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ALGORITHM FOR DIAGNOSIS AND THERAPY OF DIFFERENTIATED THYROID CANCER WITH INTEGRATION OF MOLECULAR-GENETIC PROFILING

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Abstract

The objective of this study was to evaluate the prevalence and clinicopathological relevance of BRAF-V600E, RET/PTC, TERT, RAS, and PAX8-PPAR-γ mutations in patients with differentiated thyroid carcinoma (DTC). A prospective cohort of 300 patients who underwent examination and treatment at the branch of Tashkent city of the Republican Specialized Scientific-Practical Medical Center of Oncology and Radiology, Uzbekistan, between 2014 and 2024 was analyzed. All patients underwent fine-needle aspiration biopsy with cytological interpretation according to the Bethesda system, ultrasound risk stratification using TI-RADS, and molecular testing for the selected mutations by PCR and FISH. The results demonstrated that BRAF-V600E mutation was predominant in papillary thyroid carcinoma (82.9%, $p<0.001$) and correlated with adverse Bethesda categories and TNM stages. RET/PTC was exclusively detected in PTC cases (100%, $p<0.001$), most frequently at stages T1N1M0 and T2N0M0. TERT mutation occurred more often in the follicular subtype (46.2%, $p<0.001$) and was associated with aggressive tumor behavior. All RAS and PAX8-PPAR-γ mutations (100%, $p<0.001$) were restricted to FTC, with PAX8-PPAR-γ being characteristic for macrofollicular and clear-cell variants. Correlation with TI-RADS features was weak or statistically insignificant. These findings highlight the prognostic significance of molecular profiling in DTC and its value in refining prognosis, guiding surgical and adjuvant strategies, and enabling a personalized management approach.

Keywords: Differentiated thyroid cancer, molecular-genetic analysis, prognostic markers, personalized treatment.

Introduction

Among endocrine malignant tumors, differentiated thyroid carcinoma (DTC) occupies a leading position, including papillary, follicular subtypes and Hürthle cell carcinomas, which together account for up to 95% of all cases of this pathology [1]. Since the beginning of the 21st century, there has been a marked increase in incidence. According to R.L. Siegel et al. (2021), more than 44,000 new cases and up to 2,200 deaths are recorded annually in the United States. In 2020, about 448,000 cases of DTC were reported worldwide [6], while in the United Kingdom, an increase in incidence by 68% was observed, which is partially attributed to the phenomenon of overdiagnosis [5].

Modern treatment strategies increasingly rely on molecular and genetic research. The most effective results have been demonstrated by targeted agents such as dabrafenib and trametinib in the presence of BRAF and MEK mutations [3]. In their absence, immune checkpoint inhibitors, anti-PD-1 and anti-PD-L1, are used [4]. In clinical practice, multikinase inhibitors (lenvatinib, sorafenib, sunitinib, vandetanib, and others) are also applied [2], while the combination of BRAF and MEK inhibitors has shown the highest efficacy. In parallel, new therapeutic approaches are being developed, including monoclonal antibodies, oncolytic viruses, and epigenetic modulators [8]. The first results of clinical studies with PD-1 inhibitors (spartalizumab, pembrolizumab) indicate improved survival, although accompanied by pronounced adverse reactions [2].

Objective of the study:

to improve the outcomes of diagnosis and treatment of differentiated thyroid carcinoma (DTC) through the development of an algorithm based on the application of molecular genetic analysis methods.

Materials and methods of the study

The study was conducted in the format of a prospective-retrospective cohort randomized analysis at the Tashkent City Branch of the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCO&R) of the Republic of Uzbekistan during the period from 2014 to 2024. Initially, 548 patients with clinical signs of thyroid gland neoplasms were enrolled. After completing the full diagnostic complex and the procedure of block randomization, 300 patients with a verified diagnosis of differentiated thyroid carcinoma (DTC) were included in the main group.

The number of participants met the requirements for such studies and provided sufficient statistical power to obtain objective results. Among the 300 patients with DTC, females predominated — 215 (71.7%), while males accounted for 85 cases (28.3%) (see Figure 1).

