 were benign and 4 malignant (3 were papillary thyroid carcinoma and 1 was follicular thyroid carcinoma) in histopathological diagnosis. Out of 12 cases of Bethesda 4 cytology, 4 were papillary thyroid carcinoma, 1 was minimally invasive follicular carcinoma, 2 follicular carcinoma, and 5 were benign by histopathology.

In Bethesda 3 category we had 14 patients. Out of those 14 samples 4 were malignant. 2 subjects with malignant histopathology were TIRADS 4, 1 subject with malignant histopathology as TIRADS 3, and 1 subject with malignant histopathology was TIRADS 5 by sonography. TIRADS did not help in the prediction of malignancy in patients with Bethesda 3.

Considering Bethesda 3 and 4 as indeterminate group we had 26 patients with an indeterminate cytology. In these patients, TIRADS has shown little benefit in influencing the final diagnosis. Only TR 5 had some predictive value in diagnosing malignancy.

Out of 14 cases of suspicious for malignancy (Bethesda 5), 10 cases were papillary thyroid carcinoma and 2 cases were diagnosed as follicular carcinoma by histopathology, whereas 2 were benign. Table 6 shows comparison between Bethesda
Table 1: Baseline characteristics of demographic, clinical, radiological, cytological and histopathological features

| Clinical Features          | Sub-features                  | No. (%)  |
|----------------------------|-------------------------------|----------|
| Composition                | Completely solid              | 119 (86.86%) |
|                            | Mixed solid & cystic          | 18 (13.13%) |
| Echogenicity               | Hyperechoic                   | 62 (45.25%) |
|                            | Isoechoic                     | 51 (37.22%) |
|                            | Hypoechoic                    | 24 (17.51%) |
| Calcification              | None or comet-tail artifacts  | 95 (69.34%) |
|                            | Macro calcification           | 20 (14.59%) |
|                            | Micro calcification           | 14 (10.21%) |
|                            | Rim calcification             | 8 (5.83%)  |
| Shape                      | Wider than taller             | 123 (89.78%) |
|                            | Taller than wider             | 14 (10.21%) |
| Margin                     | Smooth                        | 118 (86.13) |
|                            | Ill-defined                   | 6 (4.37)  |
|                            | Lobulated/irregular           | 13 (9.48)  |
| TIRADS Score               | TIRADS 1 (Benign)             | No FNA   |
|                            | TIRADS 2 (Not suspicious)     | No FNA   |
|                            | TIRADS 3 (Mildly suspicious)  | 91 (64.96) |
|                            | TIRADS 4 (Moderately suspicious) | 29 (22.62) |
|                            | TIRADS 5 (Highly suspicious)  | 17 (12.40) |
| BETHESDA Classification    | BETHESDA 1 (Non-diagnostic)   | 4 (2.91)  |
|                            | BETHESDA 2 (Benign)           | 85 (62.04) |
|                            | BETHESDA 3 (AUS/FLUS)         | 20 (14.59) |
|                            | BETHESDA 4 (Follicular neoplasm) | 13 (9.48)  |
|                            | BETHESDA 5 (Suspicious for malignancy) | 14 (10.21) |
|                            | BETHESDA 6 (Malignant)        | 1 (0.72)  |
| Surgical Histopathology (n=61) | Benign               | 36 (59.01) |
|                            | Malignant                     | 25 (40.98) |

Table 2: Proportion of malignancy as per TIRADS Score

| TIRADS Score | Histopathology | Total | Risk of malignancy (%) |
|--------------|----------------|-------|------------------------|
|              | Malignant      | Benign|                        |
| 3            | 5              | 17    | 22                     |
| 4            | 7              | 17    | 24                     |
| 5            | 13             | 2     | 15                     |
| Total        | 25             | 36    | 61                     |
|              | 40.98%         |       |                        |

