Review Article

What is the impact of serum molecular markers on the diagnosis of thyroid cancers? A comparison of serum molecular markers with invasive biopsy methods

ABSTRACT

Background: Tissue molecular markers have taken their place as an effective, invasive method in the diagnosis of thyroid cancers. However, if the superiority of serum molecular markers to invasive diagnostic methods as a noninvasive method can be proven, it will have a great impact on the diagnostic approach and screening programs for thyroid nodules.

Aim: The aim in this study is to investigate the effectiveness of serum molecular markers compared to invasive methods in the diagnosis of thyroid cancers.

Methods: In our study, results obtained in publications in which serum molecular markers were used in the diagnosis of thyroid cancers and absolute sensitivity was measured were compared with the absolute sensitivity rates obtained in cases where invasive methods such as fine needle aspiration biopsy and core needle biopsy were used. The results were evaluated statistically.

Results: In cases where serum molecular markers were used in the diagnosis of thyroid cancer, absolute sensitivity rates were found to be statistically significantly higher than invasive methods.

Conclusion: According to the results we have obtained, molecular markers in serum are a noninvasive method that can be used safely in thyroid cancer screening, definitive diagnosis and follow-up. Although it is a noninvasive method, its definitive diagnosis rates are higher compared to methods such as invasive fine needle aspiration biopsy and core needle biopsy. Therefore, in the near future, it is likely to take a higher priority in the diagnostic approach and screening programs for thyroid nodules.

Keywords
Thyroid cancer, diagnostic methods, molecular markers, fine needle aspiration biopsy, core needle biopsy.

1. INTRODUCTION

Thyroid nodules are a highly common disease, although many nodules do not require surgery, still due to the false or inadequate diagnosis, many cases of goiter without cancer are unduly operated, and many cases of cancer are delayed for the same reason.

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The most effective methods currently used in the definitive diagnosis of thyroid cancers (TCs) are fine needle biopsy (FNA) and core needle biopsy (CNB), such as aspiration biopsy or molecular marker (MM) expression analysis in tissue.
However, since these methods are invasive, they are not desired to be applied by many patients and cannot be used as screening tests.
In addition, it is inevitable that some complications will occur during the application. MMs have a key feature not only in diagnosis, but also in prognosis and treatment of TCs. When the molecular structure of cancer cell in TC cases is revealed by the analysis of serum MMs, the personalized treatment could be provided. Advances in research related to the molecular pathogenesis of TCs have taken an important step in the diagnosis of TCs [1]. Tissue MMs in the diagnosis of TCs have begun to be involved in algorithms related to the diagnosis of thyroid nodules [2].
In a study conducted by Rosiglono et al, they investigated micro RNA (miRNA) profiles in papillary thyroid cancer (PTC) patients, made miRNA-based molecular classification, and they showed that miRNA-146b-5p, 146b-3p, 221-3p, 222-5p, 222-3p was up-regulated, miRNA-1179,486-5p, 204-5p, 7-2-3p, 144-5p, 140-3p were down-regulated in PTC tissue [3].
Providing high sensitivity and specificity rates in Bethesda III and IV nodules, commercial products such as Afirma Gene Sequencing Classifier (multigene expression), Interpace ThyGenX + ThyraMir (7 gene panel + 10 miRNA), CBLPath ThyroSeq version 3 are available [4]. However, in the diagnosis of TCs, tissue samples are needed for tissue MMs and this can be achieved only by invasive FNA and CNB methods.
In the diagnosis of TCs, significant advances have been made in noninvasive methods. Combined two-dimensional shear wave elastography (2D-SWE) + conventional USG was performed on 31 thyroid nodules in 27 patients by Liu et al. resulting in 87.1% sensitivity [5]. Horwath et al. reported 99.6 % sensitivity and 74.35% specificity with USG in 510 nodules in 210 patients [6]. However, some authors have been reported that, multimodel diagnostic methods should be used in nodules smaller than 1 cm, since the sensitivity decrease when only USG is performed [5,7]. In a study by Ponti et al., cell-free DNA analysis was performed in fluids other than serum and an increase in many types of cancers, including TCs, was observed [8].
In recent years, some studies have been carried out in which some of the serum molecular markers are used as a noninvasive method in the diagnosis of thyroid cancers.
In a study by Ghafouri et al. in TC cases, they reported that miRNAs affect thyroid cancer activity through signaling pathways such as the membrane associated protein kinase (MAPK) pathway and rearranged during transfection (RET) genes, and that serum miRNA analyzes have a very important place in the diagnosis and treatment of thyroid cancers as a noninvasive method [9].
However, despite successful results, these tests have not yet been put into routine practice such as tissue molecular marker tests.
Therefore, the aim of this study is to reveal the importance of serum MMs and compare them with the invasive FNA and CNB methods in the diagnosis of TCs.

