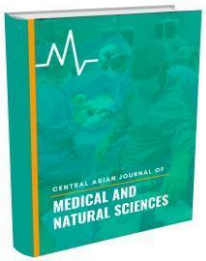

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Article

Beyond Biopsy: Could Nrf2 and microRNA-222 Redefine Thyroid Nodule Diagnosis?

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Abstract: Thyroid nodules are common and present a diagnostic challenge. Fine-needle aspiration cytology (FNAC) is frequently used but may yield indeterminate results. Molecular biomarkers such as Nuclear factor erythroid 2–related factor 2 (Nrf2) and microRNA 222 (miR 222) have emerged as promising tools to improve diagnostic accuracy. A case-control study was conducted on 87 subjects: 16 patients with papillary thyroid carcinoma (PTC), 39 patients with benign thyroid disorders, and 32 healthy controls. The study was conducted at Safeer Al-Imam Al-Hussain (A.S) Surgical Hospital and Al-Kafeel Superspeciality Hospital in Kerbala city.Serum Nrf2 levels were measured using ELISA, while miR 222 expression was analyzed using RT-PCR. Fold change (FC) of miR 222 showed the highest sensitivity (96.88%) and NPV (83.33%) in detecting simple nodules, with an AUC of 0.650. Ct miR 222 demonstrated the highest specificity (83.33%) and PPV (89.47%), making it useful as a confirmatory marker. Nrf2 exhibited poor diagnostic performance (AUC = 0.480). In multinodular cases, both Nrf2 and FC miR 222 showed fair discrimination ability (AUCs ≈ 0.636–0.637). The study confirms the high prevalence of multifocal benign nodularity in PTC patients (61.8%). Overall, the modest AUC values for all markers suggest that no single biomarker is sufficient for definitive diagnosis of thyroid nodules. The choice of biomarker depends on clinical objectives: FC microRNA-222 for maximizing detection (screening) and Ct.microRNA-222 (for simple nodules) or Nrf2 (for multi-nodules) for confirming diagnoses and minimizing false positives. Future research should focus on combining these biomarkers into panels to improve diagnostic accuracy.

Keywords: Thyroid Nodules, PTC, Benign Thyroid Diseases, Nrf2, Microrna-222

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1. Introduction

Nodules in the thyroid gland, whether solitary or multiple, are very common in clinical practice. Thyroid nodules are detected in approximately 5-7% of an adult population upon physical examination. Since modern ultrasound (US) techniques can detect small nodules, the frequency of thyroid nodules has been reported as high as 67% in unselected subjects[1].Thyroid nodules are clinically important because they can represents thyroid cancer, which occurs approximately 10-15% of nodules[2].Other consideration are the risk of thyroid dysfunction (autonomous adenoma and toxic multinodular goiter), compressive symptoms and some cosmetic concern. The main concern of patients and physicians is to diagnosed the suspected cancers as rapidly and cost effectively as possible and reduce unnecessary thyroid surgery [3].

A thyroid nodule can defined as a discrete lesion within the thyroid gland that is radiologically not be functional; accordingly, the exact morphological characteristics, thyroid functional status and pathological evaluation need to be assessed [4]. The majority of thyroid nodules derive from thyroid follicular cells. Benign follicular nodules, either solitary or as part of a multinodular goitre, are the most common masslesions. Thyroid cancer occurs in 7e15% of thyroid nodules[5].