Table 3: Comparison of TIRADS with risk of malignancy

| Pre-operative investigation | Histopathology | P   |
|-----------------------------|----------------|-----|
| Conventional USG            | Malignant      | Benign|       |
| TIRADS 4.5                  | 20 (58.3%)     | 19 (48.7%) |         |
| TIRADS 3                    | 5 (22.7%)      | 17 (77.3%) | 22 (36%) | 0.03 |
| Total                       | 25 (41%)       | 36 (59%) | 61 (100%) |       |

classifications with histopathological reports. The sensitivity, specificity, PPV, and NPV were respectively 80%, 90%, 86%, and 86%. The overall accuracy of FNAC was 85%. According to histopathological report, the risk of malignancy in case of indeterminate thyroid nodule (Bethesda 3 and 4) was 38.46%. We had 21 patients with Bethesda 2 of whom 3 harbored malignancy (papillary thyroid carcinoma) on HPE and rest were benign. In one case of malignancy TR 5 was suggestive of high risk, but other two cases were having TR 3. Despite an initial Bethesda 2 on FNAC, high risk features on USG and a higher TIRADS score compelled us to repeat this apparently benign cytology or to undergo a surgical biopsy for confirmation.

**Discussion**

Euthyroid nodule was commonly seen in females compared to males in this study, suggestive of female predominance and was almost 86.86% of total study population (N = 137). This is in accordance with earlier studies,[13] in which aprevalence of thyroid nodule in females was 86% (N = 50).

In the present study highest number of cases were reported in the 21–40 years age group (74 cases, 54%), followed by 51–60 years (34 cases, 24%). The results were in accordance to reports published earlier,[13] in which they noted that majority of the cases were between 21 and 40 years age group (80%, N = 50).

Kwak et al.[8] have proposed a TIRADS score by retrospective analysis of thyroid nodules in ultrasound and FNA, using five ultrasound criteria that can be used during thyroid evaluation.
This article describes that a malignancy risk of 0% is expected for TIRADS 2, 1.7% for TIRADS 3, a risk of 3.3-72.4% for TIRADS 4, and of 87.5% for TIRADS 5. Our study has shown 22.72% malignancy risk for TIRADS 3. The risk of malignancy in our study for TIRADS 4 and TIRADS 5 were 29.16% and 86.66%, respectively. According to another Indian study by Srinivas et al.,[12] it was concluded that the risk of malignancy for TIRADS categories 1, 2, 3A, 3B, 4A, 4B, 4C, and 5 was 0, 0, 0.64, 4.76, 66.67, 83.33, and 100%, respectively. Our results are within the range suggested by Kwak et al.[8] and two other studies based on Indian population [Table 2]. The sensitivity, specificity, PPV, and NPV of TIRADS versus histopathology were respectively 80%, 47.2%, 51.28%, and 77.27%. The overall accuracy of ultrasound was 61%.

It is intriguing that a malignancy rate of 22.7% was found in subjects with TR 3 in our study. We have used the latest ACR TIRADS criteria 2017. Other studies have used previous versions of TIRADS classification (ACR TIRADS 2009, K-TIRADS 2017 and EU-TIRADS 2017). A study using the latest ACR TIRADS 2017 criteria conducted by Barbosa et al.[11] reported a percentage of malignancy of 23.3% in subjects with TR 3. Similar finding is reported in our study also. As per ACR TIRADS criteria 2017, the four possible scenarios classified as TR3 are as follows: (i) solid and hyperechoic; (ii) solid and isoechoic; (iii) mixed solid cystic and hyperechoic with microcalcification; (iv) mixed solid cystic and hypoechoic. This might explain the higher rate of malignancy in TR3, as individual features like macrocalcification or hyperechoic nature of the nodule are not included as possible features to predict malignancy in previous systems. This might be one of the drawbacks of ACR TIRADS 2017.

Paradoxically, the specificity of TIRADS score in this study was only 47%. According to latest guidelines of TIRADS,[14] a patient with a solid nodule (2 points) which is hyperechoic (1 point) and having macrocalcification (1 point) is labelled as TIRADS 4. Sixteen such subjects in our study had a benign cytology on HPE. So this can be a limitation of the current TIRADS scoring.