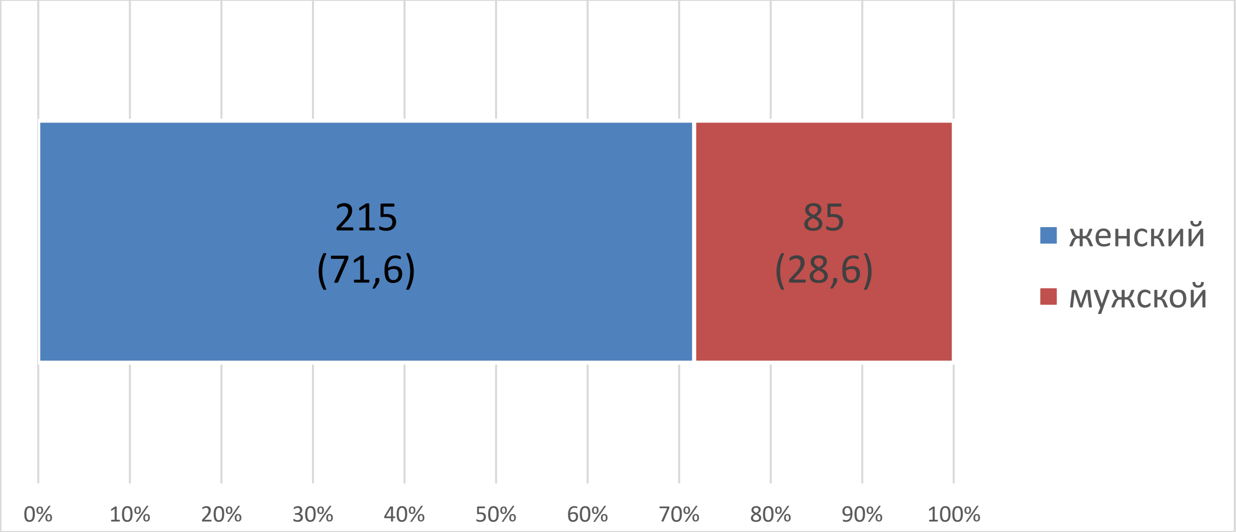


Fig. 1. Gender distribution among study participants

Table 1. Analysis of age according to gender among study participants

Indicator	Category	Age (years) Me	Q1–Q3	n	p
Gender	Female	42	38–49	215	0,489
	Male	44	39–50	85	

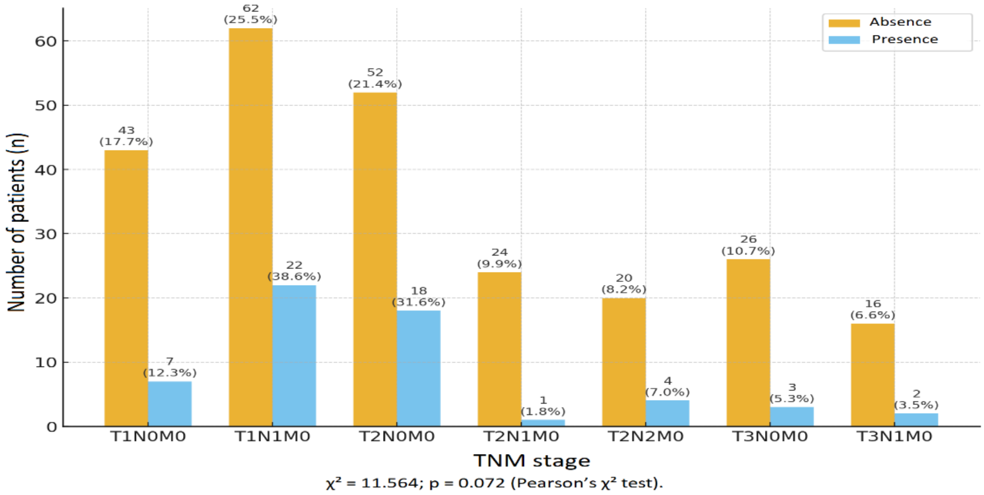
Diagnostic methods. Fine-needle aspiration biopsy (FNAB) was performed in patients with thyroid nodules ≥10 mm in size, as well as in nodules <10 mm when ultrasound signs of malignancy were present. The procedure was carried out under sonographic guidance using single-use sterile 21G needles, and in difficult-to-access cases, 20G needles were employed. Cytological interpretation of the obtained material was performed according to the Bethesda System for Reporting Thyroid Cytopathology (2010).

