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Original Research Article

The diagnostic performance of microRNAs expression levels in cellular aspirate of solitary thyroid nodule overrides that of cytology

Tawfik Ahmed T. Hussein[1]*, Emad Eldein M. Abdel Hafez[2], Yasmin M. Marie,[3] Abdel Latif Elbalshy[4], Omnia Y. Habashy[3]

[1]Department of General Surgery, Mediclinic Airport Road Hospital, Abu Dhabi, UAE
[2]Department of General Surgery, Faculty of Medicine, Benha University, Egypt
[3]Department of Medical Biochemistry, Faculty of Medicine, Benha University, Egypt
[4]Department of Pathology, Faculty of Medicine, Benha University, Egypt

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***Correspondence:**
Dr. Tawfik Ahmed T. Hussein,
E-mail: tawfikaziz@hotmail.com

ABSTRACT

Background: Evaluation of the diagnostic performance of estimated expression levels of microRNAs 203a, 206 and 576 in the cellular aspirate of solitary thyroid nodule (STN) in comparison to cytological examination for identification of malignant TN.
Methods: The 74 patients with STN underwent clinical and US evaluation and gave blood samples for estimation of serum levels of thyroglobulin (TG) and anti-thyroglobulin antibodies (ATG). Then, fine-needle aspiration was performed to obtain cellular aspirate for cytological examination and estimation of levels of microRNAs. The appropriate surgical procedure was undertaken and the excised specimens were sent for pathological diagnosis.
Results: Pathologically, 19 nodules had papillary thyroid carcinoma (PTC), 6 nodules had follicular carcinoma and one medullary carcinoma, while 48 nodules were benign nodules. Cytological examination diagnosed malignancy in 5 specimens, while specimens were suspicious of malignancy, 25 specimens were diagnosed as benign and 14 specimens as non-diagnostic. Serum levels of TG and ATG were significantly higher with malignancy. Expression levels of miR-203a and 206 were significantly lower, while miR-576 were significantly higher in malignant than benign aspirates. Multivariate regression analysis showed more significant ability for miR-576 level than cytological examination as screening and for miR-203a than miR-206 as diagnostic for malignant aspirate and for PTC
Conclusions: Estimation of expression levels of microRNAs in cellular aspirate of STN is feasible and accurate for detection of the malignancy. Down-expression of miR-203a and over-expression of miR-576 in tissue aspirate are more diagnostic for TC and can specify PTC cases out of other TC cases.

Keywords: STN, Cellular aspirate, Cytological examination, MicroRNA, Expression levels

INTRODUCTION

The worldwide incidence of thyroid cancer (TC) has dramatically increased and ranks as the 13th most common cancer diagnosis overall and the 6th most common among women.[1] PTC is the most common histological subtype among the recently diagnosed TC patients, accounts for 90% of all cases, and is characterized by a high recurrence rate and poor prognosis.[2] Medullary thyroid carcinoma (MTC), which is thyroid C cell-derived malignant lesion, is characterized by poor differentiation and being the most aggressive type of TC.[3]

MicroRNAs (miR-) are small, non-coding RNAs that regulate the expression of a target mRNA, so altered

microRNAs expression is associated with deregulation of the target RNA expression with subsequent altered function of the resultant protein and development of pathological conditions of the affected organs.[4] The role of multiple microRNAs in pathogenesis of TC was recently declared; lower expression levels of miR-30b was found to promote TC via induction of high expression levels of ubiquitin-specific protease-22 in thyroid cells and downregulation of miR-27a could facilitate the development of PTC through promoting the expression of collagen, and calcium-binding EGF domain-containing protein-1 in thyroid tissue.[5,6] On contrary, upregulated Mir-449a suppresses PTC development through binding to the 3' un-translated region of Metadherin and downregulating its expression.[7]

The widespread use of diagnostic imaging and more sensitive diagnostic tools resulted in great increase in incidence of small and localized thyroid tumors; however, this may result in over-diagnosis of STN, and needs to be refined to guard against faulty surgical-decision making.[8] The preliminary diagnosis of STN depending on the cytological examination of fine needle aspirate (FNA) had certain fallacies especially for the nodules diagnosed cytologically as indeterminate nodules.[9]

Objectives

This study tried to evaluate the ability of preoperative estimation of the expression levels of microRNAs 203a, 206 and 576 in STN aspirate for predicting and differentiating TC nodules.

METHODS

Design

Multicenter prospective non-randomized clinical trial.

Setting

Departments of general surgery and medical biochemistry and molecular biology, faculty of medicine, Benha university, Egypt in conjunction the Mediclinic hospital airport road, Abu Dhabi, Emirate and multiple private surgical centers, Egypt.

Ethical considerations

The preliminary approval of the study protocol was obtained at June 2018 before the start of case collection and after completion at March 2023, the final approval was obtained (RC: 16-6-23). Finally, the study duration was extended from June 2018 to Sep 2023.

