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The Expression of FOXE-1 and STIP-1 in Papillary Thyroid Carcinoma and Their Relationship with Patient Prognosis

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ABSTRACT

Background & objective: Most patients with papillary carcinoma of the thyroid gland (PTC) havefavorable outcome,but sinceit has severe capability to invade the nearby tissues, there isa great risk of regional and distal lymph-nodes (LNs) metastases related to poor prognostic parameters, early recurrences, and distant metastasis that lead to bad patient outcome. Discovering other prognostic biomarkers for this cancer helps to detect early recurrences, invasion, expecting patient outcome, and possible use as therapeutic-targets for it. The *fork-head-box-E-1(FOX-E-1)*, with the alternative name of *thyroid-transcription- factor-2 (TTF-2)*, is one of thetranscription factors familiesthat is huge and containsa special fork-head-domain. It has a significant role in the differentiation and maturation of thyroid-follicular cells. Stress-induced phosphor-protein-1 (STIP-1), withthe alternative name ofheat-shock-protein-(HSP)-organizing protein,is a 62.6-kD protein, with three parts of tetra-trico-peptide repeats (TPR), and is capable of interaction with heat-shock proteins forming structures that haveplethora of roles in variable cellular processes;e.g., cell cycles regulations, transcriptions, and RNA splicing.

The current study aimed at exploring the relationship between *FOXE-1* and *STIP-1* expressions, the clinicopathological parameters, prognosis, and survival of patients with PTC.

Methods: The current studyexplored *FOXE-1* and *STIP-1* expressions by the immunohistochemical methods in 36 paraffin blocks retrieved from 36 patients of PTC, analyzed the relationships between their levels of expression,clinicopathological parameters, prognosis, and survival of patients.

Results: The high expression levels forboth *FOXE-1* and *STIP-1* in PTC were associated with larger size of the tumor, extra-thyroidal extension, vessels invasion, LNs spread (P <0.001), presence of distant metastases (P values = 0.005 and 0.012, respectively) and higher stages of the cancer (P values =0.012 and 0.042, respectively).

The *FOXE-1* over-expression was associated with shortened distant metastases free survival (DMFS) and shortened five-year overall survival rates (OS) (P <0.001).

Conclusion: Patients withadvanced PTC andunfavorable prognosis had high levels of both *FOXE-1* and *STIP-1* expressions.

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Introduction

Papillary carcinoma of the thyroid gland (PTC) is the commonest among all well-differentiated thyroid gland malignancies, representing 80% to 85% of all well-differentiated cancer-thyroid and its rates of occurrence has excessively increased globally (1). Most patients with that cancer develop favorable outcomes, but as it has marked liability to invade the nearby tissues, there is a great risk of regional and distal lymph-node (LN) metastases markedly correlated with poor prognostic parameters, early recurrences liability, and

distant metastasis that lead to bad outcomes in the patients (2,3). Therefore, there are real needs to discover novel biomarkers that can expect patient prognosis, survival, and detect early cancer recurrence and possible uses as therapeutic targets for such carcinoma. The *fork-head-box-E-1 (FOXE-1)*, with the alternative name of *thyroid-transcription-factor-2 (TTF-2)*, is one of a transcription factors families that is huge and contains a special fork-head-domain. It has a significant role in the differentiation and maturation of thyroid-follicular cells (4).

Stress-induced phosphor-protein-1 (STIP-1), with the alternative name of heat-shock-protein-organizing protein, is a 62.6-kD protein with three parts of tetra-trico-peptide repeats (T-P-R) and is capable of interaction with heat-shock-proteins (HSP) forming structures with plethora of roles in variable cellular processes; e.g., cell cycles regulations, transcriptions, signal transductions, protein folding, and RNA splicing (5-7). Expression of STIP-1 is also investigated in cancers of many organs, which point that STIP-1 might play important roles in stimulating tumorigenesis (8). There is little information about the prognostic values or clinical significances of combination of both *STIP-1* and *FOXE-1* immuno-expressions in PTC.