Molecular genetic analysis included the assessment of mutations in BRAF V600E, RET/PTC, TERT, RAS, and PAX8-PPAR-γ genes. The methods used comprised polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), sequencing, and fluorescence in situ hybridization (FISH). The obtained results were compared with the clinical and morphological parameters of the tumors.

Statistical analysis was conducted using IBM SPSS Statistics v.22. To assess differences, Pearson's χ^2 test, Fisher's exact test, and the Mann–Whitney U test were applied. The odds ratio (OR) with a 95% confidence interval (CI) was calculated to estimate the strength of associations.

Results of the study:

In the analysis of the BRAF-V600E mutation, its association with the extent of the tumor process according to the TNM classification was evaluated. Among all 47 patients harboring this genetic alteration, a more aggressive course of DTC was observed. The highest frequency of the mutation occurred in stages T1N1M0 (38.6%), T2N0M0 (31.6%), and T1N0M0 (12.3%). Despite these trends, no statistically significant differences between TNM stages were identified (p=0.072; Cramer's V=0.2).

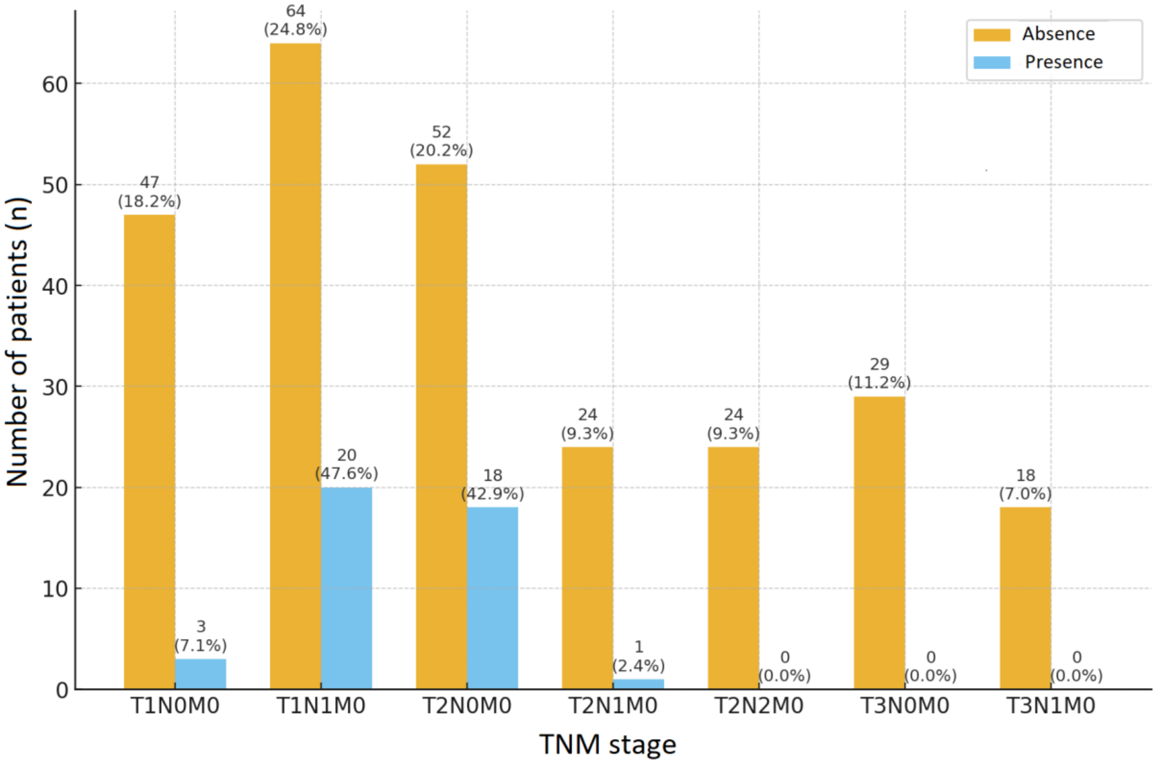


Statistical analysis method used: Pearson's χ^2 test.