Frequently tests that are used in a diagnosis of thyroid nodules include, medical history and physical examination ,ultrasound guided Fine Needle Aspiration (FNA) Scans and Xray(Ultrasound ,MRI Scan PET/CT scan) , blood tests (TSH,T3,T4,thyroglobulin, thyroglobulin antibody),and genetic testing only for an unclear diagnosis [6]. Fine needle aspiration biopsy (FNAB) is considered to be the most dependable technique for assessing thyroid nodules [7]. FNAB is highly sensitive thyroid morphological examination when used for differentiation between benign and malignant thyroid nodules [8]. However, it is, an invasive procedure and has a number of potential complications [9].The use of molecular markers in thyroid nodules has been suggested for diagnostic purpose in case of indeterminate cytological diagnosis, to assist with decision making about management option (surgical treatment). Since the majority of these nodules are benign, surgical excision led to unnecessary surgery with its associated risks and increased health care costs[10]. Molecular testing not only reduces the rate of unnecessary thyroidectomy but also may play a role in guiding the appropriate extent of surgery[11].In addition to its diagnostic role in thyroid nodules with indeterminate cytology, molecular testing also may also serve as a prognostic tool for assessing the risk of thyroid cancer recurrences and help in risk stratification of Bethesda V and VI thyroid nodules[12-13]. Some studies have demonstrated that commercially available molecular testing assays can also provide preoperative data about the risk of early recurrence and/or distant metastatic disease in thyroid cancer based on the molecular findings. MiR-222, a member of the miR-221/222 family, is located on the X chromosome p11.3 of the human genome [14]. Mature miR-222 sequences have a hairpin precursor with different arms called the 5' or 3' arm, which are also known as -5p or -3p, respectively [15-16]. Dysregulated miR-222-3p expression has been reported in various human diseases[17- 18]. In 2012, Yu and coworkers demonstrated that level of serum miR-222 was significantly higher in the PTC group than in healthy controls or those with benign nodules [19]. Nrf2 was initially cloned from the human leukemia cell line (K562) and identified as a Cap-n-collar (CNC) alkaline leucine zipper transcription factor family member [20].Nrf2 plays a crucial role in antioxidant defense and thyroid hormone synthesis, making it relevant to thyroid physiology and pathophysiology[21].Studies indicate that Nrf2 is highly expressed in Thyroid cancer tissues compared to benign nodules and normal thyroid tissue.This suggests Nrf2 could be a valuable tool for differentiating between benign and malignant thyroid nodules [22]. In this study, would examine whether Nrf2 and microRNA-222 could redefine thyroid nodule diagnosis?

2. Materials and Methods

Patients: This study includes a case-control study for a group of 87 samples: 16 samples for patients with papillary thyroid carcinoma, 39 samples for patients with benign thyroid diseases and 32 healthy control samples. Study was conducted from October 2024 to September 2025, cases of thyroidectomy for different causes were analyzed ,regarding sex, age and type of thyroid pathology ,whether benign or malignant at Safeer Al-Imam Al-Hussain(A.S)Surgical Hospital and Al-Kafeel Superspeciality Hospital in Kerbala city.The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire) including age, gender, history of family, smoking state, job, duration of disease also weights and heights. Inclusion criteria: papillary thyroid cancer, benign thyroid nodules , non nodular diseases. Exclusion criteria: Patients with other types of thyroid cancer or non-specified thyroid diseases were excluded.

Sample collection: A structured questionnaire was specifically designed to obtain information which helps to select individuals according to the selection criteria of the study. Each patient's medicinal & social data was collected by a questionnaire which including: age, gender, smoking state, family history of disease, Ultrasound finding, Fine needle aspiration(cytology report) and histopathology report. The surgical procedure was performed on all patients, and final diagnoses were based upon pathological examination whose formalin-fixed paraffin embeded confirmed PTC (based on examination of nuclear features and histopathological finding) or benign nodules was recorded. The vein samples were collected from all cases and control. 5 mL of blood was drowned from patients pre operation using 5 ml disposable syringes, blood samples were aliquot into EDTA tube then stored in deepfreeze at -80C◦for microRNA222 and gel tube for nuclear factor erythroid2-related factor2(Nrf2) biomarker measurement. Gel tube samples left for 15min at room temperature and serum was separated by centrifuging for 10 minutes at approximately 4000 x g. Serum samples were aliquot into two eppendroff and store at -80°C to avoiding multiple freezing-thawing cycles and used to check the level of(Nrf2) by ELISA test. MicroRNA-222 signatures in all samples and control was analysis using TransScript Green One-step RT/Rl Enzyme Mix (Korea) was used for total RNA mesearment according to the manufacturer's instructions. Total RNA was purified using an RNAClean XP Kit and RNase-Free DNase Set., and Nrf2 levels were measured by ELISA test using Elabscience® Human NFE2L2(Nuclear Factor ,ErythroidDerived2,like2) Elisa Kit (USA).