Therefore, the current study aimed at exploring the relationship between *FOXE-1* and *STIP-1* expressions, the clinicopathological parameters, prognosis, and survival of patients with PTC.

Materials and Methods

The current prospective cohort study included 200 patients with thyroid gland swelling admitted to General Surgery Department, Faculty of Medicine, Zagazig University Hospital, total thyroidectomy and diagnostic frozen section was done to all patients intra-operatively and the 36 cases found to have PTC were subjected to block-neck-dissection and were sent to Pathology Department Faculty of Zagazig University of Medical Sciences to complete the diagnosis and subsequent research. Routine hematoxylin and eosin (H&E) staining was attempted; then, immunohistochemical staining and evaluation of *FOXE-1* and *STIP-1* expressions were done on 36 paraffin blocks of all the 36 patients with PTC. The subjects were followed up for five years from January 2012 to January 2017 in both departments of clinical oncology and nuclear medicine as well as Medical Oncology Faculty of Zagazig University of Medical Sciences. Full patient pathological and clinical data were found in the patients` records. The current study used tumor, node and metastasis (TNM) staging system modified by the AJCC Cancer Staging Manual, the 7th edition for physicians for staging PTC (9).

The current study protocol was approved by the local ethical committee.

Immunohistochemical staining

The technique of streptavidin-biotin (10)was used for immunohistochemical staining with primary mouse monoclonal anti-FOX-E-1 antibody ab5080 diluted 1/50 (Abcam, MA, USA-Cambridge) and primary rabbit monoclonal-anti-STIP-1-antibody EPR-6606, ab126753, diluted 1:200. Human-heart tissue ovarian-carcinoma sections were used as positive controls for *FOXE-1* and *STIP-1* respectively; the negative controls were attempted by the non-immune serum instead of primary antibodies.

The current study evaluated the stained slides without previous information about patients' clinical data.

Evaluation of immunohistochemical expression of *FOXE-1*

The extent of stain in cancer cells was scored as: 1% to 33%, weak (+one); 34% to 66%, moderate (++two), 67% to 100%, strong (+++three), and 0, negative (-). Stain intensity was scored as follows: one, weak (+); two, moderate (++); three, strong (+++), and zero, negative (-).The intensity and the extent of the stain were summed to acquire the final staining index; then, such indices were scored from zero to six, and accordingly two stain-indices were used as a cutoff value above which was considered as overexpression and below which was considered as down-expression (11).

Evaluation of immunohistochemical expression of *STIP-1*

Stain-intensity was scored as: weak (one), moder-

ate (two), strong (three) or no stain (zero). The extent of stain in tumor cells was scored as follows: <25% (one), 25% to 50% (two), 50% to 75% (three), and >75% (four). Multiplication of the stain intensity by the extent allows calculating the final staining index, which scores from 0 to 12. The cutoff value of staining index was four, above which was used to define tumors as STIP-1 overexpression and the staining index and the scores of three or less as *STIP-1* down-expression (12).

Statistical analysis

All statistical analyses were conducted using SPSS version 22.0 for windows (USA; SPSS Inc., Chicago) and MedCalc-windows (Belgium MedCalc Software bvba 13, Ostend). The Mann-Whitney U test was used to compare the two groups of non-normally distributed data and a P-value <0.05 was considered significant. Representation of operation specialists (OS) and request for service (RFS) rates was made according to all the clinicopathological and immunohistochemical data and was estimated by the Kaplan-Meier curve.

Results

Patients criteria

The clinical data of the patients are summarized in Table 1.

Table 1. Demographic and Pathological Characteristics of the Study Cases

Characteristics	Number	%
Age (year)		
Mean ± SD	39.61 ± 10.21	
Median (range)	41.50 (21 – 53)	
<45 years	21	58.3%
≥45 years	15	41.7%
Gender		
Male	10	27.8%
Female	26	72.2%
Surgery		
Lobectomy	1	2.8%
Subtotal thyroidectomy	7	19.4%
Total thyroidectomy	16	44.4%
(Total thyroidectomy+BND (Block neck dissection	12	33.3%
Histopathological subtype		
Conventional	30	83.3%
Follicular variant	6	16.7%
Tumor size (cm)		
Mean ± SD	2.95 ± 1.21	
(Median (range	3 (0.50 – 5)	
≤ 4 cm	25	69.4%
>4cm	11	30.6%
Multifocality		
Absent	22	61.1%
Present	14	38.9%
Capsular invasion		
Absent	25	69.4%
Present	11	30.6%
Extrathyroid extension		
Absent	27	75%
Present	9	25%

