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Original Article

Risk of Malignancy and Management of Indeterminate Thyroid Nodules with *HRAS/NRAS* Mutations

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Abstract

Background and objectives: Molecular testing has emerged as a valuable tool for stratifying cytologically indeterminate thyroid nodules (ITNs), with Harvey rat sarcoma viral oncogene homolog/neuroblastoma RAS viral oncogene homolog (*HRAS/NRAS*) mutations being among the most prevalent molecular alterations. The study aimed to evaluate the malignancy risk of ITNs with these mutations. ***Methods:*** We conducted a retrospective study involving ITNs (Bethesda category III and IV) that underwent ThyroSeq testing between February 2016 and January 2022. A smaller subset of ITNs also underwent Afirma testing. We specifically identified nodules with *HRAS/NRAS* mutations and collected radiological, clinical, histological, and follow-up data. ***Results:*** Our analysis identified 45 ITNs with *NRAS* (29 cases) and *HRAS* (15 cases) mutations. Of the 29 nodules with *NRAS* mutations, 25 underwent surgical treatment (14 total thyroidectomies and 11 hemithyroidectomies), resulting in a surgical resection rate of approximately 86%. Among the resected nodules, six were malignant, yielding a calculated risk of malignancy (ROM) ranging from 20.6% to 25%. Three of these malignant nodules were managed with total thyroidectomy, while the other three underwent hemithyroidectomy. During a follow-up period of 43.8 months for total thyroidectomy and 32.9 months for hemithyroidectomy, no recurrence or metastasis was detected among the patients. Among the four nodules treated conservatively, three remained stable, with an average follow-up duration of 34.7 months, while one patient was lost to follow-up. Regarding *HRAS* mutations, 15 nodules were identified, with 12 of them undergoing surgical treatment (six total thyroidectomies and 6 hemithyroidectomies), resulting in an 80% surgical resection rate. Two of the resected nodules were malignant, with a calculated ROM of 13.3% to 16.7%. Both malignant nodules were managed with total thyroidectomy, and during a follow-up period of 37.9 months, no recurrence or metastasis occurred. Of the three nodules managed conservatively, all remained stable, with an average follow-up duration of 31.1 months. ***Conclusions:*** The ROM for nodules with *NRAS* (20.6–25%) or *HRAS* (13.3–16.7%) mutations was found to be low. Therefore, before opting for total thyroidectomy, conservative management, including limited resection, should be considered as a viable alternative.

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Introduction

Thyroid nodules are a prevalent clinical finding, often identified incidentally by imaging or physical examinations due to their asymptomatic nature. The detection rate of these nodules ranges from 19% to 67% in healthy individuals, with high-resolution ultrasonography being a common method of discovery.[1] Ultrasonographic assessment classifies thyroid nodules into three categories: Class I represents low-risk lesions with an expected malignancy risk of around 1%; Class II thyroid nodules are categorized as intermediate-risk lesions, with an expected malignancy risk ranging from 5% to 15%, while Class III comprises high-risk thyroid lesions with an anticipated malignancy risk of 50% to 90%.[2] Given the inherent variability of image assessment, it is important to have additional tests to further distinguish benign from malignant nodules.

In this regard, fine-needle aspiration (FNA) is considered the frontline investigation, as it is minimally invasive, safe, and accurate.[3] Over the past four decades, FNA has proven to be an efficient, cost-effective, and accurate method for determining the appropriate management approach for thyroid nodules.[4] The Bethesda System for Reporting Thyroid Cytopathology offers a standardized approach to reporting thyroid FNA results, encompassing six categories: non-diagnostic, benign, atypia of undetermined significance/follicular lesion of undetermined significance, follicular neoplasm/suspicious for follicular neoplasm, suspicious for malignancy, and malignant. Each category is associated with an appropriate recommended clinical management based on the risk of malignancy.[5] While the implementation of the Bethesda System has decreased unnecessary surgical interventions, a significant percentage of thyroid FNAs still fall into indeter-

Keywords: Thyroid nodule; Molecular test; *HRAS/NRAS* mutations; Risk of malignancy; Thyroid cancer; Thyroidectomy.

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Table 1. Risk of malignancies in *NRAS* and *HRAS* mutations positive indeterminate thyroid nodules

Molecular alteration	N	Hemithyroidectomy	Total thyroidectomy	Malignancy	ROM	Histological diagnosis
NRAS	29	14	11	6	20.6–24%	Widely invasive FTC x1; Minimally invasive FTC x 4; FVPTC x 1
HRAS	15	6	6	2	13.3–16.7%	Minimally invasive FVPTC x 1; Minimally invasive FTC x 1

After resection, the thyroid specimen was adequately sampled and examined microscopically to render the histological diagnosis. FTC, follicular thyroid carcinoma; FVPTC, follicular variant of papillary thyroid carcinoma; *HRAS*, neuroblastoma RAS viral oncogene homolog gene; *NRAS*, Harvey rat sarcoma viral oncogene homolog gene; ROM, risk of malignancy.

minate categories, including Bethesda category III and IV.[6] One study revealed that only 27% of nodules categorized as indeterminate thyroid nodules (ITNs, Bethesda III/IV) were malignant upon histologic evaluation following surgical resection.[7] Another center reported the risk of malignancy (ROM) ranged from 4.4% to 9.6% for Bethesda category III nodules and from 17.9% to 25.9% for Bethesda category IV nodules.[8] This highlights the heterogeneous nature of ROM in ITNs, suggesting a need for further sub-classification to achieve appropriate risk stratification and improve clinical management.