The study protocol

The study protocol entails two parts; firstly, all patients presenting by thyroid nodule were evaluated clinically and by thyroid US and lab investigation. Patients were prepared to obtain samples for FNAC and PCR and gave blood samples for estimation of serum levels of TG and ATG. Secondly, after surgical interference, according to surgical decision and results of preoperative investigations, the excised tissues were used for confirmatory histopathological examination using paraffin section.

Patients

All patients attending to the outpatient clinics with thyroid lesion were evaluated for the presence of any or more of the previously documented risk-factors for thyroid cancer; age younger than 20 or older than 70 years, male gender, family history of MTC or multiple endocrine neoplasia, history of previous head and neck irradiation especially during childhood, rapid growth, dysphagia, dysphonia.[10] Physical examination with special concern to findings that increase the suspicious of malignancy especially the presence of nodules >4 cm in size that showed discrepancy as regards its malignant potential, nodule is firm on palpation and/or fixed to adjacent tissue, or associated cervical lymphadenopathy especially if >1 cm or vocal cord immobility on flexible laryngoscopy.[11-13] US examination for detection of sonographic characteristics of TC especially the presence of microcalcification, increased intra-nodular flow, presence of irregular nodular margins, absence of halo or hypoechoic nodule.[14]

Exclusion criteria

Presence of synchronous or metachronous malignancies, distant metastasis, patients' fragility, uncompensated cardiac or liver diseases, autoimmune disorders, maintenance on immunosuppressant drugs, chronic renal diseases, refusal of surgery are the exclusion criteria.

Inclusion criteria

Patients with STN suspicious of being malignant, free of exclusion criteria and signed the written consent were enrolled in the study. Twenty of patients who found to have nodule of <1 cm and accepted to give blood samples and undergo FNA to give cellular samples as control group were also included in the study.

Diagnostic tools

Blood sampling were obtained and centrifuged to get serum samples that were used for ELISA estimation of serum levels of TG and ATG using abcam (abcam Inc., San Francisco, USA) ELISA kit (Cat. No. ab155441 and 178631, respectively).

Fine needle aspiration cytology (FNAC): Ultrasound-guided FNAC was performed to allow proper localization of the nodule especially small nodules and to guard against decreased number of inadequate specimens and

false negative results as previously documented.[15] The obtained aspirate specimen was divided into two parts; one part was sent for cytological examination and results were interpreted according to national cancer institute thyroid fine-needle aspiration state of the science conference as benign which is represented by the cytological findings of the colloid and cellular thyroid nodule; suspicious for malignancy on cytological finding of a follicular neoplasm and malignant if the cytological findings according to the cytomorphological criteria indicates thyroid malignant lesion or non-diagnostic.[16] The other part was put in a clean dry Eppindorff tube and kept frozen at -80ºC for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for estimation of cellular expression of genes of microRNAs 203a, 206 and 576.

Histopathological examination of the excised specimen using paraffin section to reach the definite diagnosis to be used as a gold standard for comparisons.

Management plane

Management plan was designed according to the revised American thyroid association (ATA) management guidelines for patients with thyroid nodules as follows:[17] thyroid nodule of <1 cm on US were excluded and to repeat the US 1-y later and symptomatic thyroid nodule underwent CT or MRI followed by thyroid lobectomy and was excluded from the study. However, asymptomatic thyroid nodule of ≥1 cm on US was excluded if thyroid stimulating hormone level (TSH) is low and radioactive I^{131} uptake detected hot nodule, while in case of warm/cold nodules >4 cm on US imaging, irrespective of the level of TSH, normal or high, US-guided FNAC was performed and surgical intervention was undertaken according to the surgical decision.

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR)

Total RNA including microRNA was extracted from the homogenized aspirated cells using the miRNeasy mini kit (QIAGEN, Germany). The relative quantitation of the studied microRNAs were assessed by two-step real-time PCR using maxima SYBR green (QuantiTect SYBR Green PCR Kit: QIAGEN, catalog no. 218073, Str. 1-40724) and cDNA was synthesized using miScript II RT Kit (QIAGEN, Germany). The mixture of RNA starting amounts, buffers for reverse transcription (RT) reactions and the recommended RNA input were incubated for 60 min at 37ºC, for 5 min at 95ºC to inactivate the miScript RT Mix then placed on ice and later diluted by 40 µl RNase-free water to the 10 µl RT reaction and mixed gently then briefly centrifuged and continued with real-time PCR by the use of QuantiTect SYBR Green PCR Kit3 according to manufacturer's instructions (SYBR). The PCR reaction mix was prepared in a total volume of 25µl/tube (12.5 µl of 2× QuantiTect SYBR green PCR master Mix, 2.5 µl of 10x miScript primer assay, 2.5 ml of 10× miScript universal primer, template cDNA up to 250 ng and RNase-free water). The real-time cycler was programmed using ABI 7900HT fast real-time PCR system, (Applied biosystem, Singapore). The amplification level former-155 was programmed with a denaturation step at 95°C for 30 sec, followed by 40 cycles at 95°C for 10 sec and 60°C for 30 sec and the process is repeated for 40 cycles. The expression levels of microRNAs in each sample were determined after correction with the GADPH expression level. Controls were chosen as the reference samples, and fold changes in the levels of microRNAs were determined by the $2^{-\Delta\Delta}$CT (cycle threshold) method and expressed as fold change (FC) using Step One software (Applied biosystems, USA).