2. MATERIALS AND METHODS

2.1. Study design, inclusion and exclusion criteria

In order to compare the absolute sensitivity rates of serum MM, which is a non-invasive method in the diagnosis of TCs, and FNA and CNB, which are the most frequently used invasive diagnostic methods today; the results obtained in studies conducted with serum MMs and in which absolute sensitivity (AS) rates were investigated in the literature, and the results of 4 studies in which the AS was investigated, including a large number of cases with invasive FNA and CNB were included in our study.
There are not many studies in the literature in which AS rates were measured with serum MM in the diagnosis of TC. Therefore, the results of studies with large numbers of serum MM could not be included in the study.
Although there are many studies in which AS rates were measured and invasive methods were used in the diagnosis of TCs, the results of the studies in which a limited number of invasive methods were used and AS measured were included in our study in order not to negatively affect the research results.

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Since our study was compared only to the diagnostic methods used in thyroid cancer cases, AS rates of these methods in non-cancer cases (such as non-toxic multinodular goiter) were not evaluated.
The reason why only serum MMs were included in our study as a noninvasive method; it is more effective than other noninvasive methods as a definitive diagnosis and screening method.
The reason for including FNAand CNB as an invasive method; it is still the most commonly used method in definitive diagnosis.
The reason for comparing only the AS rates results in our study; the most appropriate measure in comparison of definitive diagnosis and screening methods.
In addition, no comparison was made with sensitivity and specificity rates.
In this study, only histopathologically definitive diagnosis (gold standard) reported articles in which invasive diagnostic methods used for the diagnosis of TCs were included and when comparing diagnostic methods, AS (the rate of cancer cases detected by MM or FNA in cases with TCs with definite histopathological diagnosis) was taken as basis.
In the diagnosis of TCs, the group that includes studies evaluating MMs in serum and the studies performed on FNA and CNB were designated as GpA and GpB, respectively.
The AS rates obtained in the studies performed in GpA (The studies using serum MMs for diagnosis [10-13] and GpB (The studies using invasive FNA and CNB methods for diagnosis [14-17]) were compared with each other and evaluated statistically.

2.2. Statistical Analysis

SPSS 23.0 package program was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages. In comparison of categorical variables, Chi-square test and Fischer's Accuracy Test were applied. In all tests, a p-value <0.05 was considered statistically significant.

3. RESULTS

The studies included in GpA and GpB, the reference numbers of the studies and the number of cases are shown in (Table 1).

Table 1. Studies conducted in GpA and GpB, number of cases and reference number.

Studies on MMs in Serum (GpA)	Number of TC Cases	Ref. No.
miRNA 95, miRNA 190	79	10
miR-221, miR-222, miR-146b	42	11
miR-222	30	12
miR-451a, miR1-25-3p	50	13
Studies with FNB and/or CNB in Tissues (GpB)		
FNA	756	14
CNB	325	15
FNA	369	16
FNAand CNB	318	17

GpA: Group A; GpB: Group B; FNA: Fine needle aspiration biopsy; CNB: Core needle biopsy; MM: Molecular marker; TC: Thyroid cancer

The absolute sensitivities, in cases of TC with GpA and GpB, were found to be 97.55±3.40 % and 82.10±11.71 %, respectively (Table 2).