Fig. 2. Frequency of detection of the BRAF-V600E mutation depending on the stage of the tumor process (TNM).

The BRAF-V600E mutation was more frequently observed in patients with less favorable clinical tumor characteristics. However, no statistically significant association was found with TNM category, histological type, variant of differentiated thyroid carcinoma (DTC), or with the TI-RADS and Bethesda classifications.

In the analysis of the RET/PTC mutation, its frequency was evaluated depending on the TNM stage of the tumor process. The mutation was detected in all 42 patients (100%) and was most frequently registered at stages T1N1M0 (47.6%) and T2N0M0 (42.9%). A moderate-strength association was established between the presence of the RET/PTC mutation and TNM stage (p<0.001; Cramer's V=0.32).

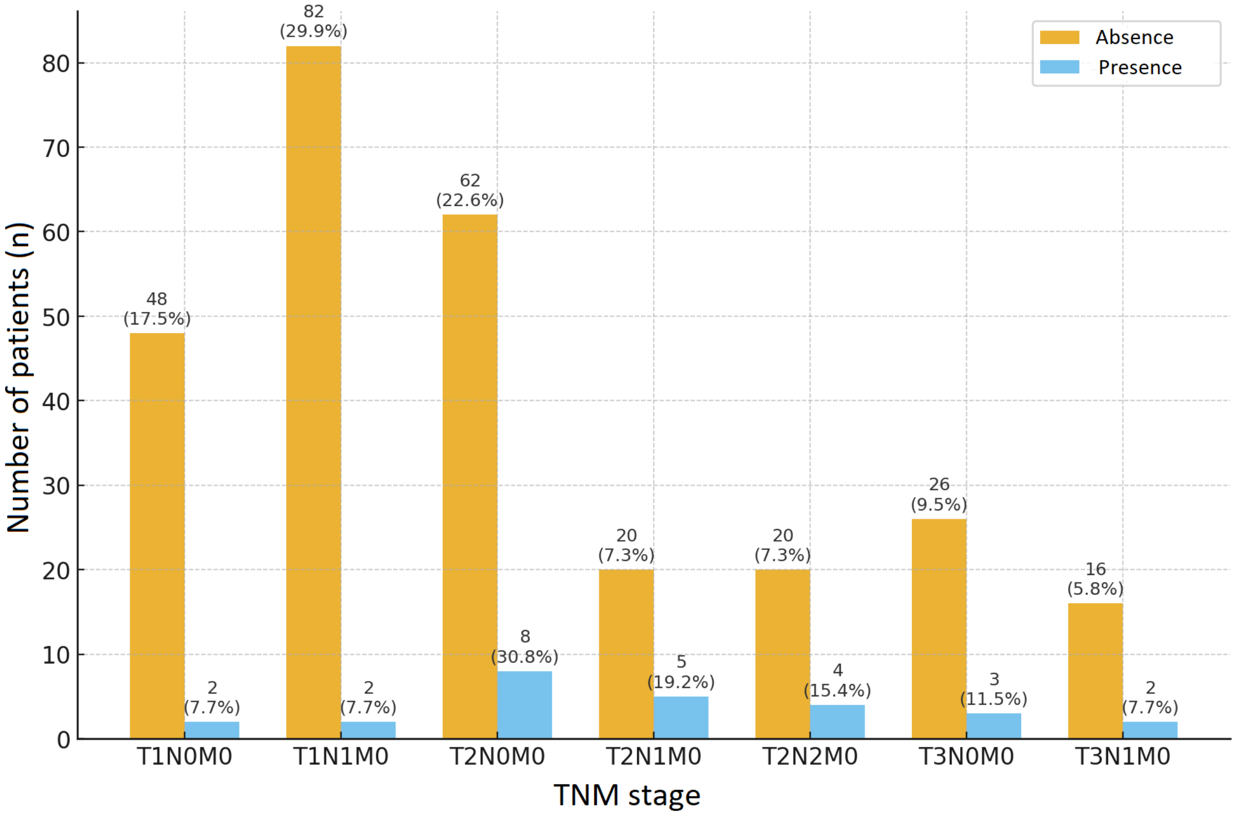


χ^2=30,984; $p<0{,}001$* (Pearson's χ^2 test).