The sequences of the used primers for the detection of the expression levels of the studied microRNA.

Table 1: The sequences of the used primers.

Items	Sequences
miR-206-F	TGACAAAGGCAG-GAGGTA
miR-206-R	ATCTCTGGGTGCTGG-TGAAGG
miR-203a-F	ATCCAGTGCGTGTCGTG
miR-203a-R	TGCTGTGAAATGTTTAGGA
miR -576-F	TTGGGTCAAGAGTCAGAAGTTT
miR 576-R	TGGCTTCTACTTGTCCTTTCC
GAPDH-F	CCACCCATGGCAAATTCCATGGCA
GADDH-R	TCTAGACGGCAGGTCAGGTCCAC

Statistical analysis

One-way ANOVA test was used to determine the significance of the results using p at cutoff point of <0.5 as significant. Regression analysis and ROC curve analysis were performed to determine the diagnostic value for each variate. Paired analysis of difference of area under curve (AUC) was performed for each two variate to determine the best predictor. Kaplan-Meyer analysis was used to determine the cutoff point for the studied variate that might predict the cumulative hazard for malignancy diagnosis. Performance characters of lab variate were evaluated versus pathological diagnosis using the determined cutoff points. IBM® SPSS® statistics (Version 22, 2015; Armonk, USA) was for Windows statistical package was the applied system.

RESULTS

During the study duration 124 patients had STN; 5 patients had systemic diseases, 3 patients had autoimmune disorders, 2 patients had other malignancy and one patient had coagulopathy. Also, 31 patients had nodule of <1 cm and 8 patients had hot nodule and were excluded from the study and 74 patients were included in the study. Histopathological diagnosis of the excised specimens was 26 malignant nodules; 19 were diagnosed

as papillary carcinoma, 6 nodules were diagnosed as follicular carcinoma and one medullary carcinoma. The other 48 nodules were diagnosed as benign nodules; 31 nodules were diagnosed as follicular adenoma and 17 nodules were diagnosed as colloid adenoma (Figure 1). Enrolment data of the studied patients showed non-significant difference in comparison to that of controls (Table 1).

Cytological examination of FNA specimens diagnosed malignancy in 5 specimens (6.8%), 30 specimens (40.5%) were suspicious of malignancy, 25 specimens (33.8%) were diagnosed as benign and 14 specimens (18.9%) were diagnosed as non-diagnostic (Table 2).

The ROC curve analysis for serum levels of TG and ATG showed significantly high screening ability for both variate (Figure 2 A) and the paired analysis for AUC defined non-significant (p=0.821) difference between their AUCs. Multivariate regression analysis defined estimation of serum TG as the more significant (β=0.427, p<0.001) screening test for malignant STN in comparison to serum ATG levels (β=0.380, p=0.002). Comparative diagnostic ability of cytology versus estimation of the expression levels of microRNAs in cellular aspirate using ROC curve analysis showed significant (p<0.001) screening ability for both variates to detected malignancy (Figure 2 B) with non-significant (p=0.145) difference between both AUCs. However, multivariate regression analysis showed more significant ability for miR-576 level (β=0.593, p<0.001) than cytological examination (β=0.238, p=0.016) as screening for malignant cellular aspirate.

Analysis of the estimated levels of the studied variate using ROC curve analysis to define the best predictors for malignancy in the aspirates of STN showed high AUC for the cellular expression levels of miR-203a and 206 with high diagnostic ability, but paired analysis for AUC defined the expression levels of miR-203a as the superior diagnostic variate with significant (p=0.003) difference for its AUC in comparison to that of miR-206 (Table 4, Figure 2 C). Also, multivariate regression analysis for both variate defined low expression levels of miR-203a in cellular aspirate of STN as the more significant predictor (β=0.733, p<0.001) for malignancy than the low expression levels of Mir-206 (β=0.228, p=0.008).

Mean serum levels of TG and ATG were significantly higher in samples of patients than control samples and according to the results of the pathological examinations serum levels of both variates were significantly higher in samples of patients had malignant than in samples of patients had benign nodules. The mean levels of expression of genes of miR-203a and 206 in cellular aspirate of thyroid nodules of enrolled patients were significantly higher than levels estimated in cellular aspirate of controls with significantly lower levels in cellular aspirate of malignant nodules than that of benign nodules. On contrary, the mean expression level of miR-576 in cellular aspirate of STN was significantly higher than in cellular aspirate of controls with significantly higher levels in aspirate of malignant than in benign nodules (Table 3).