Table 2. Absolute sensitivity in GpA and GpB

Diagnosis	GpA (MMs)	GpB (FNA/CNB)	Total	P
TC diagnosis (-)	6 (2.9)	433 (17.9)	439 (16.75)	< 0.05
TC diagnosis (+)	201 (97.1)	1981 (82.1)	2182 (83.25)	-
AS ± SD	97.55 ± 3.40	82.10 ± 11.71	-	< 0.05
Range	92.8 - 100.0	66.1 - 90.9	60.1 - 100.0	-

GpA: Group A; GpB: Group B; FNA: Fine needle aspiration biopsy; CNB: Core needle biopsy; TC: Thyroid cancer. Values in parentheses represent the percent of that group to the total number of patients in the same group

The percent absolute sensitivity values versus study groups plot, in GpA and GpB, is shown in (Fig 1).

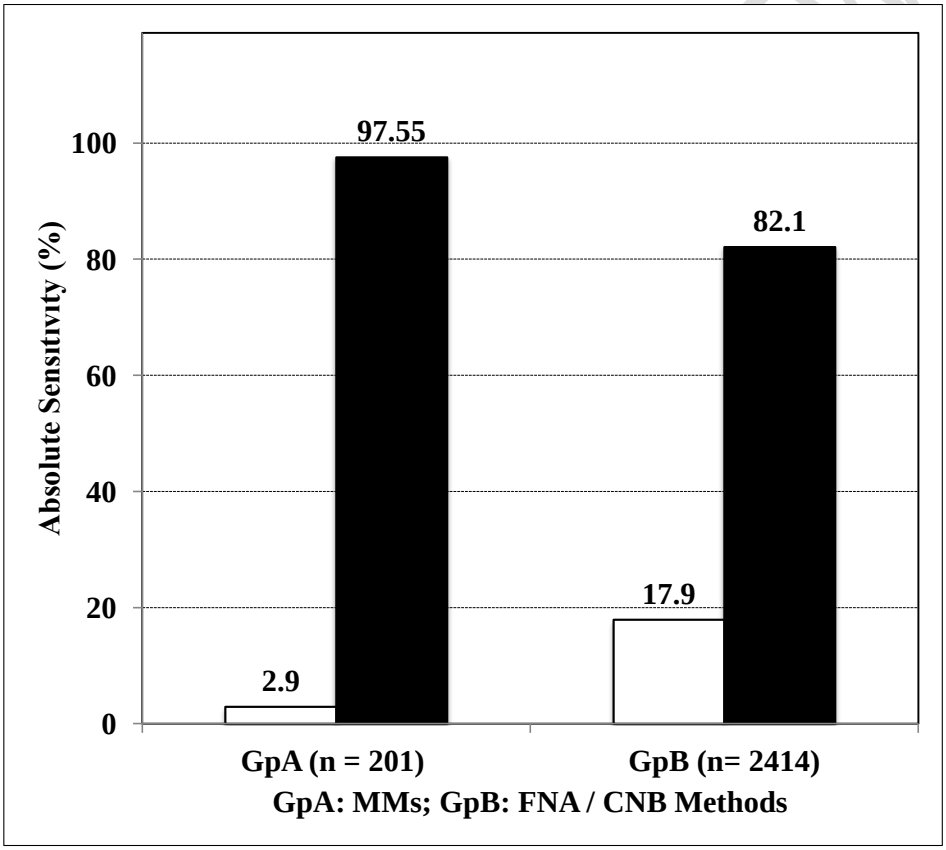

Fig. 1. Absolute sensitivities (%) of GpA (MMs) and GpB (FNA/CNB) versus studies. Empty columns % MD (misdianosed) and filled columns % CD (corectly diagnosed)

The results clearly reveal that AS in GpA is significantly higher ($p < 0.05$) than GpB (Table 2, Fig 1). According to the results we obtained, the highest absolute sensitivity rates among the studies conducted in GpA were obtained in A1 (97.4%), A2 (100%), A3 (100%) and A4 (92.8%) studies (Table 3, Fig 2). In the studies performed in Gp B, the highest absolute sensitivity rate was obtained in B1 (90.9%) (Table 3, Fig 2).