The ethical approval : The hospital ethics committee approved the study plan, and all patients or their relatives were informed. The Ethical Committee at Kerbala University- College of Medicine issued an ethics certificate(No. 24-64 dated October10, 2024), The kerbala health directorate approved our study ,No.3672 on October 21, 2024 and Al-Kafeel Superspeciality Hospital in Kerbala city gave their approval to study plan, No. 4562 on December 2. Verbal approval was taken from all patients included in the study.

Statistical Analysis: Information from the questionnaire from all participants was entered into a data sheet and was assigned a serial identifier number. Multiple entries were used to avoid errors. The data analysis for this work was generated using graphical pad presim 9. Descriptive statistics were performed on the participants' data of each group. Values were illustrated by n (%) for categorical. The distribution of the data was checked using the Shapiro-Wilk test as numerical means of assessing normality.

3. Results

The demographic characteristics of the study groups, including healthy controls, benign hyperplasia patients, and papillary thyroid carcinoma (PTC) patients.

The Healthy Control Group consisted of individuals with a mean age of 39.09 ± 14.33 years The gender distribution in this group was predominantly female, comprising 26 individuals (81.3%), while males accounted for 6 individuals (18.7%). The Benign Hyperplasia Group comprised 39 participants, accounting for 44.8% of the total study population. For the Papillary Thyroid Carcinoma (PTC) Group, 16 individuals were classified as PTC Stage 1, representing 18.4% of the total study population, with a mean age of 44.81 ± 10.26 years. The gender distribution within this group showed a strong female predominance, with 48 individuals (86%) being female and 8 individuals (14%) being male.

The majority of patient samples 39, were obtained from AL-Safeer Hospital. ALKafeel Hospital contributed 13 samples while Al-Hussaini Hospital provided 3 samples, as presented in Figure (1).

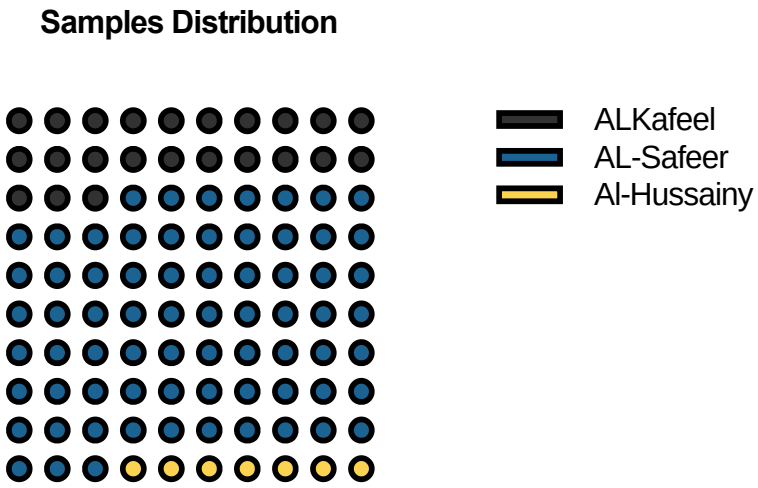

Figure 1. Distribution of samples collection from different hospitals.

Among the papillary thyroid carcinoma patients, the distribution of nodule types is presented in Table 1. The majority of patients had multiple nodules, accounting for 61.8%. Patients with a simple nodule represented 21.2% of the group. This finding indicates that multifocal benign nodularity is very common in patients who are subsequently diagnosed with papillary thyroid carcinoma.

Table 1. Frequency of the nodule's types among benign lesion & papillary thyroid carcinoma patients group.