The ROC curve analysis for the performance of serum variate for distinguishing PTC among aspirates of malignant STN showed higher screening ability for serum TG levels than ATG levels, despite of the non-significant (p=0.551) difference between AUCs for both variate (Figure 3 A). Also, multivariate regression could not differentiate between serum TG (β=0.433, p<0.001) and serum ATG (β=0.378, p<0.001) levels as serum screening variate for PTC. Similarly, ROC curve analysis defined high ability for cytological examination and estimation of the expression levels of miR-576 as screening for PTC and paired analysis of difference of AUC showed non-significant (p=0.455) difference between their AUCs (Figure 3 B). However, multivariate regression analysis defined higher screening ability for expression level of Mir-576 (β=0.430, p<0.001) than for FNAC (β=0.296, p=0.004), but could not exclude FNAC as predictor for PTC.

On contrary, ROC curve analysis defined significantly (p<0.001) high AUC for the expression levels of miR-203a and 206 in cellular aspirate of STN as diagnostic variate for PTC, but paired sample analysis defined significant (p=0.006) difference between AUCs of both variate in favor of miR-203a (Table 5, Figure 3 C). Further, multivariate regression analysis showed higher significance of the standardized coefficient for level of miR-203a (β=0.608, p<0.001) than for level of miR-206 (β=0.242, p=0.007) in one model and in another statistical model assured the diagnostic ability of miR-203a for the presence of PTC cells in cellular aspirate (β=0.703, p<0.001).

Kaplan-Meyer regression analysis for the diagnostic level of expression of gene of miR-203a defined risk of malignancy of 100% in absence of expression of gene of miR-203a, but this risk decreased to about 80% at expression level of 0.15 and to 70% at level of 0.20, and decreased to 60% at level of 0.55 (Figure 4). Evaluation of the diagnostic performance of estimated level of miR-203a at cutoff point of ≤0.2 showed a sensitivity, specificity and accuracy rates of 92%, 91.8% and 92% with PPV and NPV for malignancy of 85.2% and 95.7%. On contrary, using cutoff point ≤0.55, the sensitivity rate and NPV were 100%, while specificity and accuracy rates and PPV were 30.6%, 54.1% and 42.4%.

Using Kaplan-Meyer regression analysis for the diagnostic ability of estimated expression level of miR-576 in cellular aspirate of STN showed at cutoff point of ≤4.5 the hazard for STN to be malignant is zero, but the hazard increases to 20% and 40% at cutoff points of ≥5 and ≥6 (Figure 5).

The diagnostic performance characters of these cutoff points were higher with cutoff point of ≥5 (Table 6).

Table 2: Enrolment data of studied patients.

Data		Control (n=20) (%)	Patients, (n=74) (%)	P value
Age (In years)	<30	1 (5)	1 (1.3)	0.079
	30-39	3 (15)	7 (9.5)	
	40-49	11 (55)	24 (32.4)	
	≥50	5 (25)	42 (56.8)	
	Mean (±SD)	45.5±7.5	48.5±6.5	0.075
	Range	26-55	29-58	
Gender	Male	6 (30)	25 (33.8)	0.749
	Female	14 (70)	49 (66.2)	
Body mass index (kg/m^2)	Average (<24.9)	1 (5)	5 (6.8)	0.165
	Overweight (25-30)	7 (35)	42 (56.8)	
	Obese (>30)	12 (60)	27 (36.4)	
	Mean (±SD)	30.1±2.9	29±2.8	0.121
	Range	23.05-33.5	23.4-34.4	

Table 3: Cytological and pathological diagnosis of the excised nodules.

Histopathological diagnosis, n (%)		Cytological diagnosis, n (%)			
		Benign	**Suspicious**	**Malignant**	**Non-diagnostic**
Colloid adenoma	17 (23)	10 (13.5)	4 (5.4)	0	3 (4.1)
Follicular adenoma	31 (41.9)	14 (18.9)	7 (9.5)	2 (2.7)	8 (10.8)
Follicular carcinoma	6 (8.1)	0	4 (5.4)	0	2 (2.7)
Papillary carcinoma	19 (25.7)	0	15 (20.3)	3 (4)	1 (1.4)
Medullary carcinoma	1 (1.4)	1 (1.4)	0	0	0
Total	74 (100)	25 (33.8)	30 (40.5)	5 (6.8)	14 (18.9)

Table 4: Mean levels of TG and ATG estimated in patients' serum samples and mean levels of expression of genes of miR-203a, 206 and 576 in nodular aspirate.