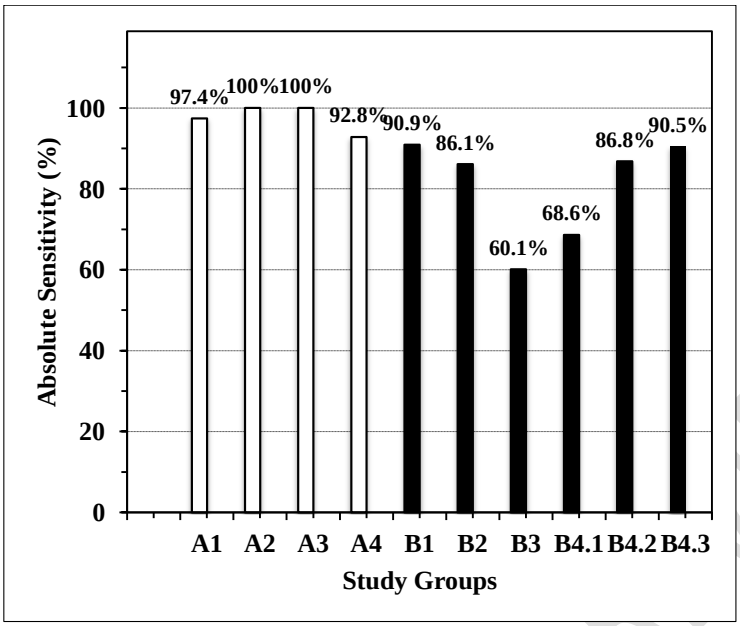

Fig. 2. Absolute sensitivities (%) of GpA (MMs) and GpB (FNA/CNB) versus studies

All the absolute sensitivity rates obtained in the studies within the groups are shown in (Table 3).

Table 3. Absolute sensitivities in studies conducted in GpA and GpB

AG Code	TC (-)	TC (+)	Total	AS	MMs/MU	Ref. No.
A1	2 (2.5)	77(97.5)	79	97.4	miRNA29b,miRNA95, miRNA190,miRNA579	10
A2	0 (0.0)	42 (3.6)	42	100.0	miR222, miR146b	11
A3	0 (0.0)	30 (100)	30	100.0	miR222	12
A4	4 (7.1)	52 (92.9)	56	92.8	miR451a,miR25-3p	13
B1	69 (15.5)	697 (31.8)	766	90.9	FNA	14
B2	45 (10.1)	280 (12.8)	325	86.1	CNB	15
B3	147 (33.1)	222 (10.1)	369	60.1	FNA	16
B4.1	100 (22.5)	218 (10.0)	318	68.6	FNA	17
B4.2	42 (9.5)	276 (12.6)	318	86.8	CNB	17
B4.3	30 (6.8)	288 (13.2)	318	90.5	FNA+CNB	17

AG Code: Author's Group Code; GpA: Group A; GpB: Group B; MMs:Molecular markers; MU: Method used; FNA: Fine needle aspiration biopsy; CNB: Core needle biopsy. TC (-): Misdiagnosed (Although histopathologically cancer (gold standard) but missed by the methods used); TC (+): Correctly diagnosed; Values in paranthesis represent the percent of that group to the total number of patients in the same group.

4. DISCUSSION

MMs used in cancer diagnosis have many important advantages. The first advantage is that even in exceedingly small tumors their expression increases in blood or tissue [7], and the second advantage is that some of these markers are tumor-specific, thus providing tumor-specific information to the patient

[18]. In a study performed by Ye et al., circFOXM1 expression increased [18], in another study conducted by Zhang et al., circ_0067934 expression increased [19], while in down-regulation; tumor growth inhibition has been observed.

With the FNA method, more successful results are obtained in the diagnosis of TCs. In a retrospective study of 137 patients with thyroid nodules, Huong et al. reported that most of the nodules could be diagnosed with clinical findings, USG findings and FNA, and molecular diagnostic methods are not needed [20]. Paja et al. reported that in 4112 thyroid nodule cases, FNA sensitivity rate could be increased up to 96% when CNB was performed [16]. In a study conducted by Mais, during his interviews with 878 laboratories where the results of FNA were evaluated, there was an increasing demand for molecular diagnostic methods in FNA cases with insufficient results [21]. FNA was performed in a large series of 13351 cases by Ke et al., they reported sensitivity rates as 95.0% and specificity rates as 63.9% [22].