Nodule's Types	Percent%
Multiple	61.8
Simple	21.2

Diagnostic performance of studied biomarkers in discriminating between simple nodules patients and healthy controls

Nuclear factor erythroid2-related factor2 (Nrf2), and Folding Change microRNA222 were used for differentiating between patients with simple nodules and healthy controls. The evaluation included optimal cutpoints, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the Area Under the Receiver Operating Characteristic Curve (AUC), presented in Table 2 & Figure 2. Comparing the diagnostic abilities of the three markers, FC microRNA-222 demonstrated the highest discriminatory power with an AUC of 0.650, suggesting a fair ability to distinguish between the simple nodule group and healthy controls. Ct.microRNA-222 followed with an AUC of 0.598, indicating slightly better than chance performance. Nrf2 showed the lowest discriminatory power with an AUC of 0.480, which is close to random chance, suggesting limited diagnostic utility.

In terms of sensitivity, FC microRNA-222 stood out with an exceptionally high sensitivity of 96.88%, meaning it was highly effective at correctly identifying individuals with simple nodules. Nrf2 and Ct.microRNA-222 had considerably lower sensitivities of 56.25% and 53.12%, respectively.

Regarding specificity, Ct.microRNA-222 exhibited the highest specificity of 83.33%, indicating its strong ability to correctly identify healthy individuals. Nrf2 had a moderate specificity of 58.33%, while FC microRNA-222 had the lowest specificity at 41.67%.

For positive predictive value (PPV), Ct.microRNA-222 showed the highest PPV (89.47%), suggesting that a positive test result for Ct.microRNA-222 was most likely to indicate a simple nodule. FC microRNA-222 followed closely with a PPV of 81.58%, and Nrf2 had a PPV of 78.26%.

Conversely, for negative predictive value (NPV), FC microRNA-222 had the highest NPV (83.33%), indicating that a negative test result for FC microRNA-222 was highly likely to rule out a simple nodule. Nrf2 and Ct.microRNA-222 had significantly lower NPVs of 33.33% and 40%, respectively. The high NPV (83.33%). However, the relatively low specificity of FC microRNA-222 (41.67%) indicates that it may frequently misclassify healthy individuals as having a simple nodule, leading to a higher rate of false positives. Its moderate AUC (0.598) indicates some utility, but its lower sensitivity means it would miss a significant number of simple nodule cases.

Nrf2, with its AUC of 0.480, appears to have very limited diagnostic value in distinguishing simple nodule patients from healthy controls. Its low sensitivity, specificity, and NPV suggest it is not a reliable standalone biomarker for this purpose. The low NPV of Nrf2 (33.33%) is particularly problematic, as a negative result would not be very reassuring for ruling out a simple nodule.. Nrf2 followed very closely with an AUC of 0.636, essentially demonstrating comparable performance. Ct.microRNA-222 exhibited the lowest discriminatory power with an AUC of 0.475, which is close to random chance, suggesting minimal diagnostic utility.

In terms of sensitivity, FC microRNA-222 had the highest (65.62%), implying it was most effective at correctly identifying individuals with multi-nodules. Nrf2 had a sensitivity of 56.25%, and Ct.microRNA-222 had the lowest at 50%.

Regarding specificity, Nrf2 showed the highest (67.44%), indicating its best ability to correctly identify healthy individuals. FC microRNA-222 had a specificity of 62.79%, while Ct.microRNA-222 had the lowest at 58.14%.

For positive predictive value (PPV), FC microRNA-222 had the highest (56.76%), suggesting that a positive test result for FC microRNA-222 was slightly more likely to indicate multi-nodules. Nrf2's PPV was 56.25%, and Ct.microRNA-222 had the lowest at 47.06%.

Conversely, for negative predictive value (NPV), FC microRNA-222 had the highest (71.05%), indicating that a negative test result for FC microRNA-222 was most likely to rule out multi-nodules. Nrf2 followed with an NPV of 67.44%, and Ct.microRNA-222 had the lowest at 60.98%.