Characteristics	Number	%
Vascular invasion		
Absent	29	80.6%
Present	7	19.4%
LN involvement		
Absent	22	61.1%
Present	14	38.9%
Involved nodes		
Neck nodes	13	36.1%
Mediastinal nodes	1	2.8%
Distant metastasis		
Absent	31	86.1%
Present	5	13.9%
Site of DM		
Lung	1	2.8%
Lung+Bone	4	11.1%
T		
T1	7	19.4%
T2	17	47.2%
T3	3	8.3%
T4	9	25%
N		
N0	22	61.1%
N1	14	38.9%
M		
M0	31	86.1%
M1	5	13.9%
Stage		
Stage I	22	61.1%
Stage II	10	27.8%
Stage III	2	5.6%
Stage IV	2	5.6%
STIP-1		
Low	20	55.6%
High	16	44.4%
FOXE-1		
Low	22	61.1%
High	14	38.9%
STIP-1/FOXE-1		
Low/Low	20	55.6%
Low/High	0	0
High/Low	2	5.6%
High/High	14	38.9%

Continuous variables are expressed as mean ± SD and median (range); categorical variables are expressed as number (percentage). BND(Block neck dissection)

The current study included 10 (27.8%) males and 26 (72.2%) females with the age range of 21 to 53 years and the mean age of39.61 ± 10.21; in addition, 30 (83.3%) cases were conventional-PTC and 6 (16.7%) were follicular variant of PTC.

- High nuclear expression of *FOXE-1* was detected in 14 out of 36 (38.9%) cases of PTC (tables 2 and 3; Figure 1).

- High cytoplasmic expression of STIP-1 was detected in 16 out of 36 (44.4%) cases of PTC (tables 2 and 3; Figure 2).

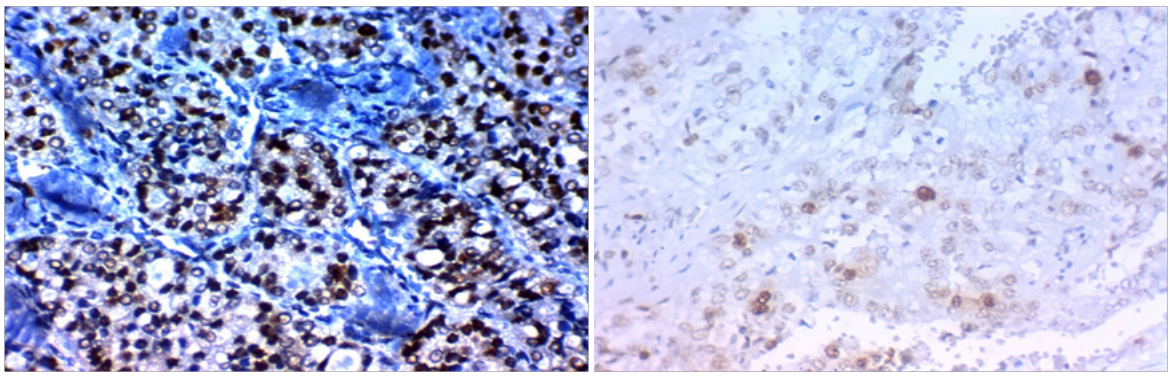


Fig 1 A **Fig 1 B**

Figure1. Immunohistochemical staining of FOXE-1 in papillary thyroid carcinoma (PTC) :(A) High immunohistochemical expression in the nucleus of PTC grade III stage III X400; (B) Low immunohistochemical expression in the nucleus of PTC grade II, stage II X400. Note: High *FOXE-1* immunohistochemical expression in high grade and stage PTC and low expression in low grade and stage PTC; A& B the original magnification was X400

