Original Article

Serum TSH Level as a Simple Efficient Tool to Assess the Risk of Thyroid Malignancy in Euthyroid Patients with Indeterminate Cytology - A Cohort Study

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Abstract

Context: A significant number of fine-needle aspiration cytology (FNAC) for thyroid nodules is reported as indeterminate. Expensive molecular testing can give a clue to the possibility of malignancy in this group. The effectiveness of serum thyroid-stimulating hormone (TSH) levels as a diagnostic tool in euthyroid patients with indeterminate cytology has not been previously studied, especially in the Indian population. **Aims:** This study was conducted to evaluate the predictive efficacy of serum TSH in the early diagnosis and treatment of malignancy. **Settings and Design:** This is a retrospective cross-sectional study on a cohort of patients who presented to our department with complaints of thyroid swelling and underwent thyroidectomy. **Methods and Material:** Euthyroid patients who underwent thyroid surgery for newly diagnosed thyroid nodules with FNAC reported as indeterminate cytology were included in our study. Based on the histopathological report, the patients were divided into two groups and into quartiles based on TSH values. **Statistical Analysis Used:** The mean difference in the numerical variables between groups was compared using the independent two-sample 't' test for parametric data and Mann–Whitney 'u' test for non-parametric data. A logistic regression analysis was done with age, sex, TSH level and nodule size as dependant variables and malignancy as the independent variable. **Results:** There were 211 patients in group A and 93 in group B. Patients with malignancy confirmed on final histopathology showed higher serum TSH levels compared to benign nodules (2.93 ± 1.067 vs 1.73 ± 1.051, $P = <0.001$). The mean TSH levels of all types of malignant nodules correlated with our test model (>2.185 mIU/L). **Conclusions:** Serum TSH above 2.185 mIU/mL is a good predictor of malignancy in indeterminate nodules. It is an inexpensive, safe and reliable diagnostic screening test for the risk of malignancy in an indeterminate nodule.

Keywords: Indeterminate nodule, malignancy, thyroidectomy, TSH

Introduction

Thyroid disorders are very common in India and thyroid nodules represent 12% of endocrine disorders seen at major referral centres in India.[1,2] These patients are subjected to fine-needle aspiration cytology (FNAC) reported using the Bethesda system for reporting thyroid cytopathology.[3] The sensitivity of FNAC to predict malignancy in thyroid nodules ranges between 67% and 98%.[4] Bethesda IV, V and VI report is suspicious, suggestive or confirmed malignancy, respectively, and therefore an indication for surgical removal of the thyroid gland.[5] With adequate treatment, a thyroid malignancy has the best long-time disease-free survival, approaching a cure for the condition.[6] However, thyroid surgery has a few rare but serious complications.[7] A dilemma in management occurs in those patients with FNAC reported as indeterminate cytology (Bethesda III).[5] Molecular testing in such situations may yield results. However, the high cost of the tests and false-negative results reduces their use in day-to-day clinical practice. There have been studies suggesting the usefulness of serum thyroid-stimulating hormone (TSH) as a

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predictor of malignancy in thyroid nodules but is deficient in the sub-group, indeterminate nodules. It has been shown that a higher serum TSH level is associated with a higher risk of thyroid malignancy.[8] This study is a retrospective analysis of patients diagnosed with thyroid nodules of indeterminate cytology and euthyroid status. We aimed to evaluate the predictive efficacy of serum TSH in the early diagnosis and treatment of malignancy.

Subjects and Methods

The study was conducted at a tertiary referral care centre in Southern India. A retrospective cross-sectional study on a cohort of patients who presented to our department with complaints of thyroid swelling and underwent thyroidectomy between January 2015 and January 2020 was done. The data was collected from the hospital database of the patients with thyroid nodules who have already undergone thyroidectomy.

The study was conducted following the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The data was collected over three months from May to July 2021 after obtaining ethical clearance from the Institutional Research Board (ECASM-AIMS-2021-226). The study was registered with the clinical trials registry of India with registration number CTRI/2021/10/037654. Informed consent had been obtained from all patients as a routine at admission for their enrolment into a study and for subsequent use of the data.