Despite the positive results reported, a meta-analysis indicated of non-diagnostic results at 12.9% based on the Bethesda system, suspicious for malignancy, 22.4 % of cases with FNA [7]; nondiagnostic results, in 4.2-13.7% of patients [23]; Seningen et al. reported that, out of 1,945 cytologic results with FNA, 9.3%, 26.3%, 1.4%, 37.5%, and 25.6% were nondiagnostic, negative for malignancy, atypical, suspicious for malignancy and positive for malignancy, respectively [14].

However, as can be seen in our results (Table 2), these risks were not found in diagnostic methods with MMs, and high ASs has been observed compared to GpB studies. The analysis of MMs in serum provides an additional advantage since it is not as risky as invasive FNA and CNB methods. An important risk of FNA in TCs is the inoculation, embolism, or infarction of cancer cells along the needle path. Hayashi et al. in 11,745 FNA cases; they reported that they revealed tumors due to tumor cell implantation occurring along the needle pathway in 22 cases and lymph node FNA in 8 cases [24]. It has been reported that newly formed tumors are more aggressive than the primary tumors; 53% of the cases were new and in 10 years, 4 of these cases died due to cancer [24]. Kini et al. found that tumor cells caused infarction, as a result of FNA, and 28 of the cases had to be operated [25]. Given that FNA is performed annually in hundreds of thousands of patients, it can be said that tumor inoculation or infarction is an important risk in cancer patients.

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MMs in TCs do not only function in diagnosis, but also serve as key to personal prognosis and molecular therapy. In a study by Gomez et al. in 60 different types of PTC patients, they measured the levels of miR-146, miR-221 and miR-222 in serum, and reported that their levels increased in cases with poor prognosis [26].

RET mutations are more common in hereditary type medullary thyroid carcinoma (MTC). Hereditary type MTC cases where RET mutations exist show a more aggressive clinical course [27]. In a study by Shabani et al, in 25 patients with RET(+) and RET(-), miR-144 and miR-34a levels in serum showed a relative increase in patients with MTC compared with normal control samples and also in RET(+) versus RET(-) patients [27].

Fanfone et al. reported high expression of galectin-1 (Gal-1) in TCs in a study in mice for the diagnosis of TC [28]. In a prospective study by Makki et al., serum values of 5 proteins were measured in patients with PTC, and they were disclosed that galectin-3 (Gal-3) and tissue inhibitor of metalloproteinase-1 (TIMP-1) values in malignant cases were elevated [29]. Abooshahab et al. revealed that increased serum values of citrate and lactate are important in the diagnosis of TCs [30]. Bircan et al. reported that mitochondrial DNA defects have a role in cancer formation. They performed a study with 48 PTC patients and showed that mtDNAD310 instability in the blood may play a role in PTC tumorigenicity [31].

Sedaghati et al. stated that the detection of long noncoding RNAs in serum has an important role in the initiation and prognosis of TCs [32]. In a study conducted by Li et al. they reported that serum Gal-3 level is increased in TCs as well as in many other cancer types [33].

In this study, comparison of ASs of the serum MMs with the other risky invasive methods (FNA and CNB) revealed that ASs of MMs in serum are significantly higher ($p < 0.05$) and more effective in the diagnosis of TC (Table 3, Fig 2).

In a study by Oloomi et al., in TC the levels of some biomarkers (Carcinoembroyonic Antigen (CEA), Estrogen Reseptor (ER) beta, cytokeratin19 and proto-oncogene) were determined in tissue and serum and it was found that although the tissue levels of these markers remain the same, serum levels increased thus it was considered that they may have high potential in the diagnosis of TCs [34].