Table 2. Correlation between Pathological Features, *STIP-1* and *FOXE-1*Expressions in the Study Patients

Characteristics	All (N=36)		STIP-1				P-value	FOXE-1				P-value
			Low (N=20)		High (N=16)			Low (N=22)		High (N=14)		
	No	(%)	No	(%)	No	(%)		No	(%)	No	(%)	
Age (year)												
Mean ± SD	39.61	± 10.21	42.10	±10.29	36.50	±9.51	0.082•	41.18	±10.27	37.14	±9.97	0.199•
Median (range)	41.50	(21-53)	46.50	(22-53)	35	(21-50)		45.50	(22-53)	37	(21-50)	
<45 years	21	(58.3%)	9	(42.9%)	12	(57.1%)	0.070‡	11	(52.4%)	10	(47.6%)	0.302‡
≥45 years	15	(41.7%)	11	(73.3%)	4	(26.7%)		11	(73.3%)	4	(26.7%)	
Gender												
Male	10	(27.8%)	6	(60%)	4	(40%)	1.000‡	6	(60%)	4	(40%)	1.000‡
Female	26	(72.2%)	14	(53.8%)	12	(46.2%)		16	(61.5%)	10	(38.5%)	
Histopathological subtype												
Conventional	30	(83.3%)	18	(60%)	12	(40%)	0.374‡	20	(66.7%)	10	(33.3%)	0.181‡
Follicular variant	6	(16.7%)	2	(33.3%)	4	(66.7%)		2	(33.3%)	4	(66.7%)	
Tumor size (cm)												
Mean ± SD	2.95	± 1.21	2.32	±1.11	3.75	±0.81	<0.001‡	2.45	±1.16	3.75	±0.82	0.001*
Median (range)	3	(0.50-5)	2.50	(0.50-4)	4	(2.5-5)		2.50	(0.5-4.5)	4	(2.5-5)	
≤ 4 cm	25	(69.4%)	18	(72%)	7	(28%)	0.004‡	19	(76%)	6	(24%)	0.010‡
>4cm	11	(30.6%)	2	(18.2%)	9	(81.8%)		3	(27.3%)	8	(72.7%)	

Characteristics	All (N=36)		STIP-1				P-value	FOXE-1				P-value
			Low (N=20)		High (N=16)			Low (N=22)		High (N=14)		
	No	(%)	No	(%)	No	(%)		No	(%)	No	(%)	
Multifocality												
Absent	22	(61.1%)	18	(81.8%)	4	(18.2%)	<0.001‡	19	(86.4%)	3	(13.6%)	<0.001‡
Present	14	(38.9%)	2	(14.3%)	12	(85.7%)		3	(21.4%)	11	(78.6%)	
Capsular invasion												
Absent	25	(69.4%)	19	(76%)	6	(24%)	<0.001‡	21	(84%)	4	(16%)	<0.001‡
Present	11	(30.6%)	1	(9.1%)	10	(90.9%)		1	(9.1%)	10	(90.9%)	
Extrathyroid extension												
Absent	27	(75%)	20	(74.1%)	7	(25.9%)	<0.001‡	22	(81.5%)	5	(18.5%)	<0.001‡
Present	9	(25%)	0	(0%)	9	(100%)		0	(0%)	9	(100%)	
Vascular invasion												
Absent	29	(80.6%)	20	(69%)	9	(31%)	0.001‡	22	(75.9%)	7	(24.1%)	<0.001‡
Present	7	(19.4%)	0	(0%)	7	(100%)		0	(0%)	7	(100%)	
LN involvement												
Absent	22	(61.1%)	18	(81.8%)	4	(18.2%)	<0.001‡	18	(81.8%)	4	(18.2%)	0.001‡
Present	14	(38.9%)	2	(14.3%)	12	(85.7%)		4	(28.6%)	10	(71.4%)	
Distant metastasis												
Absent	31	(86.1%)	20	(64.5%)	11	(35.5%)	0.012‡	22	(71%)	9	(29%)	0.005‡
Present	5	(13.9%)	0	(0%)	5	(100%)		0	(0%)	5	(100%)	
Stage												
I	22	(61.1%)	14	(63.6%)	8	(36.4%)	0.042§	16	(72.7%)	6	(27.3%)	0.012§
II	10	(27.8%)	6	(60%)	4	(40%)		6	(60%)	4	(40%)	
III	2	(5.6%)	0	(0%)	2	(100%)		0	(0%)	2	(100%)	
IV	2	(5.6%)	0	(0%)	2	(100%)		0	(0%)	2	(100%)	
STIP-1												
Low	20	(55.6%)						20	(100%)	0	(0%)	<0.001‡
High	16	(44.4%)						2	(12.5%)	14	(87.5%)	
FOXE-1												
Low	22	(61.1%)	20	(90.9%)	2	(9.1%)	<0.001‡					
High	14	(38.9%)	0	(0%)	14	(100%)						