All euthyroid patients who underwent thyroid surgery for newly diagnosed thyroid nodules with FNAC reported as indeterminate cytology (Bethesda III) were included in our study.

Patients, other than Bethesda class III or those already diagnosed with thyroid malignancy were excluded from the study. Other exclusion criteria were patients on thyroid hormone supplementation and serum TSH level not within the euthyroid range.

A single fasting blood sample from patients was collected for measurement of serum TSH at their first visit to the outpatient department (OPD). Serum TSH level estimation was done using electrochemiluminescence immunoassay (ECLIA, Roche Diagnostica, So Paulo, Brazil) with a 3.01% interassay coefficient of variation. A serum TSH level between 0.4 and 4.5 mIU/L was considered to be within the normal range of laboratory values (The same reference interval was used for all age groups).

FNAC is done by a dedicated cytologist at the pathology department in our institution. The FNAC diagnosis was by the Bethesda classification of thyroid nodules and those nodules with indeterminate cytology were reported as Bethesda III. The thyroidectomy specimen was subjected to histopathological analysis and the final histopathology report was considered to be the reference standard for diagnosis and division of the sample into benign and malignant groups. The charts of these patients were reviewed and the data collected were tabulated into a data sheet. Observer bias in FNAC reporting was eliminated since this was a retrospective study, FNAC was done by a dedicated cytologist and all patients with indeterminate nodules were included in the study. Based on the histopathological report of the surgical specimen, the patients were divided into two groups - Group A (Benign disease) and Group B (Malignant disease) [Figure 1]. To further analyse the predictive role of serum TSH, the patients were then sub-divided into four equal quartiles (Q) based on the serum

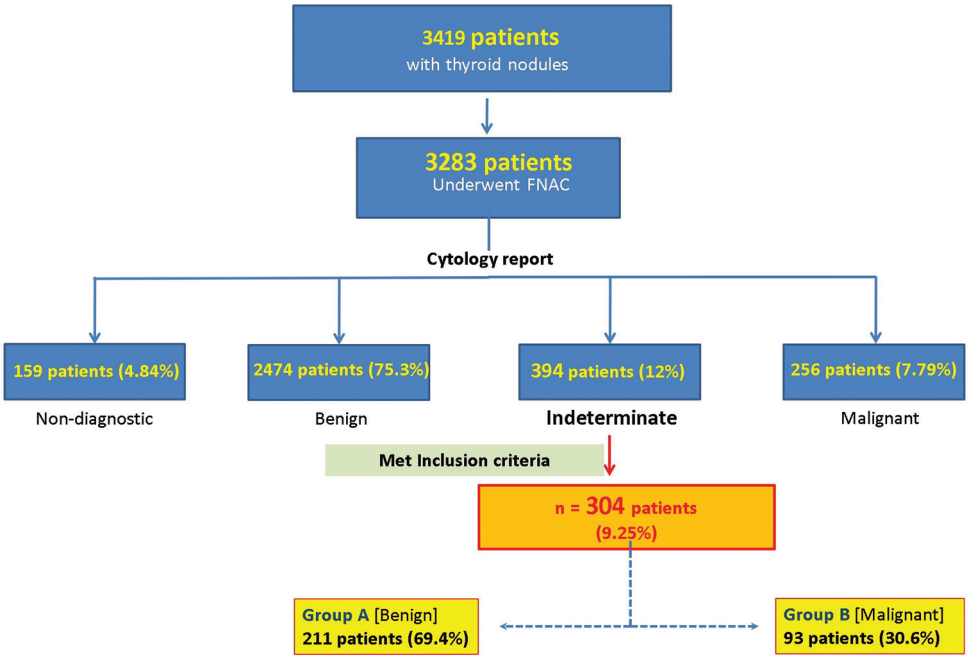

Figure 1: Flow diagram for selection criteria

TSH values (Q1: 0.4–1.42 mIU/L, Q2: 1.43–2.44 mIU/L, Q3: 2.45–3.46 mIU/L and Q4: 3.47–4.5 mIU/L).

The work has been reported in line with the STROCSS (Strengthening the reporting of cohort, cross-sectional and case-control studies in surgery) criteria.[9]

Statistical analysis

The medical records of the patients included in the study were reviewed and the data so collected were tabulated into a data sheet. Statistical analysis was done using IBM SPSS version 20.0 (SPSS Inc, Chicago, USA).