Results were shown that both FC microRNA-222 and Nrf2 illustrated a comparable and modest diagnostic utility in distinguishing multi-nodule patients from healthy controls, as indicated by their similar AUC values (approximately 0.636-0.637). An AUC in this range suggests that these markers have some discriminatory ability, but they are not highly accurate on their own for this purpose.

Table 2. AUC, optimal threshold, Sensitivity, and specificity of Serum Levels Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222 among Simple nodule Group and Healthy Control.

	Cutpoint	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
Nrf2	0.061	56.25%	58.33%	78.26%	33.33%	0.480
microRNA222	23.88	53.12%	83.33%	89.47%	40%	0.598
FC	3.72e-06	96.88%	41.67%	81.58%	83.33%	0.650

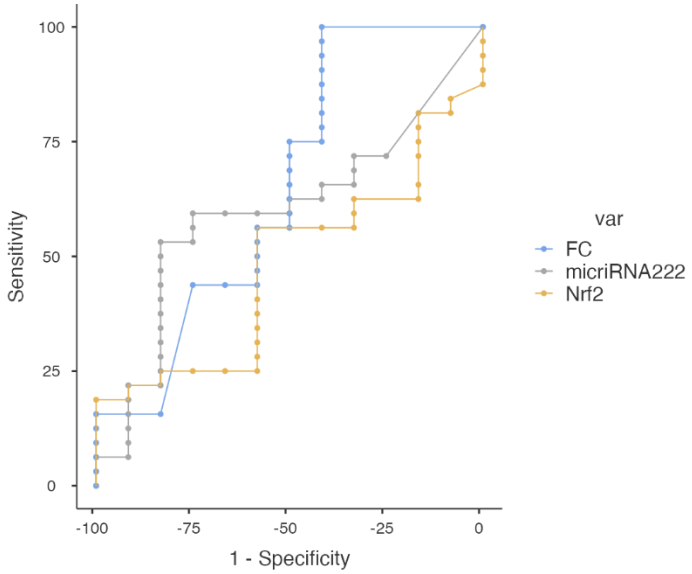

Figure 2. ROC curves of Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222 serum levels among Simple nodule patients group to analyze the optimal diagnostic points for predicting such cases compared to control group.

Comparing the performance of these markers in classifying patients within the simple nodule group, FC microRNA-222 demonstrated the highest number of true positives (19 TP), suggesting it was slightly more effective at correctly identifying individuals with simple nodules among those classified as positive. Nrf2 followed closely with 18 TP, and Ct.microRNA-222 had 17 TP, as presented in Table 3

In terms of minimizing false negatives (FN), Nrf2 showed the lowest count (14 FN), meaning it missed fewer actual simple nodule cases compared to Ct.microRNA-222 (15 FN) and FC microRNA-222 (13 FN).

For true negatives (TN), Ct.microRNA-222 had the highest count (10 TN), indicating it was best at correctly identifying individuals without the nodule within the negative group. FC microRNA-222 followed with 9 TN, while Nrf2 had the lowest true negative count (7 TN).

Regarding false positives (FP), Ct.microRNA-222 exhibited the lowest number (2 FP), suggesting it was most accurate in avoiding misclassifying healthy individuals as having a nodule. FC microRNA-222 had 3 FP, and Nrf2 had the highest number of false positives (5 FP).

Table 3. Distribution of Simple nodule Group according to Suggested Cutoff Value of Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222.

Cutoff value Nrf2			**Cutoff value: microRNA222**			**Cutoff value:FC**		
	0.061			23.88			3.71897e-06	
	Negative	Positive		Negative	Positive		Negative	Positive
Negative	7 (TN)	5 (FP)	Negative	10 (TN)	2 (FP)	Negative	9 (TN)	3 (FP)
Positive	14 (FN)	18 (TP)	Positive	15 (FN)	17 (TP)	Positive	13 (FN)	19 (TP)

Diagnostic performance of studied biomarkers in discriminating between multi-nodules patients and healthy controls

In order to differentiated between patients with multi-nodules and healthy controls, an evaluation of the diagnostic performance of serum levels of Nuclear factor erythroid2-related factor2 (Nrf2), Ct. microRNA-222, and Folding Change (FC) microRNA-222 was performed and presented in Table 4 and Figure 3.