Categorical variables are expressed as number (percentage);
continuous variables are expressed as mean ± SD and median (range)
* Independent samples and Student t tests; • the Mann-Whitney U test;
‡ Chi-square test; § Chi-square test for trend; P <0.05 was significant.
LN, lymph node

Table 3. Correlation between Pathological Features and Expression of Both Markers Together in the Study Patients

Characteristics	All (N=36)		STIP-1/FOXE-1						P-value
			Low/Low (N=20)		High/Low (N=2)		High/High (N=14)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Age (year)									
Mean ± SD	39.61	± 10.21	42.10	± 10.29	32	± 4.24	37.14	± 9.97	0.186•
Median (range)	41.50	(21-53)	46.50	(22-53)	32	(29-35)	37	(21-50)	
<45 years	21	(58.3%)	9	(42.9%)	2	(9.5%)	10	(47.6%)	0.144‡
≥45 years	15	(41.7%)	11	(73.3%)	0	(0%)	4	(26.7%)	
Gender									
Male	10	(27.8%)	6	(60%)	0	(0%)	4	(40%)	0.663‡
Female	26	(72.2%)	14	(53.8%)	2	(7.7%)	10	(38.5%)	
Surgery									
Lobectomy	1	(2.8%)	0	(0%)	0	(0%)	1	(100%)	
Subtotal thyroidectomy	7	(19.4%)	3	(42.9%)	1	(14.3%)	3	(42.9%)	0.538‡
Total thyroidectomy	16	(44.4%)	8	(50%)	1	(6.3%)	7	(43.8%)	
Total thyroidectomy +BND (Block neck dissection)	12	(33.3%)	9	(75%)	0	(0%)	3	(25%)	
Histopathological subtype									
Conventional	30	(83.3%)	18	(60%)	2	(6.7%)	10	(33.3%)	0.291‡
Follicular variant	6	(16.7%)	2	(33.3%)	0	(0%)	4	(66.7%)	
Tumor size (cm)									
Mean ± SD	2.95	± 1.21	2.32	± 1.11	3.75	± 1.06	3.75	± 0.82	0.002•
Median (range)	3	(0.50-5)	2.50	(0.50-4)	3.75	(3-4.50)	4	(2.50-5)	
≤ 4 cm	25	(69.4%)	18	(72%)	1	(4%)	6	(24%)	0.011‡
>4 cm	11	(30.6%)	2	(18.2%)	1	(9.1%)	8	(72.7%)	
Multifocality									
Absent	22	(61.1%)	18	(81.8%)	1	(4.5%)	3	(13.6%)	<0.001‡
Present	14	(38.9%)	2	(14.3%)	1	(7.1%)	11	(78.6%)	
Capsular invasion									
Absent	25	(69.4%)	19	(76%)	2	(8%)	4	(16%)	<0.001‡
Present	11	(30.6%)	1	(9.1%)	0	(0%)	10	(90.9%)	
Extrathyroid extension									
Absent	27	(75%)	20	(74.1%)	2	(7.4%)	5	(18.5%)	<0.001‡
Present	9	(25%)	0	(0%)	0	(0%)	9	(100%)	