For all the continuous variables, the results are given in mean ± standard deviation (SD), and for categorical variables as a percentage. The mean difference in the numerical variables between groups was compared using the independent two-sample 't' test for parametric data and Mann–Whitney 'u' test for non-parametric data. To find out the association between two categorical variables, a Chi-square test was applied. Receiver operating characteristic (ROC) curve analysis was used to plot the true positive rate (sensitivity) in the identification of malignancy and the false positive rate (specificity) for different cut-off points of TSH. A logistic regression analysis was done with age, sex, TSH level and nodule size as dependant variables and malignancy as the independent variable. To train the model, 80% of the data was used and the remaining 20% data was used to test the model. A confusion matrix was created to test the data output and to calculate the sensitivity and specificity.

To compare the outcome (sensitivity, specificity, positive predictive value, negative predictive value and accuracy) of the two groups, the McNemar test was used. *P* value <0.05 was considered as statistically significant.

Results

In the period from January 2015 to January 2020, FNAC was done for 3,283 out of a total of 3419 patients that presented to our department with thyroid nodules. On further analysis of FNAC results, 159 (4.84%) lesions were reported as non-diagnostic, 2474 (75.3%) nodules were reported as benign, 394 (12%) nodules were reported as having indeterminate cytology and 256 (7.79%) nodules were reported as malignant.

Among the 394 cases having indeterminate cytology, 304 cases that underwent surgery, met our inclusion criteria and hence were taken into the study. There were 211 patients (69.4%) in group A and 93 patients (30.6%) in group B [Figure 1].

The demographics, mean TSH levels of the patients and comparison with histopathological characteristics are shown in Table 1. The mean age of the study population was 47.245 ± 13.5 years. The study population showed a female predominance (76.3%). The mean TSH value of the study group was 2.33 ± 1.059 mIU/L (within the euthyroid range) and the mean size of the nodule was 26.34 ± 11.66 mm. There was no significant difference in age and gender in comparing the two groups. Mean TSH was higher in group B and was statistically significant ($P < 0.001$) [Table 1].

When a box plot for the size of the nodule against the TSH level was plotted, we found no statistically significant difference [Figure 2a]. However, patients with malignancy confirmed on final histopathology showed higher serum TSH levels compared to benign nodules (2.93 ± 1.067 vs 1.73 ± 1.051, $P = <0.001$) [Figure 2b].

The distribution of patients into four quartiles based on serum TSH values is shown in Figure 3a. The first quartile had a low prevalence of malignancy with only 10.75% of malignancies falling into the first quartile while the last quartile showed a high prevalence of malignancy accounting for 46.23% of the malignancies. When benign cases were taken into consideration, their prevalence was higher in the first quartile [Figure 3a]. Quartile-based sub-categorisation analysis of benign lesions is shown in Figure 3b and of malignancies in Figure 3c.

Table 1: Demography, histopathology and mean TSH levels of the cohort

Characteristics	Benign	Malignant	*P*
Gender			
Female	166 (77.7%)	68 (73.1%)	0.335
Male	47 (22.3%)	25 (26.9)	
Age (years)	48.48±13.33	46.01±13.67	0.14
Nodule size (mm)	26.06±10.23	26.63±13.09	0.41
TSH levels (mIU/L)	1.73±1.051	2.93±1.067	<0.001