Molecular testing for ITNs emerges as a cost-effective and convenient alternative to diagnostic lobectomy when other indications for thyroidectomy are absent.[9] These molecular tests can reclassify over half of the patients with ITNs as benign, sparing them from unnecessary diagnostic surgery and optimizing initial management for thyroid cancers lacking preoperative evidence of high-risk disease.[10] The majority of ITNs with negative or benign molecular test results remain stable over the years, demonstrating the high sensitivity of molecular tests in ruling out malignancy in these nodules and reducing the risk of unnecessary surgical intervention.[11]

In our earlier research, we highlighted that mutations within the neuroblastoma RAS viral oncogene homolog (*NRAS*) and Harvey rat sarcoma viral oncogene homolog (*HRAS*) genes stood out as notably prevalent molecular alterations in ITNs.[8] Building upon this foundation, the current study took a focused approach, delving into the calculated ROM associated with ITNs harboring these mutations and proposing a management strategy based on these findings and follow-up data.

Materials and methods

Study design: This retrospective study aimed to evaluate the ROM and clinical outcomes of thyroid nodules with *HRAS/NRAS* mutations in cases of cytologically indeterminate thyroid nodules that underwent molecular testing between February 2017 and January 2022. The study adhered to the ethical guidelines of the Helsinki Declaration (as revised in 2013). This study was approved by the Institutional Review Board of Wake Forest University School of Medicine (IRB00060592). The individual consent for this retrospective analysis was waived.

Case selection and analysis

We included cases of ITNs categorized as Bethesda III and IV. The *HRAS/NRAS* mutations were identified by ThyroSeq (V2 for cases before 08/2017 and V3 for cases after 08/2017) or Afirma testing. Furthermore, radiographic impressions, details of the surgical course, and follow-up data were meticulously compiled to facilitate robust clinical correlation. Estimates of the risk of malignancy (ROM) in thyroid nodules were calculated according to a previously published method.[12] Briefly, the lower-bound estimate was calculated by dividing the number of confirmed malignancies by the total number of ITNs. The upper-bound estimate was calculated by dividing the number of confirmed malignancies by the number of ITNs selected to undergo surgery.

Results

We identified 45 thyroid nodules meeting the selection criteria, encompassing NRAS mutations in 30 cases (excluding one patient from further analysis due to the loss of follow-up) and HRAS mutations in 15 cases.

NRAS mutations

Surgical resection was performed in approximately 86% (25/29) of *NRAS*-positive cases, with 56% (14/25) undergoing hemithyroidectomy and 44% (11/25) total thyroidectomy. Out of the 29 *NRAS*-positive cases, six were found to be malignant upon surgical resection. The overall ROM in *NRAS*-positive cases was estimated to be between 20.6% and 24%. Bethesda III cases accounted for 33.3% of *NRAS*-positive malignancies (two cases), all diagnosed as minimally invasive follicular thyroid carcinomas, while Bethesda IV cases made up 66.7%, with varied pathology including minimally invasive follicular carcinomas (two cases), widely invasive follicular carcinomas (one case), and minimally invasive follicular variant papillary thyroid carcinomas (one case). Three of these malignant nodules were managed with total thyroidectomy, while the other three underwent hemithyroidectomy. During an average follow-up period of 43.8 months for total thyroidectomy and 32.9 months for hemithyroidectomy, no recurrence or metastasis was detected among the patients. Among the four nodules treated conservatively, three remained stable, with an average follow-up duration of 34.7 months, while one patient was lost to follow-up (Table 1).

HRAS mutations

For *HRAS*-positive cases, 12 underwent surgical treatment (six total thyroidectomies and six hemithyroidectomies), resulting in an 80% surgical resection rate. Two out of 15 were malignant upon surgical resection, resulting in a calculated ROM between 13.3% and 16.7%. Both malignant nodules were managed with total thyroidectomy, and during an average follow-up period of 37.9 months, no recurrence or metastasis occurred. In the three cases managed conservatively, all remained stable, with an average follow-up duration of 31.1 months (Table 1).

Bethesda categories

In Bethesda category III, a subset comprising 32 cases, a

Table 2. Malignant rate and mutation in nodules with different Bethesda category

Bethesda category	Total cases	Malignant cases	Malignant rate
III	32	4 (NRAS = 2, HRAS = 2)	12.5%
IV	13	4 (NRAS = 4)	31%

HRAS, Neuroblastoma RAS viral oncogene homolog gene; *NRAS*, Harvey rat sarcoma viral oncogene homolog gene.

malignancy rate of 12.5% (4/32) was observed upon surgical resection. Notably, within these malignant cases, two harbored *NRAS* mutations, and the other two featured *HRAS* mutations. In Bethesda category IV, encompassing 13 cases, a higher malignancy rate of 31% (4/13) was identified. All malignancies within Bethesda IV cases exhibited *NRAS* mutations (Table 2).