Variables		Control	Benign	Malignant	Total
TG (ng/ml)	Levels	21±2.3	24.2±1.75	27.7±2.94	25.4±2.79
	P vs. control		<0.001	<0.001	<0.001
	P vs. benign			0.0002	
ATG (IU/ml)	Levels	42.5±11.14	65.85±12.24	86.66±18	73.17±17.54
	P vs. control		<0.001	<0.001	<0.001
	P vs. benign			<0.001	
miR-203a	Levels	0.899±0.187	0.44±0.138	0.156±0.071	0.338±0.18
	P vs. control		<0.001	<0.001	<0.001
	P vs. benign			<0.001	
miR-206	Levels	3.81±1.537	1.649±0.388	1.082±0.527	1.45±0.516
	P vs. control		<0.001	<0.001	<0.001
	P vs. benign			<0.001	
miR-576	Levels	1.33±0.61	4.077±1.247	5.946±1.181	4.73±1.513
	P vs. control		<0.001	<0.001	<0.001
	P vs. benign			<0.001	

Table 5: Statistical analyses for estimated variate as predictors for malignancy in cellular aspirate of STN.

Statistical analyses	ROC curve				Paired-sample area difference under ROC curves			
	AUC	**Std. error**	**P**	**95% CI**	**AUC difference**	**Std. error**	**P**	**95% CI**
Serum TG	0.267	0.058	0.001	0.154-0.380	0.022	0.353	0.821	[-0.166]-0.209
Serum ATG	0.289	0.065	0.003	0.162-0.416				
FNAC	0.253	0.065	<0.001	0.125-0.381	-0.114	0.332	0.145	[-0.268]-0.040
miR-576	0.139	0.047	<0.001	0.047-0.230				
miR-203a	0.950	0.030	<0.001	0.891-1.000	-0.181	0.303	0.003	[-0.301]-[-0.062]
miR-206	0.768	0.062	<0.001	0.646-0.890				

Table 6: Statistical analyses for estimated variate as predictors for PTC among cellular aspirates of malignant STN.

Statistical analyses	ROC curve				Paired-sample area difference under ROC curves			
	AUC	Std. error	P	95% CI	AUC difference	Std. error	P	95% CI
Serum TG	0.227	0.056	<0.001	0.117-0.336	0.063	0.367	0.551	[-0.144]-0.270
Serum ATG	0.290	0.075	0.006	0.143-0.436				
FNAC	0.223	0.064	<0.001	0.098-0.347	-0.063	0.342	0.455	[-0.228]-0.102
Mir-576	0.160	0.056	<0.001	0.051-0.269				
miR-203a	0.981	0.012	<0.001	0.957-1.000	-0.197	0.286	0.006	[-0.336]-[-0.058]
Mir-206	0.784	0.069	<0.001	0.649-0.919				

Table 7: The diagnostic performance characters of cutoff points of estimated gene expression levels of miR-203a and 576 for malignancy in cellular aspirate of STN.

miR	Cutoff point		Sensitivity	Specificity	NPV	PPV	Accuracy
203a	≤0.20	%	92	91.8	95.7	85.2	91.9
		95% CI	74-99	80.4-97.7	85.6-98.8	69-93.7	83.2-97
	≤0.55	%	100	30.6	100	42.4	54.1
		95% CI	86.3-100	18.3-45.4		37.9-47	42-98.7
576	≤4.5	%	88.5	54.2	89.7	51.1	66.2
		95% CI	69.9-97.6	39.2-68.6	74.3-96.3	42.7-59.4	54.3-76.8
	≥5	%	80	79.6	88.6	66.7	79.7
		95% CI	59.3-93.2	65.7-89.8	77.9-94.5	52.7-78.2	68.8-88.2
	≥6	%	64	93.9	83.6	84.2	83.8
		95% CI	42.5-82	83.1-98.7	75.1-89.7	63.2-94.3	73.4-91.3