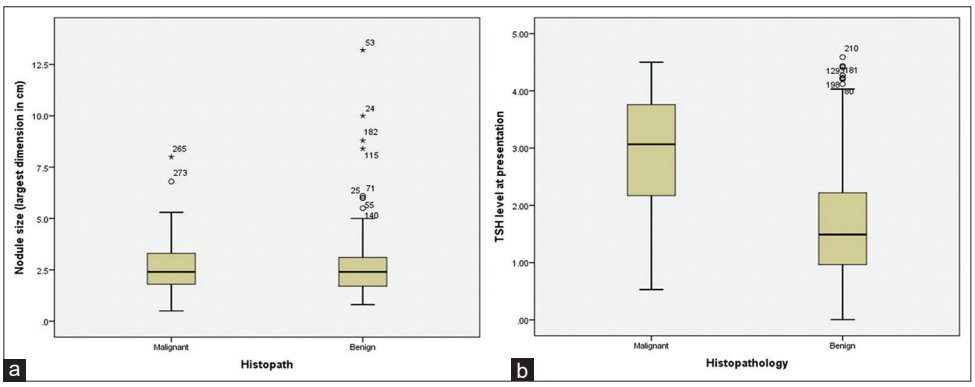

Figure 2: Box plot of size and histopathology vs TSH. (a) Box plot – Nodule size vs Histopathology. (b) Box plot – Serum TSH vs Histopathology

ROC curve analysis [Figure 4a] shows a sensitivity of 95.7% and specificity of 27.51% at a serum TSH value of 1.009. At a serum TSH value of 3.0, the sensitivity is 53.8% and the specificity is 84.45%. A TSH value of >/=2.185 mIU/L can identify patients having malignancy with a 74.19% sensitivity and 73.93% specificity. The model accuracy was found to be 0.75 (75%) which suggests that this is a good model for the prediction of malignancy [Figure 4b].

The different histopathological types of the benign and the malignant nodules were compared with their corresponding TSH levels and the mean TSH level was plotted as a histogram [Figure 5]. The distribution of different histological types of benign nodules was colloid nodules (45.49%), follicular adenoma (31.2%), nodular hyperplasia (10.42%), dominant nodule of multinodular goitre (7.58%) and hurthle cell adenoma (5.21%). The mean TSH levels of all types of benign nodules correlated with our test model (<2.185 mIU/L). An analysis within the group showed no statistically significant difference between the different histological types [Figure 5].

The distribution of different histological types of malignant nodules was papillary carcinoma (79.56%), follicular carcinoma (18.27%) and hurthle cell carcinoma (2.15%). The mean TSH levels of all types of malignant nodules also correlated with our test model (>2.185 mIU/L). An analysis within this group showed that the mean TSH levels of hurthle cell carcinoma (3.3934 ± 0.2945 mIU/L) were higher than papillary carcinoma (2.7838 ± 1.0913 mIU/L) and this result achieved statistical significance ($P < \mathbf{0.001}$). Though the mean TSH levels in follicular carcinoma (3.5533 ± 0.7304 mIU/L) were higher than those with papillary carcinoma (2.7838 ± 1.0913 mIU/L), this result was not found to be statistically significant ($P = 0.41$).

The significant association between the highest TSH quartile (Q4) and the increased incidence of malignancy was as per our study model [Figure 3b and c].

Discussion

Indeterminate nodules may be atypia of undetermined significance (AUS) or follicular lesions of undetermined

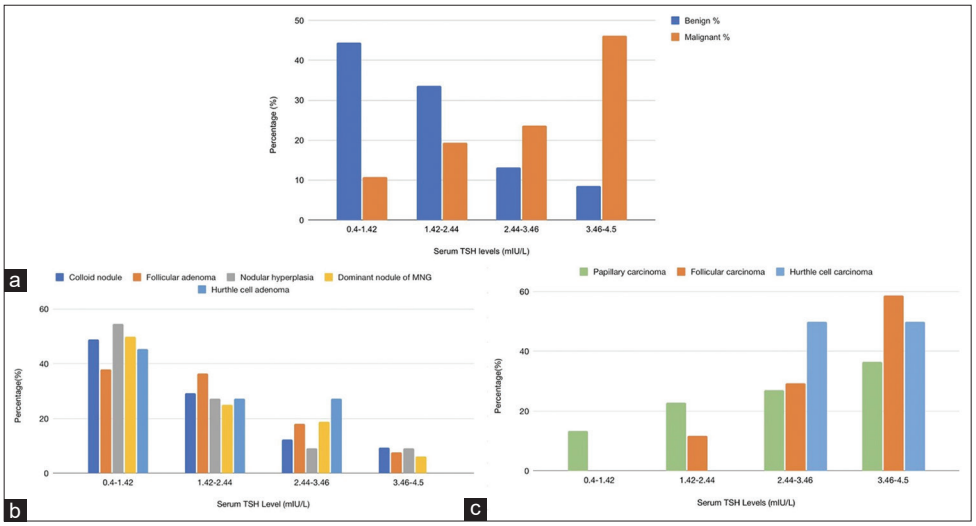

Figure 3: Quartile-based serum TSH distribution. (a) Quartile stratification of serum TSH. (b) Quartile distribution of TSH levels in benign and (c) malignant disease