Table 4. AUC, optimal threshold, Sensitivity, and specificity of Serum Levels Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222 among Multi-nodule Group and Healthy Control.

	Cutpoint	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
Nrf2	0.061	56.25%	67.44%	56.25%	67.44%	0.636
microRNA222	24.3	50%	58.14%	47.06%	60.98%	0.475
FC	0.1965	65.62%	62.79%	56.76%	71.05%	0.637

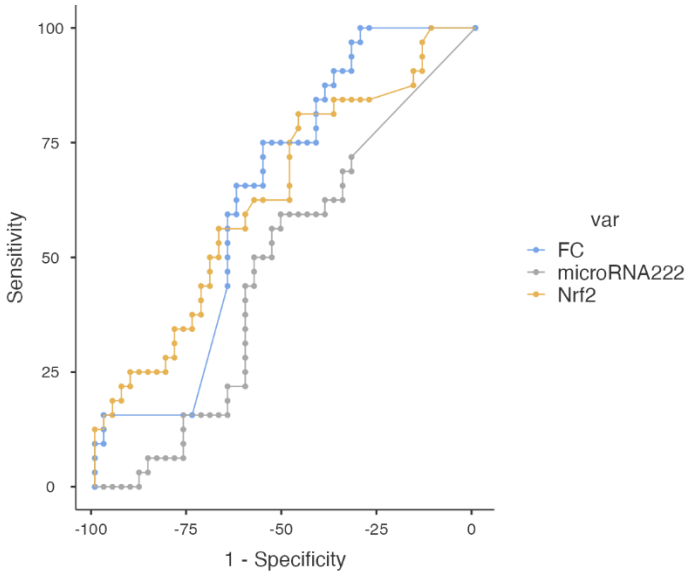

Figure 3. ROC curves of Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222 serum levels among Multi-nodule patients group to analyze the optimal diagnostic points for predicting such cases compared to control group.

The performance of these markers in classifying patients within the multi-nodule group was compared, FC microRNA-222stands out for its highest number of true positives (21 TP), indicating it's most effective at correctly identifying individuals with multi-nodules among those who test positive. Nrf2 followed with 18 TP, and Ct.microRNA-222 had the lowest at 16 TP.

In terms of minimizing false negatives (FN) which means not missing actual multi-nodule cases FC microRNA-222also performed best with only 11 FN. Nrf2 had 14 FN, while Ct.microRNA-222 had the highest number of false negatives at 16.

For correctly identifying individuals without multi-nodules (true negatives, TN), Nrf2 led with 29 TN. FC microRNA-222 had 27 TN, and Ct.microRNA-222 had 25 TN.

Regarding false positives (FP) incorrectly identifying someone without multi-nodules as having them Nrf2 had the lowest count at 14 FP. FC microRNA-222 followed with 16 FP, and Ct.microRNA-222 had the highest at 18 FP, presented in Table 5.

Table 5. Distribution of Multi-nodule Group according to Suggested Cutoff Value of Nuclear respiratory factor 2 (Nrf2), Ct.microRNA222 and Folding Change microRNA222.

Cutoff value Nrf2			**Cutoff value: micr0RNA222**			**Cutoff value:FC**		
	0.061			24.3			0.196	
	Negative	Positive		Negative	Positive		Negative	Positive
Negative	29 (TN)	14 (FP)	Negative	25 (TN)	18 (FP)	Negative	27 (TN)	16 (FP)

Positive	14 (FN)	18 (TP)	Positive	16 (FN)	16 (TP)	Positive	11 (FN)	21 (TP)

4. Discussion

The findings suggest that FC microRNA-222, at its optimal cutpoint, holds the most promise as a biomarker for identifying simple nodules, primarily due to its exceptionally high sensitivity and good negative predictive value. A sensitivity of nearly 97% means that very few simple nodule cases would be missed, making it a valuable tool for screening or ruling out the condition.