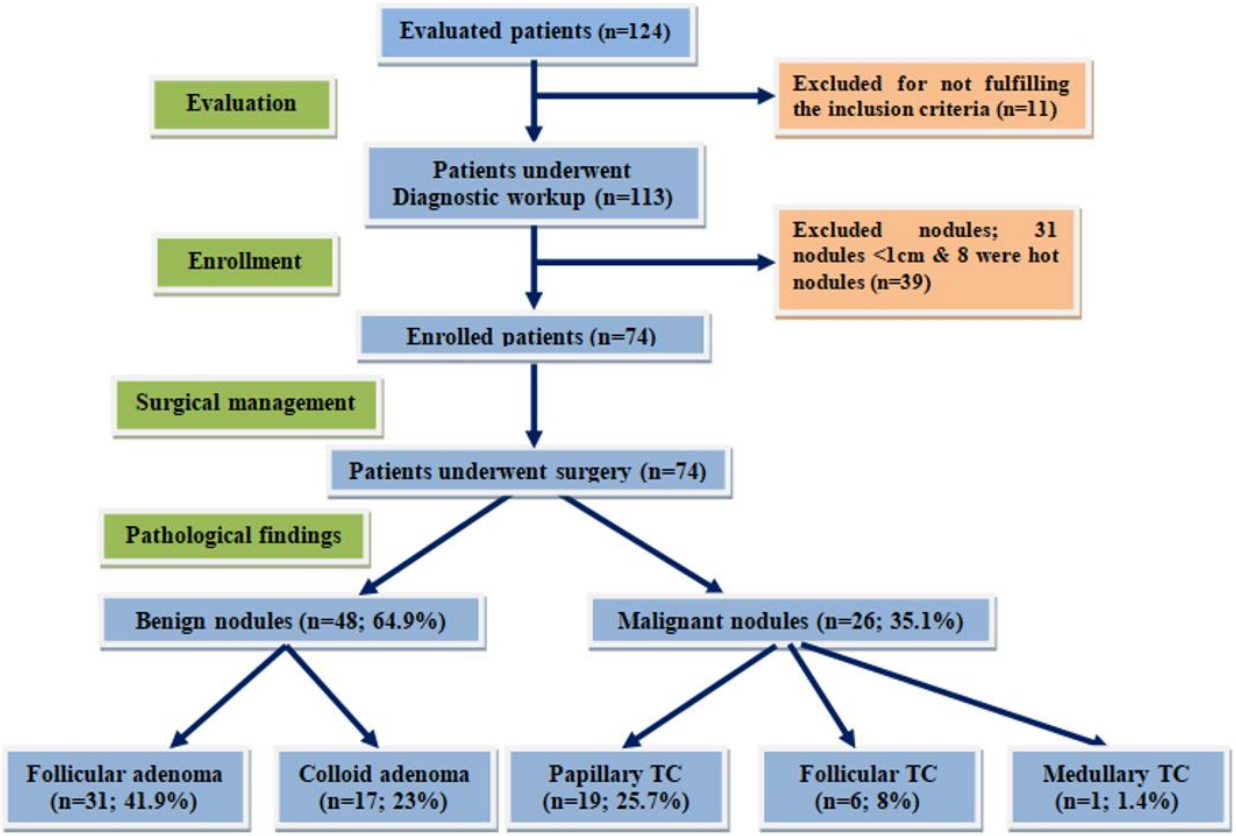

Figure 1: Study flow chart.

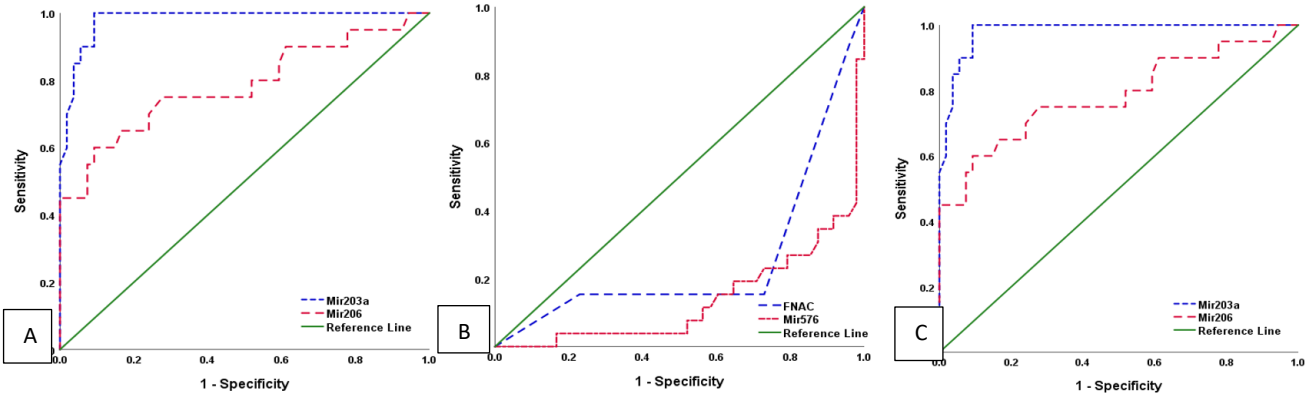

Figure 2 (A-C): ROC curve analysis for screening ability of serum levels of TG versus ATG for malignant STN. ROC curve analysis for screening ability of cytological examination versus estimation of expression levels of Mir-576 in cellular aspirate of STN. ROC curve analysis for diagnostic ability of expression levels of miR-203a and 206 in cellular aspirate of STN.