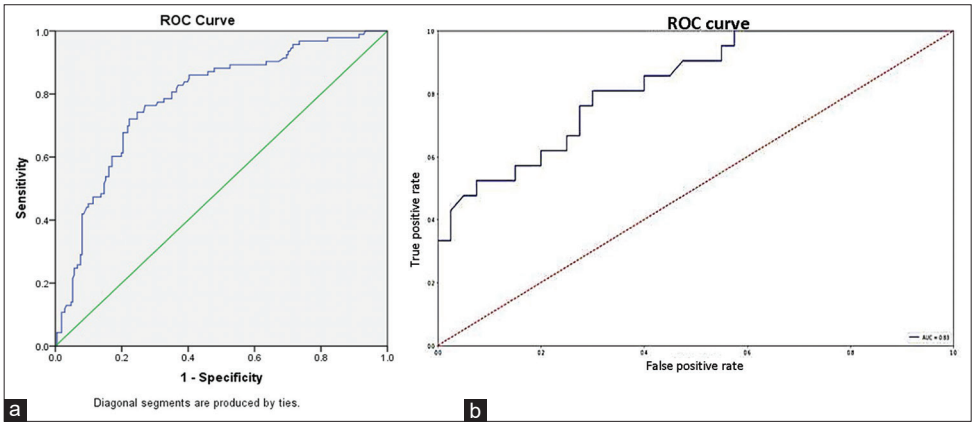

Figure 4: ROC curve and logistic regression analysis. (a) ROC curve analysis based on serum TSH level. (b) ROC curve analysis based on the logistic regression test model

	BENIGN NODULES	
Histopathology	Number of cases (%)	Mean TSH
Colloid nodule	96 (45.49%)	1.691 ± 1.082
Follicular adenoma	66 (31.27%)	1.854 ± 1.024
Nodular hyperplasia	22 (10.42%)	1.62 ± 1.048
Dominant nodule of MNG	16 (7.58%)	1.691 ± 1.008
Hurthle cell adenoma	11 (5.21%)	1.719 ± 1.043
Total	211 (100%)	**1.73 ± 1.051**

	MALIGNANT NODULES	
Histopathology	Number of cases (%)	Mean TSH (mIU/L
Papillary carcinoma	74 (79.56%)	2.7838 ± 1.0913
Hurthle cell carcinoma	2 (2.15%)	3.3945 ± 0.2945
Follicular carcinoma	17 (18.27%)	3.5533 ± 0.7304
Total	93 (100%)	**2.93 ± 1.067**

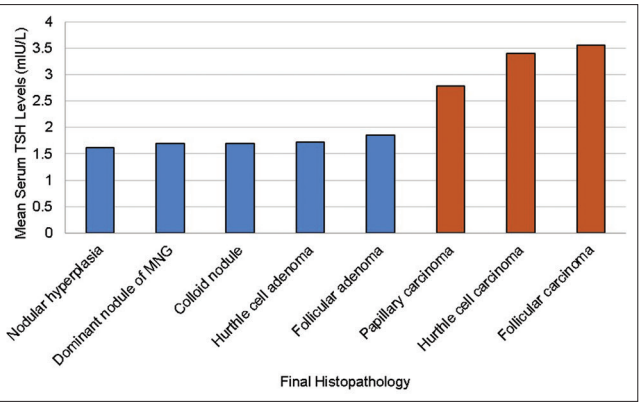

Figure 5: Comparison of mean TSH levels of different types of benign and malignant nodules

significance (FLUS) and are based on the architectural and cytological atypia in the FNAC sample.[5] In an indeterminate nodule, there is a 10–30% risk of malignancy, and is a reason for apprehension for both the surgeon and the patient. The problem with indeterminate nodules is that patients with benign diseases will undergo unnecessary surgery while a patient who truly has malignancy may not receive early and optimal treatment.