Ct.microRNA-222, while having a lower sensitivity than FC microRNA-222, demonstrated the highest specificity and a strong positive predictive value. This suggests that Ct.microRNA-222 is particularly good at confirming the presence of a simple nodule when the test is positive and at correctly identifying healthy individuals.

In a clinical context, the choice of biomarker depends on the desired outcome. when the goal is to identify nearly all simple nodule cases, even at the expense of some false positives, FC microRNA-222 would be the preferred marker due to its high sensitivity. while, when the aim is to confirm a diagnosis and minimize false positives (e.g., before invasive procedures), Ct.microRNA-222, with its high specificity and PPV, might be more suitable.

The overall modest AUC values for all markers suggest that none of them are perfect diagnostic tools when used alone. This highlights the complex nature of simple nodule diagnosis. The analysis of these markers for the simple nodule group highlights their distinct strengths and weaknesses.

FC microRNA-222 appears to be a robust indicator for identifying positive cases, as it yields the highest number of true positives. This suggests that a positive result for FC microRNA-222, at its given cutoff, is highly indicative of a simple nodule. However, it also has a relatively higher false negative rate compared to Nrf2, implying it might miss a few actual simple nodule cases.

Nrf2 demonstrates a strong ability to correctly identify positive cases with 18 true positives and has the lowest number of false negatives. However, its lower true negative count and higher false positive count suggest it might be less specific, potentially leading to more healthy individuals being misclassified as having a nodule. This makes Nrf2 a potentially good screening tool where the goal is to capture as many true cases as possible, even at the expense of some false alarms.

Ct.microRNA-222 stands out for its high true negative count and remarkably low false positive count. This implies that Ct.microRNA-222 is highly specific in identifying individuals who genuinely do not have a simple nodule. However, its higher false negative count suggests it might miss a considerable number of actual simple nodule cases, making it less suitable as a primary screening tool.

When the clinical priority is to not miss a simple nodule, Nrf2 seems promising due to its low false negative rate. If the priority is to avoid misclassifying healthy individuals as having a nodule, Ct.microRNA-222 appears to be the most accurate. FC microRNA-222 strikes a balance, offering good true positive identification.

The performance of these individual markers suggests that a single biomarker might not be sufficient for comprehensive diagnosis or risk stratification in simple nodule cases. comparing the diagnostic capabilities of these markers in differentiating multi-nodule patients from healthy controls, FC microRNA-222 showed the highest discriminatory power with an AUC of 0.637, indicating a fair ability to distinguish between the two groups

FC microRNA-222 emerges as having a slight edge due to its higher sensitivity, meaning it's better at identifying actual multi-nodule cases. Its best NPV also makes it somewhat useful for ruling out the presence of multi-nodules. This implies that if a test for FC microRNA-222 is negative, there's a reasonable chance the individual does not have multi-nodules.

Nrf2, while having slightly lower sensitivity than FC microRNA-222, demonstrated the highest specificity. This means Nrf2 is better at correctly identifying healthy individuals, reducing the number of false positives. Its comparable AUC to FC microRNA-222 indicates that it could also be a relevant marker.

Ct.microRNA-222 performed poorly across all metrics, with an AUC very close to 0.5, signifying that its diagnostic ability is nearly equivalent to random chance. Its low sensitivity, specificity, PPV, and NPV indicate that it is not a reliable biomarker for distinguishing multi-nodule patients from healthy controls in this study.

Overall, while FC microRNA-222 and Nrf2 show some promise, their individual performances are not sufficiently robust for definitive diagnosis. The relatively low sensitivity of Nrf2 and the modest specificity of FC microRNA-222 mean that both markers would lead to a notable number of misclassifications when used alone. For instance, Nrf2 would miss a significant proportion of actual multi-nodule cases (low sensitivity), while FC microRNA-222 would incorrectly identify some healthy individuals as having multi-nodules (modest specificity). This analysis reveals varying strengths and weaknesses among Nrf2, Ct.microRNA-222, and FC microRNA-222 when used to classify patients within the multi-nodule group.

