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# ROLE OF MOLECULAR MARKERS IN DIFFERENTIAL DIAGNOSIS OF THYROID NODULES WITH INDETERMINATE CYTOLOGY

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Relevance. Fine-needle aspiration (FNA) biopsy is the gold standard in detecting malignancy of thyroid gland. Up-to 30% cases fall in the category of indeterminate cytology (Bethesda class III and IV) where the morphological features are insufficient to classify the type of neoplasm. This necessitates implementation of new approaches, which can help to stratify patients according to the risk of malignancy in order to avoid overdiagnosis. Recent advances in molecular studies of thyroid cancer have allowed molecular testing as a new approach.

Objective: The term «indeterminate cytology» refers to Bethesda class III or class IV findings (estimated malignancy rate of 10% to 30% and 25% to 40%.) The options suggested for identifying these nodules include repeat FNA, but it provides a definitive diagnosis for only 40% of class III nodules. Class IV refers to so-called follicular tumors, which include both benign and malignant lesions.

Material and Methods. Data analysis on the issue of thyroid nodes was carried out on the basis of Pubmed, Cochrane Library and Scopus. Molecular studies of thyroid cancer were conducted

Results. Thyroid Nodules (TN) prevalence in iodine-sufficient populations is around 5%. More common is accidental finding in patients with undetected nodules. Estrogen (estrogen receptors are found in thyroid follicular cells in normal and neoplastic tissue), affecting thyroid-stimulating hormone and may have a role in TNs formation.

According to PubMed, Cochrane Library, and Scopus databases, up to 60% of adults in the general population harbour one or more thyroid nodules, but the actual prevalence of cancer ranges from 1 to 5%. Examination of patients with thyroid nodules consists of initial evaluation, lab tests, thyroid ultrasound and FNA as a definite diagnostic procedure, which helps to recognize nodule morphology. Nonetheless, FNA in up to 30% of patients may result in false or indeterminate results.

Conclusion. TNs were found in one of every four people in the general population. AlarminglyTNs have become a pandemic irrespective of country development and economic status. TNs have a strong etiological association with iodine nutrition, the prevalence of TNs is affected by sex, age, body mass index, & head-and-neck radiation exposure history. Based on the present results, women had a 1.5-fold greater TN prevalence than men.

Estrogen-induced increase in thyroid follicular cell proliferation have been shown in vitro, Furthermore, the prevalence of TNs has is 4 times higher after 70 years.

Keywords:fna biopsy; indeterminate cytology; molecular testing

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## RELEVANCE

The term «indeterminate cytology» refers to Bethesda class III or class IV findings, that's associated with an estimated malignancy rate of 10% to 30% and 25% to 40%. The options suggested for identifying these nodules include repeat FNA, but it provides a definitive diagnosis for only 40% of class III nodules [3]. Class IV refers to so-called follicular tumors, which include both benign and malignant lesions, and in this situation cytology can't help. On performing 2nd cytological study; if indeterminate results persist or follicular tumor is found, diagnostic lobectomy is traditionally done to get a confirmatory pathological diagnosis. This procedure is costly and possess certain risks. Often reoperation (complete thyroidectomy) is done if nodule is found to be malignant. Hence, patients up to 60% that undergo lobectomy for an indeterminate nodule are often under- or overtreated at initial surgery [5].

The purpose of the study – The term «indeterminate cytology» refers to Bethesda class III or class IV findings (estimated malignancy rate of 10% to 30% and 25% to 40%.) The options suggested for identifying these nodules include repeat FNA, but it provides a definitive diagnosis for only 40% of class III nodules [4]. Class IV refers to so-called follicular tumors, which include both benign and malignant lesions.

## MATERIALS AND METHODS OF RESEARCH

Thyroid Nodules (TN) prevalence in iodine-sufficient populations is around 5%. More common is accidental finding in patients with undetected nodules.