Over five years, our centre had a 12% incidence of indeterminate cytology from FNAC for thyroid nodules in 3283 patients. These results conform with results published elsewhere in the world, with the incidence of indeterminate nodules ranging from 10%–30% of all FNAC for thyroid nodules.[4,10]

The risk of malignancy is higher in older individuals with many studies showing a favourable prognosis in younger females (<45) and a higher risk of aggressive thyroid cancer in older males (>45).[11,12] In our series, the mean age of the patients with an indeterminate nodule having a confirmed malignancy was 46 years. However, there was no correlation of age or gender, for the risk of malignancy in an indeterminate nodule.

Studies show that there is a higher risk of follicular and rare variants of cancer being detected from nodules of large size.[13] However, when indeterminate nodules alone are considered, size does not seem to affect the rate of cancer detection.[14] There was no correlation between the size of the nodule and the risk of malignancy in indeterminate nodules in our series of patients.

A significant relation was found between serum TSH levels and the risk of malignancy in euthyroid patients with thyroid nodules having indeterminate cytology in our study. The role of TSH as a thyroid cell growth factor is well established. The significance of serum thyrotropin levels in thyroid cancer growth and progression is still debated.[8]

The mean serum TSH value of patients with benign nodules was 1.73 ± 1.051. Among these 2474 patients, 2343 patients had a TSH value below 2.185, while the remaining 131 had a value slightly more than 2.185. Hence, if we take serum TSH level as the only deciding criteria for thyroidectomy, then 131 patients with benign nodules (5.29%) will undergo thyroidectomy.

The development of thyroid malignancy can be affected by TSH-stimulated signalling pathways. Multiple receptors and pathways are affecting the development, progression, invasion and metastasis of thyroid cancers. Src family kinase (SFK), Janus kinase (JAK)-signal transducer and thyroid-stimulating hormone receptor (TSHR) are a few of the important signalling pathways in thyroid cancer. TSH is a key thyroid cell growth factor, and thyrotropin-activated signalling pathways are known to play a role in thyroid carcinogenesis. Each of these pathways is interlinked and can independently affect thyroid cells or can affect the other thyroid pathways. Any flaw in one of these pathways can lead to the development of and progression to invasion and metastasis in thyroid cancer.[15-17]

In the therapy of thyroid cancers, TSH suppression plays an important role.[14] However, the role of TSH as a diagnostic tool is still controversial and some *in-vivo* transgenic mouse models show that TSH has a role in goitrogenesis but is insufficient to cause carcinogenesis by itself.[18] TSH levels in the upper limits of the normal range (last quartile) are associated with a higher risk of malignancy.[5]

In past years, numerous studies have investigated the use of serum TSH levels as a predictor of malignancy in thyroid nodules, with conflicting results. An association between differentiated thyroid cancer (DTC) and elevated TSH levels was first reported by Haymart *et al*.[19] The authors found a trend for higher TSH levels among subjects with a final pathological diagnosis reported as malignancy in a series of 845 patients with thyroid nodules undergoing surgical treatment. Another study from Brazil by Golbert *et al*.[8] found a significant relationship between malig nant thyroid nodules and higher TSH levels, among different categories of Bethesda classification. Castro *et al*.[20] did not find any significant association between serum TSH levels and risk of malignancy. However, they reported an increased incidence of malignancy in patients taking thyroid hormone supplementation. Muniz

Fighera *et al*.[21] reported higher TSH levels in a follicular lesion in comparison to patients with the benign cytological diagnosis.[22] A link between elevated thyrotropin levels and increased risk of thyroid cancer has also been reported.[14]

In our study, a significant positive relationship was found between higher serum TSH levels and an increased risk of thyroid malignancy.

To the best of our knowledge, no previous studies have investigated the association between serum TSH levels that is within the normal range and different types of benign and malignant thyroid lesions. We analysed this risk, stratifying patients based on the serum TSH levels divided into quartiles. The risk for malignancy increases as we move to the higher quartiles. The different types of malignant nodules are associated with higher serum TSH levels.