The results of this study support the data reported by Oloomi et al. (Table 2, Fig 2).
Another important advantage of MMs in TCs is that their level in serum could be measured even in the existence of small (2 cm or less) TC nodules and significatly high ASs is obtained. In a study by Rezai et al., in patients with 2-cm-small diameter TCs, miR-22 levels were found significantly higher than that of the control group [12]. However, in cases where FNA was performed, the accuracy rates may decrease when the nodule diameter falls below 1 cm [5,7]. Another advantage of diagnostic tests with MMs in TCs is that its cost-effectivenes. In a study by Li, diagnostic tests with MMs in indeterminate nodules have been shown to be more cost-effective [35].
According to the results of Nylen et al.'s study, it has been reported that the sensitivity and specificity of the results obtained with miRNA panels in the diagnosis of thyroid cancers are higher than the single tests [36]. When evaluated in terms of AS rates, the results obtained in our study are compatible with the results obtained by Nylen (Table 3)
In another study by Rosignalo et al., by examining serum miRNA profiles in PTCs, reported that a more conservative approach can be used in the follow-up of patients [37]. In their study, 754 miRNA expression profiles were first examined in 11 PTC patients. In this study, promising miRNAs were re-evaluated in 44 PTC patients and 20 healthy control cases by absolute quantitative polymerase chain reaction analysis. 20 PTC patients were followed for 2 years. When the expressions of miRNAs in serum before and after 30 days, 1 year and 2 years were measured, it was revealed that miRNA-146a-5p and miRNA -221-3p were consistent with ATA responses in all patients [37]. According to the results obtained by Rosignolo, miRNA-146a-5p and miRNA -221-3p are superior to other MMs in the definitive diagnosis and follow-up of thyroid cancers [37].
The results we obtained in our study are consistent with the results of Rosignolo et al. (Table 3).
Mahmoudian et al. reviewed studies in the literature investigating the effect of serum MMs in the diagnosis of TCs; they reported that miRNA-375,34a, 145b, 221, 222, 155, Let-7, 181b were found effective [38].
Fan et al. performed circMAN 1A2 analysis in serum with quantitative real-time PCR test in many types of cancer and TCs, and reported that the diagnostic value was high in TCs [39].
In a study by Pilli et al., the roles of miRNA-95 and 190 were investigated in a large series of 1000 patients and compared with the FNA results [40]. According to their results, the sensitivity and specificity of miRNAs were higher than FNA results, however, they reported that the highest sensitivity and specificity rates could be achieved when performed together with FNA [40].
Zhang et al. investigated serum miRNA expressions in 47 PTC, 35 benign thyroid nodules, and 40 healthy control cases [41]. They reported that expressions of miRNA-222, miRNA-221, miRNA-146b and miRNA-21 were higher than the benign thyroid nodules (BTN) and control group in TCs, sensitivity and specificity ratios increased when combined with USG, and miRNA expressions were in parallel with poor prognosis [41].

The inventions in the analysis of serum MMs in TCs have had a significant impact on the diagnostic and follow-up methods of TCs. Serum MMs are noninvazive and not risky at all; no inoculation, embolism, or infarction of TC cells (like in FNA and CNB) will occur.

In addition, serum MM is more cost-effective in the diagnosis of TCs.
The limited aspects of our study; there are very few publications in the literature in which absolute sensitivity rates are measured and costs are evaluated in the diagnosis of thyroid cancers. The superior aspect of our study; This is the first study comparing serum molecular markers with invasive FNA and CNB methods as a non-invasive screening and definitive diagnostic method.

5. CONCLUSION

According to our results, in serum MMs; it is a noninvasive method that can be used safely in screening, definitive diagnosis and follow-up of TC. Although it is a noninvasive method, its definitive diagnosis rates are higher compared to methods such as invasive FNA and CNB. Therefore, in the near future; it is likely to take a higher priority in the diagnostic approach and screening programs for thyroid nodules.

6. ABBREVIATIONS

AS: Absolute Sensitivity; MMs: Molecular Markers; CNB: Core Needle Biopsy; 2D-SWE: Two-Dimensional Shear Wave Elastography; FNA: Fine Needle Aspiration Biopsy; IGF1: Insulin-like Growth Factor 1; IGF2: Insulin-like Growth Factor 2; MR: Magnetic Resonance; MTC: Medullary Thyroid Carcinoma; PTC: Papillary Thyroid Cancer; TCs: Thyroid Cancers; USG: Ultrasonography

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