TNs were found in one of every four people in the general population. AlarminglyTNs have become a pandemic irrespective of country development and economic status. TNs have a strong etiological association with iodine nutrition, the prevalence of TNs is affected by sex, age, body mass index, & head-and-neck radiation exposure history.Based on the present results, women had a 1.5-fold greater TN prevalence than men.

Estrogen (estrogen receptors are found in thyroid follicular cells in normal and neoplastic tissue), affecting thyroid-stimulating hormone and may have a role in TNs formation. Estrogen-induced increase in thyroid follicular cell proliferation have been shown in vitro, Furthermore, the prevalence of TNs has is 4 times higher after 70 years [2].

## THE RESULTS OF THE STUDY AND THEIR DISCUSSION

Recent advances in molecular studies of thyroid cancer have allowed to use new approach – molecular testing - to solve the above problem. It has reduced the number of diagnostic surgeries performed. They are based on three principle molecular approaches:

1. Evaluation of gene expression.
2. Somatic mutation testing.
3. Classifiers based on microRNA [1].

Few of them are also currently used in rule- in and rule- out testing as they have enough positive and negative predictive values.

How does thyroseq work? ThyroSeq: looks for unique genetic alterations in your thyroid nodule. If the nodule is benign (non-cancerous) or malignant (cancerous). ThyroSeq uses an innovative technology called Next Generation Sequencing to analyse genes important for cancer development. ThyroSeq uses cells already collected during FNA procedure and sequences the hundreds of gene regions in one tube. Pathologists interpret genetic alterations found to determine the risk of cancer in your thyroid nodule. FNAB samples are carried out using 2 commercial panels. One of them assesses the mRNA levels for 142 genes. The negative predictive value for samples with uncertain significance in FNAB using this panel has been reported to be 96%. Therefore, in individuals with benign lesions, it may be helpful in avoiding

needless surgical procedures. Seven molecular markers (BRAF, KRAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PPAX8/PPARγ) that are frequently found in thyroid tumors are evaluated by another commercial panel. Differentiated thyroid carcinomas emerge as a result of genetic alterations. Primary sites of RAS mutation observation, BRAF mutations are more frequently seen in papillary thyroid cancer.

## CONCLUSION

However, the currently available data differs significantly from one another in cohort selection criteria, sample sizes, malignancy rates, study design, and applied reference standards; there are no direct head-to-head comparisons. Nevertheless, major molecular approaches proved to be considerably more cost-effective than diagnostic lobectomy.

*Table 1. Molecular testing in global clinical practice (Bethesda III, IV) Hemithyroidectomy: $9,000–12,000 (USA, 2018)*

| The test system | Markers | Sensitivity % | Specificity % | PPV % | PV % | Cost $ |
|---|---|---|---|---|---|---|
| Afirma GSC | Expression of 1115genes+905 mutations and 235 translocations for 593 genes | 91 | 68 | 49ss | 96 | 4875-6400 |
| ThyroSeq v3 | 112 genes (12135 mutations), 150 translocations | 94 | 82 | 64 | 98 | 3200 |
| ThyGeNEXT/ ThyraMIR | Mutations of 10 genes, 38 translocations, 10 microRNAs | 95 | 90 | 76 | 98 | 2600-5000 |
| mir-THYpe | 11 microRNAs | 95 | 81 | 62 | 98 | 1200 |
| Thyroid Print | Expression of 10 genes | 91 | 87 | 70 | 97 | 4000 |
| Thyroid-INFO | Expression of 2 genes, 5 microRNAs, 1 mutation, mtDNA | 89 | 93 | 81 | 96 | 185 |

It's still debatable if a comprehensive molecular profile of thyroid nodules can provide predictive information and guide the extent of surgery. But, if all clinical, imaging and cytological findings indicates a requirement of diagnostic surgery then molecular testing should be certainly considered.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## PERSONAL CONTRIBUTION OF THE AUTHORS

Upadhyay Aryan – preparation of the draft of the article, processing of the material;

Suresh Aishwarya – editing, design of the final version of the article;

S.U. Yakubouski – analysis and generalization of data, formulation of conclusions.

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