Fig. 3. Frequency of detection of the RET/PTC mutation depending on the stage of the tumor process (TNM).

The RET/PTC mutation showed a statistically significant association with papillary thyroid carcinoma (PTC) and the tumor stage according to the TNM classification. At the same time, no statistically significant correlations were found with the histological variant of the tumor, TI-RADS categories, or the Bethesda system.

In the analysis of the TERT mutation according to the TNM classification, it was found that the mutation most frequently occurred at stages T2N1M0 (30.8%), T2N0M0 (19.2%), T2N2M0 (15.4%), and T3N0M0 (11.5%). However, the association between the presence of the mutation and the TNM stage was weak and did not reach statistical significance (p=0.052; Cramer's V=0.2).



χ^2=12,479; p=0,052 (Pearson's χ^2 test).

Statistical analysis method: Pearson's χ^2 test.

Fig. 4. Frequency of detection of the TERT mutation depending on the stage of the tumor process (TNM).

The TERT mutation was more frequently observed in follicular thyroid carcinoma (FTC) as well as in microfollicular and follicular variants of carcinoma. It was also commonly found in patients classified as TR-5 according to the TI-RADS system and in category V according to the Bethesda system. However, a statistically significant association was confirmed only with the type of differentiated thyroid carcinoma ($p<0.001$).

In the analysis of the **RAS mutation**, it was found that its presence was significantly associated with the tumor stage according to the TNM classification ($p<0.001$). The highest detection rates were observed in stages T3N1M0, T3N0M0, and T2N2M0 (26.7% each). In all cases (100%), this genetic alteration was found exclusively in follicular thyroid carcinoma (FTC), confirming its type-specific nature ($p<0.001$).

At the morphological level, the mutation was most frequently identified in follicular and macrofollicular carcinoma forms (26.7% each, p=0.020). No association was observed with ultrasound features according to the TI-RADS system (p=0.791). Within the Bethesda classification, the mutation was more often recorded in category V (60.0%), but the risk was significantly higher in Bethesda VI (OR=3.801; 95% CI: 1.547–6.136; p=0.004).

Table 2. Frequency of RAS gene mutation detection depending on clinicomorphological characteristics

Indicator	Category	Absence of mutation, n (%)	Presence of mutation, n (%)	χ^2 / Fisher's exact test	p	Cramer's V / OR (95% CI)
TNM stage	T3N1M0, T3N0M0, T2N2M0	–	4 (26,7%) each	χ^2=36,153	<0,001*	V=0,35
Type of DTC	PTC / FTC	237 (83,2)/ 48 (16,8)	0/ 15 (100%)	χ^2=59,398	<0,001*	V=0,44; OR=5,713 (4,356–7,492)
Histotype	Follicular / Macrofollicular / others	83/ 107/	4 (26,7%)/ 4 (26,7%)/	χ^2=19,642	0,020*	V=0,26
TI-RADS	TR-4/ TR-5	119 (41,8)/ 166 (58,2)	7 (46,7%)/ 8 (53,3%)	FET=0,791	0,791	V=0,02
Bethesda	V/ VI	248 (87,0)/ 37 (13,0)	9 (60,0%)/ 6 (40,0%)	χ^2=8,471	0,004*	V=0,17; OR=3,801 (1,547–6,136)

** Statistically significant differences at $p<0.05$.*

RAS gene mutations were fully associated with follicular thyroid carcinoma (FTC) and showed a statistically significant relationship with unfavorable prognostic parameters, specifically TNM stage and Bethesda classification. The influence on the histotype was moderate, while no association with TI-RADS was observed.