The limitation of FNAC includes false-negative result and false positive results. A comparative study was done by Bloch[15] between FNAC and histopathology and found that the accuracy of FNAC was 91.6%. Handa et al.[16] have a similar study in which FNAC revealed a sensitivity of 97%, specificity 100% a PPV of 96% and a NPV of 100%. Mundasad et al.[17] had done similar study and identified that FNAC had a sensitivity (52.6%), specificity (86.6%) and accuracy (79.1%) for thyroid malignancy. According to histopathological diagnosis the risk of malignancy was calculated in case of indeterminate thyroid nodule (Bethesda 3 and 4) was 38.46%. In our study sensitivity of FNAC was 80%, specificity was 90%, positive and negative predictive value was 86%, and the overall diagnostic accuracy was 85%.

**Table 4: Major Ultrasound features and histopathology results**

| Major ultrasound Features | Histopathology (n=61) | Total | \( P \) |
|---------------------------|-----------------------|-------|-------|
|                            | Malignant (n=25)      | Benign (n=36) |       |
| Taller than Wider          | 9                     | 3     | 12    | 0.01 |
| Present                   | 16                    | 33    | 49    |      |
| Hypoechoegenecity          | 17                    | 8     | 25    | <0.01|
| Present                   | 8                     | 28    | 36    |      |
| Microcalcification         | 20                    | 5     | 25    | 0.0001|
| Present                   | 5                     | 31    | 36    |      |
| Irregular margin           | 7                     | 4     | 11    | 0.17 |
| Absent                    | 18                    | 32    | 50    |      |

**Table 5: Diagnostic attributes of individual USG finding**

| Features                  | Sensitivity | Specificity | PPV   | NPV   | OR (95%CI) | Likelihood Ratio |
|----------------------------|-------------|-------------|-------|-------|------------|------------------|
| Taller than wider          | 36%         | 92%         | 75%   | 67.4% | 6.19 (1.57 to 22.78) | 4.32 |
| Hypoechoegenecity          | 68%         | 78%         | 68%   | 77%   | 7.44 (2.47 to 22.61) | 3.06 |
| Microcalcification         | 80%         | 86.11%      | 80%   | 86%   | 24.80 (6.129 to 86.37) | 5.76 |
| Irregular margin           | 28%         | 89%         | 64%   | 64%   | 3.11 (0.78 to 10.33) | 2.52 |

**CONCLUSION**

We can conclude that FNAC and TIRADS both are highly sensitive (80%) but FNAC is more specific (90%) and accurate test (85%) in identifying thyroid cancer. Among individual USG parameters, microcalcification was most sensitive (80%) and specific (86%). Irregular margin and taller-than-wider shape had a specificity of 89% and 92%, respectively. In patient having high risk feature on USG (TIRADS 5), a benign cytology dose not completely rule out risk of malignancy and they should undergo surgical biopsy for further confirmation.

A benign FNAC diagnosis should be viewed with caution as false-negative results do occur and these subjects should be followed up and any clinical suspicion of malignancy even in the presence of benign FNAC requires surgery. USG features like taller-than-wide and irregular margins are specific for malignancy but have poor sensitivity. The suspicious indeterminate results prove to be an area of uncertainty which can be resolved by surgical resection and biopsy.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate
participant consent forms. In the form, the participants have given their consent for clinical information to be reported in the journal. The participants understand that their names will not be published and due efforts will be made to conceal their identity.

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Conflicts of interest
There are no conflicts of interest.

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Table 6: Comparison of FNAC with histopathology

| Fine-needle aspiration assay | Malignant | Benign | Total |
|-----------------------------|-----------|--------|-------|
| BETHESDA 5,6                | 12 (86%)  | 2 (14.28%) | 14 (4%) |
| BETHESDA 2                  | 3 (14.28%) | 18 (86%) | 21 (6%) |
| Total                       | 15 (43%)  | 20 (57.14%) | 35 (100%) |