FC microRNA-222 appears to be the most promising marker in this context. Its highest number of true positives and lowest number of false negatives indicate it's superior at correctly identifying individuals who actually have multi-nodules and minimizing missed cases. This high sensitivity is particularly valuable in screening scenarios where it's critical not to overlook a condition. However, its false positive rate, though not the highest, suggests some healthy individuals might still be incorrectly classified.

Nrf2 demonstrates excellent specificity, as evidenced by its highest number of true negatives and lowest number of false positives. This makes Nrf2 a strong candidate for ruling out multi-nodules, meaning a negative test result is highly reliable. While it identifies a decent number of true positives, its false negative rate is higher than FC microRNA-222, suggesting it might miss more actual cases.

Ct.microRNA-222 generally shows the weakest performance among the three markers for this group. It has the lowest true positives, highest false negatives, and highest false positives. This indicates that, at the given cutoff, Ct.microRNA-222 is less effective at both identifying multi-nodules and correctly ruling them out.

In a clinical setting, the choice of which marker to prioritize would depend on the diagnostic goal. when the primary objective is to detect as many multi-nodule cases as possible to ensure no patient is missed (high sensitivity), FC microRNA-222would be the preferred marker. While when the goal is to minimize false alarms and confidently identify individuals who don'thave multi-nodules (high specificity), Nrf2 would be more suitable.

while these biomarkers show some potential, a single marker might not be entirely sufficient for a definitive diagnosis of multi-nodules. The finding that the majority of papillary thyroid carcinoma patients (61.8%) presented with multiple nodules aligns with existing literature, which frequently reports multifocality as a common characteristic of PTC, with some studies indicating its presence in 30-85% of cases [23]. This high prevalence of multifocal benign nodularity in patients subsequently diagnosed with PTC underscores the diagnostic challenge posed by thyroid nodules and highlights the need for effective screening and discriminatory tools.

Nrf2 showed limited diagnostic utility in this context, with an AUC of 0.480, close to random chance. Its low sensitivity (56.25%), specificity (58.33%), and particularly low NPV (33.33%) suggest it is not a reliable standalone biomarker for differentiating simple nodule patients from healthy controls. While Nrf2 is known to be activated in papillary thyroid carcinoma and plays a role in antioxidant responses [24]. its performance as a diagnostic marker in this specific study for simple nodules was not robust.

The findings regarding microRNA-222 are consistent with broader research indicating its involvement in thyroid cancer. MicroRNA-222, often studied alongside microRNA-221, has been identified as a potential biomarker for papillary thyroid carcinoma, with various studies reporting high AUC values, sensitivity, and specificity, particularly when used in combination with other miRNAs [25]. The AUC values observed in this study for individual microRNAs, while modest, contribute to the growing body of evidence on their diagnostic potential, albeit highlighting the need for further exploration into optimal cut-off values and combinations.

5. Conclusion

The overall modest AUC values for all markers across both simple and multi-nodule groups suggest that none of them are perfect diagnostic tools when used alone. This highlights the complex nature of thyroid nodule diagnosis, which often requires a multi-faceted approach. In a clinical setting, the choice of biomarker would depend on the specific diagnostic objective. For instance, if the goal is to maximize detection and minimize missed cases (e.g., for screening), biomarkers with high sensitivity like FC microRNA-222 would be preferred. Conversely, if the aim is to confirm a diagnosis and minimize false positives (e.g., before invasive procedures), markers with high specificity like Ct.microRNA-222 (for simple nodules) or Nrf2 (for multi-nodules) might be more suitable.

The findings underscore the potential of these biomarkers, particularly FC microRNA-222 and Nrf2, but also emphasize the limitations of single-marker approaches. Future research could explore the diagnostic performance of panels combining these and other promising biomarkers to achieve higher accuracy, sensitivity, and specificity, ultimately improving the diagnosis and management of thyroid nodules.

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