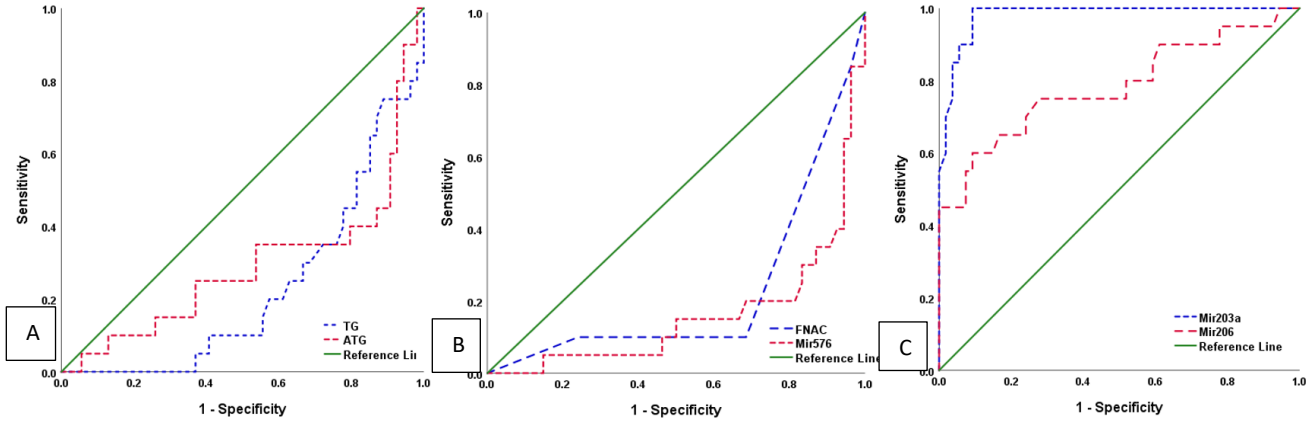

Figure 3 (A and C): ROC curve analysis for serum levels of TG versus ATG for identification of PTC. ROC curve analysis for FNAC versus expression level of Mir-576 for identification of PTC. ROC curve analysis for expression levels of genes of Mir-203 versus Mir-206 for the identification of PTC.

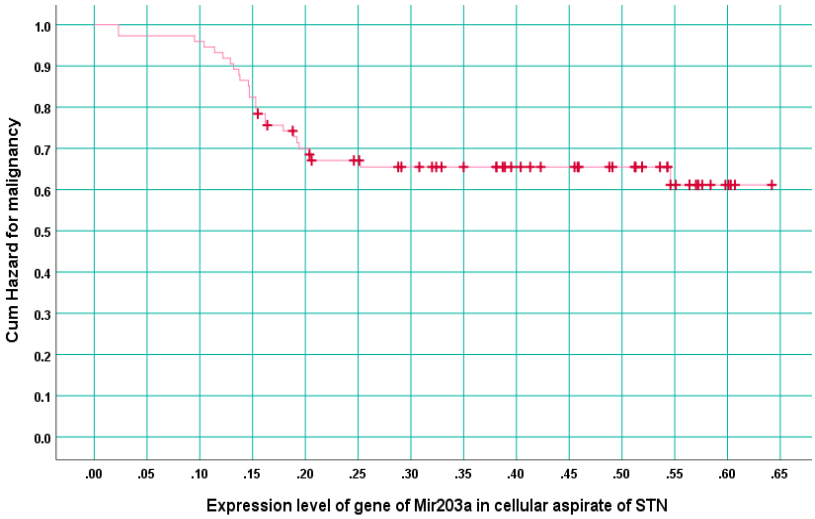

Figure 4: Hazard curve using Kaplan-Meyer analysis for the estimated expression levels of miR-203a for malignancy in cellular aspirate of STN.

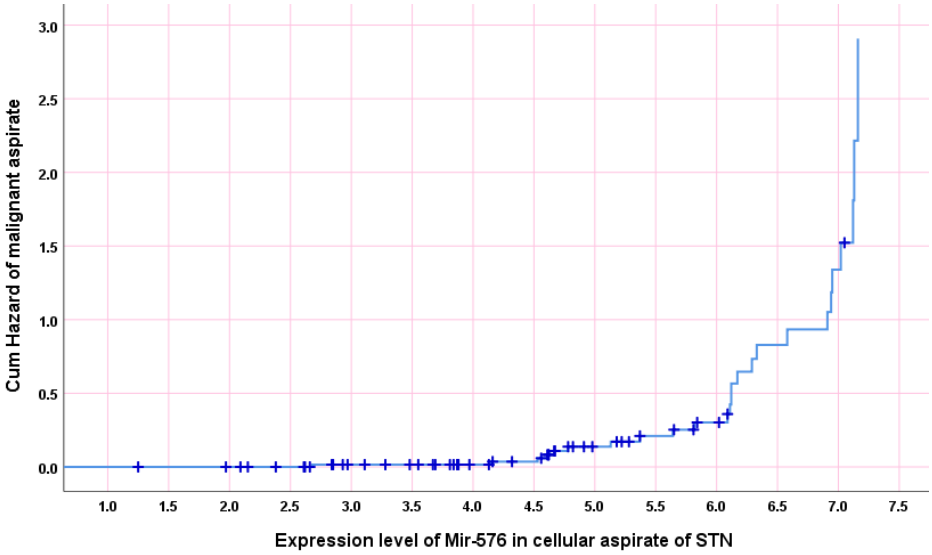

Figure 5: Hazard curve using Kaplan-Meyer analysis for the estimated expression levels of miR-576 for malignancy in cellular aspirate of STN.