Husniye Baser *et al*. investigated the association between concentrations of TSH and malignancy in euthyroid patients harbouring thyroid nodules with different Bethesda categories. Patients with benign cytology had TSH levels significantly lower than those with other cytology results. Moreover, TSH levels increased as the Bethesda category did, rising from Bethesda categories II to VI.[23]

Duccini and colleagues verified this in prospective cross-sectional research. Higher TSH levels within the reference range were shown to be linked with the diagnosis of differentiated thyroid carcinoma in thyroid nodules.[24]

Though many studies have found a consistent link between higher TSH levels and malignant nodules, an ideal TSH cut-off value for predicting cancer risk is yet to be discovered.[25-27]

The use of serum TSH levels as a malignancy predictor has been limited due to a paucity of previous research and a lack of verifying nomograms or equations designed to identify an acceptable TSH cut-off value. Using the best point of a ROC curve during the research period, we identified the TSH cut-off value of 2.185 mIU/L to be a good cut-off value for predicting malignancy.

Many new thyroid cancer molecular biomarker-based diagnostic methods have been developed, and some have already been implemented in clinical settings. B-rapidly accelerated fibrosarcoma proto-oncogene (BRAF) and Rat sarcoma gene (RAS) point mutations, Rearranged during transfection oncogene (RET)/ Papillary thyroid carcinoma/papillary histotype (PTC) and Paired-box gene 8 (PAX8)/Peroxisome proliferator-activated receptor (PPAR) rearrangements, as well as Tropomyosin receptor kinase (TRK) rearrangements, are currently the most effective panels for testing for mutations in thyroid FNAC samples. Although the use of these molecular techniques has been confirmed in certain studies, these tests are costly and not available in all centres depending on the prevalence of thyroid cancer. Their impact on patient care is still disputed.[28-33]

Though the 2016 American Thyroid Association (ATA) guidelines acknowledge that higher-than-normal serum TSH levels are linked to a higher risk of malignancy and more advanced stage of thyroid cancer, TSH measurement is not yet recommended as a tool for stratifying the risk of malignancy in patients with newly diagnosed thyroid nodules.[3]

The difficulty in decision-making for a surgeon in AUS/FLUS can be overcome with a simple TSH measurement that can suggest an increased risk for malignancy rather than the more expensive molecular testing options. Our study strongly advocates the use of serum TSH levels as a cost-effective adjunct for risk stratification and follow-up of patients with indeterminate thyroid nodules in combination with clinical and ultra-sonography (USG) findings. The authors also suggest the inclusion of serum TSH in the ATA/National Comprehensive Cancer Network (NCCN) guideline algorithm for the evaluation and management of AUS/FLUS. TSH is an excellent, easily available option in screening patients for thyroid malignancy.

The strength of the study is the large number of patients taken for analysis.

Limitations – This is a retrospective study from a single institution. A prospective multicentre study may improve the accuracy of the findings.

Sonographic features of the thyroid nodule and serum levels of thyroid antibodies were not taken into account in our study. Further studies combining these parameters along with interquartile TSH levels to form a risk assessment scoring system may be useful for better clinical decision-making.

Conclusion

Serum TSH value of more than 2.185 mIU/L is a good cut-off value for predicting malignancy in indeterminate nodules. In all euthyroid patients with indeterminate nodules, serum TSH in the upper range of normal (2.185–4.5 mIU/L) is suggestive of an increased likelihood of malignancy. Surgeons can counsel such patients for early total thyroidectomy rather than go for a thyroid lobectomy as the initial approach. In a large nation like India, this test can be an inexpensive, safe and reliable diagnostic screening test for the risk of malignancy in thyroid nodules and particularly the dilemma of the indeterminate nodule.

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Key messages

- Serum TSH value of more than 2.185 mIU/L is a good cut-off value for predicting malignancy in indeterminate nodules.
- Serum TSH in the upper range of normal (2.185–4.5 mIU/L) is suggestive of an increased likelihood of malignancy.
- Receiver operating characteristic (ROC) curve analysis and logistic regression model accuracy were found to be

0.75 (75%) which suggests that this is a good model for the prediction of malignancy.

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Conflicts of interest

There are no conflicts of interest.

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