Clinical outcomes

Following total thyroidectomy for patients with ITNs and *HRAS/NRAS* mutations, the survival rate was 100% (11/11 survived) with a follow-up of 37.9–43.8 months and no instances of recurrence or metastasis. Similarly, patients undergoing partial thyroidectomy also exhibited a 100% survival rate (7/7 survived) with a follow-up of 32.9 months and no recorded recurrence or metastasis. For patients with ITNs that were not resected, all (6/6) remained stable with follow-up of 31.1–34.7 months (Table 3).

Discussion

Molecular testing has emerged as a critical tool for risk assessment in cytologically ITNs, particularly in Bethesda categories III and IV. Employing these tests in a reflex manner offers an accurate prediction of the risk of malignancy in ITNs (Bethesda categories III and IV), thereby reducing the number of unnecessary thyroid surgeries.[8] Among the various mutations, rat sarcoma (*RAS*) gene mutations, including *KRAS, NRAS,* and *HRAS*, have been previously identified as the most common genetic alteration in these nodules.[13,14] Previous research has explored the association of *RAS* mutations with malignancy, revealing variable results. Some studies have demonstrated that *HRAS* and *NRAS* mutation were associated with a substantial risk of cancer,[15] while others have shown varying risks of malignancy for *HRAS*, *NRAS,* and *KRAS* mutations.[16–18] Hence, the current study focused on the ROM in nodules harboring *NRAS* and *HRAS* mutations.

Our study delved into the clinical significance of *NRAS* and *HRAS* mutations in these nodules and their implications for patient management. One noteworthy finding is the low risk of malignancy associated with thyroid nodules harboring *HRAS/NRAS* mutations. Specifically, our study showed that the calculated ROM for nodules with *HRAS* mutations ranged from 13.3% to 16.7%, while for *NRAS* mutations, it ranged from 20.6% to 25%. These ROMs are slightly lower than the malignancy rates typically associated with ITNs based on FNA alone. Our findings align with the growing body of evidence that underscores the low ROM associated with *RAS* mutations in ITNs. Guan *et al.*[19] recently demonstrated that only 16 out of 80 *RAS*-positive ITNs were thyroid carcinoma and concluded that *RAS* mutations were not helpful markers to identify malignancy among Bethesda III/IV cytologies but might predict favorable behavior.

Histopathological assessments of malignant nodules with *RAS* mutations, including our study, consistently reveal low-grade neoplasms such as minimally invasive follicular variant of papillary thyroid carcinoma and minimally invasive follicular thyroid carcinoma. This aligns with the hypothesis that *RAS* mutations may serve as indicators of a more favorable prognosis in malignant nodules. We have previously reported that follicular adenoma and nodular hyperplasia were the most common histologic findings in nodules with NRAS mutation.[8] Building on our results and those of previous studies,[19,20] it suggests that isolated *NRAS/HRAS* mutations in indeterminate thyroid nodules may warrant more conservative approaches, potentially avoiding routine total or near-total thyroidectomy to reduce the risk of complications associated with aggressive interventions.

It is important to acknowledge the limitations of our study. Firstly, our research was retrospective, and the sample size was relatively small. Future studies with larger cohorts could further validate our findings and provide more robust statistical analyses. Additionally, the follow-up period in our study was limited, and longer-term monitoring of patients is needed to assess the durability of our observed outcomes.

Conclusions

Our study emphasizes the low risk of malignancy associated with *NRAS* (20.6–24%) or *HRAS* (13.3–16.7%) mutations in indeterminate thyroid nodules. Conservative management, including limited resection, should be considered before opting for total thyroidectomy. Future endeavors should focus on larger, multi-institutional prospective studies to validate and enhance our understanding of predicting malignancy risk in *NRAS/HRAS*-positive thyroid nodules.

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Table 3. Management and clinical outcome of nodules with *NRAS/HRAS* mutations

Management	Survival/stability rate	Follow-up, months
Total thyroidectomy	100% (5/5)	37.9–43.8
Hemithyroidectomy	100% (3/3)	32.9
Observation	100% (6/6)	31.1–34.7

There was no recurrence or metastasis in the malignant nodules (n = 8) that were resected surgically. All nodules (n = 6) that were not resected remained stable. *HRAS*, neuroblastoma RAS viral oncogene homolog gene; *NRAS*, Harvey rat sarcoma viral oncogene homolog gene.

Conflict of interest

The authors declare no conflict of interest related to this publication.

Author contributions

Study concept and design (WL, AB), acquisition of data (AB, SC, TS), analysis and interpretation of data (WL, JW, TS), drafting of the manuscript (AB), and critical revision of the manuscript for important intellectual content (WL). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

The study adhered to the ethical guidelines of the Helsinki Declaration (as revised in 2013). This study was approved by the Institutional Review Board of Wake Forest University School of Medicine (IRB00060592). The individual consent for this retrospective analysis was waived.

Data sharing statement

The data used in support of the findings of this study are included within the article.

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