DISCUSSION

Comparative analysis of the cytological and the microRNAs expression levels in the aspirate of the STN illustrated the high screening and diagnostic performance of the expression levels of microRNAs in comparison to cytological examination for distinguishing aspirates of malignant nodules. In support of these data, recent studies assured the diagnostic performance of microRNAs in thyroid tissue aspirates; wherein, Hua et al reported an association between TC tumorigenesis and deregulated expression levels of miR-211 and suggested upregulation of the long non-coding RNA Down syndrome cell adhesion molecule-antisense 1 suppressed the tumorigenesis of TC via regulating miR-211 expression levels.[18] Also, Toraih et al found upregulation of miR-146b-5p and miR-221-3p in thyroid tissue could predict lymph node metastases and recurrence of TC with high accuracy but miR-146b was more sensitive and specific than miR-221.[19] Further, Zhang et al detected significant upregulation of circ_0002111 in PTC samples and cells and mechanistically, tumorigenesis occurs through down regulating miR-134-5p with induction of PTC cell proliferation, migration and glycolytic metabolism.[20]

The reported down-expression of miR-206 and miR-203a could distinguish aspirates of PTC from other aspirates of STN, either benign or malignant with high specificity than cytological examination of cellular aspirate as judged by their high AUC. However, down-expression of miR-203a was found to be more diagnostic than down-expression of miR-206 for TC and PTC samples of STN aspirate as shown by the significant difference between the AUCs for both micro-RNAs.

In line with these findings, Liu et al found miR-206 was significantly down-regulated in PTC tissues and Lu et al identified downregulation of 5 microRNAs including miR-206 and found their expression levels were associated with the overall survival of PTC patients.[21,22] Recently, Yuan et al detected significantly decreased expression level of miR-206 in TC FNAC with high sensitivity and specificity and found negative relation between miR-206 expression levels and TNM staging and lymph node metastasis.[23]

Regarding miR-203a, detected down-regulated expression levels of miR-203a supported that previously reported by Wu et al who detected significantly lower expression levels of miR-203 in PTC tissues in comparison to normal controls and concluded that miR-203 could function as biomarker for PTC.[24] Also, Zhu et al detected decreased levels of miR-203 in relation to adjacent normal tissues and normal thyroid tissues and found miR-203 was implicated in tumor stage and lymph-node metastasis and can detect PTC with high sensitivity and specificity rates of 73.7%, and 84%, respectively and concluded that miR-203 satisfactory diagnostic value in PTC and its upregulation can inhibit PTC cell growth and promote cell apoptosis.[25] Also, Dai et al detected significantly decreased levels of miRNA-203a-3p in PTC tissues and Stojanović et al found down-regulated miR-203a-3p indicates presence of either PTC/follicular thyroid adenoma with high sensitivity and specificity.[26,27]

On contrary, the expression levels of miR-576 were upregulated in malignant than in benign FNAC and in aspirates of PTC and aspirates of other TC and were found to be more diagnostic than cellular findings. These data are in accordance with Hai et al who detected significant increase in the expression level of miR-576-5p in PTC tissues.[28]

Multiple studies tried to unearth the mechanisms underlying the roles of the studied microRNAs in TC in general and especially PTC, where Liu et al found up-

regulated miR-206 could inhibit the proliferation, induce apoptosis, suppress the expressions of multidrug resistance-related proteins in euthyrox-resistant PTC cells through blockage p38 mitogen-activated protein kinase and the c-Jun n-terminal kinase signalling pathway by targeting mitogen-activated protein 4-kinase 3.[21]

Thereafter, Wu et al using PTC cell lines found miR-203 regulates the expression of Bcl-2 via its downstream regulator survivin and inhibition of miR-203 histone acetylation was associated with high expression levels of miR-203 in PTC tissue samples.[24] Also, You et al found upregulated miR-203 levels might antagonize PTC through suppression of the epithelial-mesenchymal transition, proliferation, migration and invasion of PTC and induction of expression of apoptotic factors with subsequent apoptosis of PTC cells via downregulated AKT serine/threonine kinase 3, a protein coding gene, which is a key regulator of head and neck squamous cell carcinoma.[29,30] Also, Dai et al reported that miR-203a-3p inhibits cell proliferation with arresting the cell cycle process, preventing the metastatic abilities, activating autophagy and enhancing cell apoptosis via interaction with mitogen-activated protein 3-kinase 1.[26] Further, Hai et al *in vitro*, found overexpression of miR-576-5p promoted the proliferation, migration, and invasion of human PTC cell line-1 and attributed these findings to the ability of miR-576-5p to promote cellular proliferation by enhancing expression of mitogen-activated protein kinase-4 and activating the protein kinase B pathway.[28]

Limitation

The low prevalence of cancer thyroid in the study localities limited the number of cases.

CONCLUSION

Estimation of expression levels of microRNAs in tissue aspirate of STN is feasible and could be considered as more accurate diagnostic modality than detection of the malignant cells in the aspirate. Down-expression of miR-203a and over-expression of miR-576 in tissue aspirate are more diagnostic for TC and can specify PTC cases out of other TC cases.

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