Characteristics	All (N=36)		STIP-1/FOXE-1						P-value
			Low/Low(N=20)		High/Low (N=2)		High/High (N=14)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Vascular invasion									
Absent	29	(80.6%)	20	(69%)	2	(6.9%)	7	(24.1%)	0.001‡
Present	7	(19.4%)	0	(0%)	0	(0%)	7	(100%)	
LN involvement									
Absent	22	(61.1%)	18	(81.8%)	0	(0%)	4	(18.2%)	<0.001‡
Present	14	(38.9%)	2	(14.3%)	2	(14.3%)	10	(71.4%)	
Involved nodes									
No	22	(61.1%)	18	(81.8%)	0	(0%)	4	(18.2%)	0.002‡
Neck nodes	13	(36.1%)	2	(15.4%)	2	(15.4%)	9	(69.2%)	
Mediastinal nodes	1	(2.8%)	0	(0%)	0	(0%)	1	(100%)	
Distant metastasis									
Absent	31	(86.1%)	20	(64.5%)	2	(6.5%)	9	(29%)	0.010‡
Present	5	(13.9%)	0	(0%)	0	(0%)	5	(100%)	
T									
1	7	(19.4%)	7	(100%)	0	(0%)	0	(0%)	<0.001§
2	17	(47.2%)	13	(76.5%)	1	(5.9%)	3	(17.6%)	
3	3	(8.3%)	0	(0%)	1	(33.3%)	2	(66.7%)	
4	9	(25%)	0	(0%)	0	(0%)	9	(100%)	
N									
0	22	(61.1%)	18	(81.8%)	0	(0%)	4	(18.2%)	<0.001‡
1	14	(38.9%)	2	(14.3%)	2	(14.3%)	10	(71.4%)	
M									
0	31	(86.1%)	20	(64.5%)	2	(6.5%)	9	(29%)	0.010‡
1	5	(13.9%)	0	(0%)	0	(0%)	5	(100%)	
Stage									
I	22	(61.1%)	14	(63.6%)	2	(9.1%)	6	(27.3%)	0.025§
II	10	(27.8%)	6	(60%)	0	(0%)	4	(40%)	
III	2	(5.6%)	0	(0%)	0	(0%)	2	(100%)	
IV	2	(5.6%)	0	(0%)	0	(0%)	2	(100%)	
Radioiodine therapy dose (mCi)									
Mean ± SD	197.50	±183.04	195	± 207.12	80	± 0	217.85	± 157.87	0.196•
Median (range)	100	(60-870)	105	(60-870)	80		110	(80-540)	

The Kruskal-Wallis H test; ‡ Chi-square test; § Chi-square test for trend;
LN, lymph node BND(Block neck dissection)