In the analysis of the **PAX8-PPAR-γ mutation** according to the TNM classification, statistically significant differences were identified ($p<0.001$). The highest number of positive cases occurred in stages T3N0M0 (27.3%), T3N1M0 (22.7%), T2N2M0 (22.7%), and T2N1M0 (22.7%). The strength of the association with stage was assessed as moderate (Cramer's V=0.39).

All identified PAX8-PPAR-γ mutations (100%) were observed exclusively in follicular thyroid carcinoma (FTC), confirming their type specificity ($p<0.001$). The risk of mutation presence in FTC was 6.577 times higher (95% CI: 4.935–8.765).

From a histological perspective, the mutation was more frequently found in macrofollicular (36.4%) and clear-cell (22.7%) carcinomas, with a moderate association strength (Cramer's V=0.34). No statistically confirmed association with ultrasound features according to the TI-RADS classification was detected ($p=0.315$). Within the Bethesda system, the mutation was more frequently observed in category V (68.2%), though the risk significantly increased in category VI (OR=2.457; 95% CI: 1.241–4.867; $p=0.015$).

Table 3. Frequency of detection of the PAX8-PPAR-γ mutation depending on clinicomorphological characteristics

Indicator	Category	Absence of mutation	Presence of mutation	χ^2	p	V / OR (95% CI)
TNM stage	T3N0M0/ T3N1M0/ T2N2M0/ T2N1M0/ др.	258 (–)	22 (100%)	$\chi^2=45{,}219$	<0,001*	V=0,39
Type of DTC	PTC / FTC	237 (85,3%)/ 4 (14,7%)	0 / 22 (100%)	$\chi^2=89{,}311$	<0,001*	V=0,55; OR=6,577 (4,935–8,765)
Histotype	Macrofollicular / Clear-cell / others	257 (–)	22 (100%)	$\chi^2=33{,}746$	<0,001*	V=0,34
TI-RADS	TR-4/ TR-5	119 (42,8%)/ 15 (57,2%)	7 (31,8%) / 1 (68,2%)	$\chi^2=1{,}01$	0,315	V=0,06
Bethesda	V/ VI	242 (87,1%)/ 3 (12,9%)	15 (68,2%) / (31,8%)	$\chi^2=5{,}911$	0,015*	V=0,14; OR=2,457 (1,241–4,867)

Note: statistically significant differences at $p<0.05$.

The PAX8-PPAR-γ mutation showed a strong association with follicular thyroid carcinoma (FTC), being detected in all cases of this tumor type. The highest frequency of detection was observed in macrofollicular and clear-cell variants of carcinoma. A statistically significant association was found with certain TNM stages. The correlation with ultrasound features according to the TI-RADS classification was weak and statistically insignificant, while a moderate association was observed according to the Bethesda system.

Conclusion

The present study evaluated the frequency of genetic mutations BRAF-V600E, RET/PTC, TERT, RAS, and PAX8-PPAR-γ in patients with differentiated thyroid carcinoma (DTC) and analyzed their relationships with the clinicomorphological characteristics of the tumor process.

It was established that the BRAF-V600E mutation occurred more frequently in papillary carcinoma and at early TNM stages (T1–T2); RET/PTC was identified exclusively in papillary carcinoma, whereas the TERT mutation was mainly associated with the follicular type and corresponded to a more aggressive clinical course. The RAS and PAX8-PPAR-γ mutations demonstrated a 100% association with follicular carcinoma, with PAX8-PPAR-γ being more commonly observed in macrofollicular and clear-cell variants of carcinoma.

The highest prognostic significance was confirmed for BRAF-V600E, RET/PTC, RAS, and PAX8-PPAR-γ mutations, which showed a consistent association with tumor type and several morphological features. At the same time, the TI-RADS system and Bethesda classification exhibited only limited prognostic value and a weak correlation with mutation status.

Thus, the obtained results demonstrate the high diagnostic and prognostic value of molecular genetic analysis in DTC. Its implementation enables more accurate prognosis, individualization of surgical and adjuvant treatment strategies, and substantiates the integration of personalized therapeutic approaches into the management of patients with differentiated thyroid carcinoma.

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