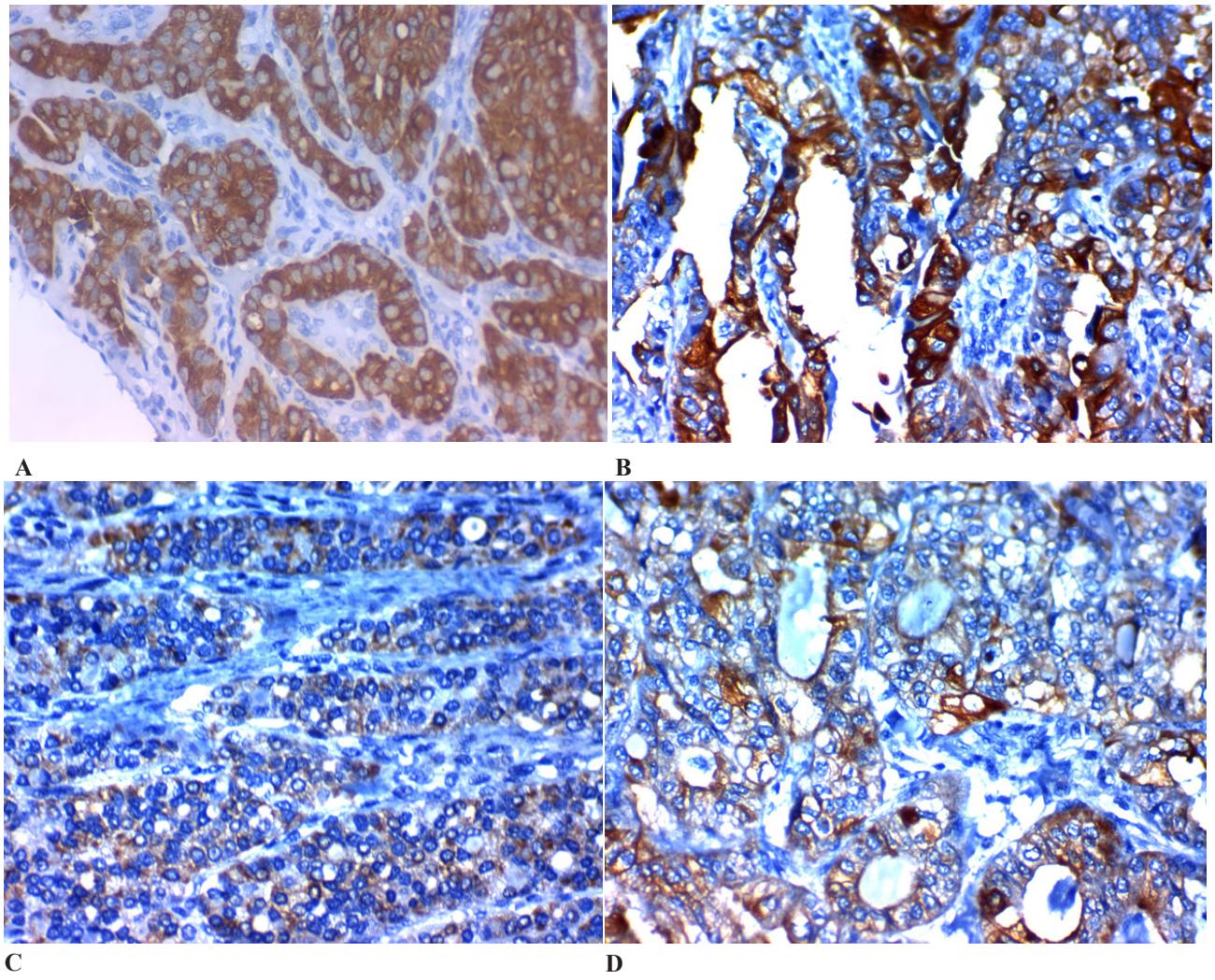


Figure 2. Immunohistochemical staining of STIP-1 in PTC:

(A) High Immunohistochemical expression in the cytoplasm of PTC grade III stage III X400
(B) High Immunohistochemical expression in the cytoplasm of PTC grade IV, stage III X400
(C) Low Immunohistochemical expression in the cytoplasm of PTC grade II, stage IIB X400
(D) Low Immunohistochemical expression in the cytoplasm PTC grade II, stage II X400
Note: High STIP-1 immunohistochemical expression in high grade and stage PTC and low expression in low grade and stage PTC;
the original magnification was X100 for A, and X400 for B, C and D.

- The overexpression of both *FOXE-1* and *STIP-1* in PTC was associated with larger size of the cancer, multifocality, capsular invasion, extra-thyroidal extension, vascular invasion, LN spread (P <0.001), presence of distant metastases (P-values =0.005 and 0.012, respectively), and stage of the tumor (P-values =0.012 and 0.042, respectively).

- No significant correlations were observed between the markers expression and age, gender of the patients, histopathological sub-type of the PTC, type of performed surgery or dose of radioactive iodine used for the patients.

None of the marker expressions had significant correlation with histopathological subtype of PTC, but both had significant correlations with survival and prognosis; it was the explanation of note 14 (I think it is necessary to explain disaffiliation of *STIP-1* and *FOXE-1* expression with different histologic types of PTC, as it is inconsistent with the expression of the two markers with prognosis and survival).

Recurrence and survival analysis

tables 4 and 5; Figure 3

Table 4. Correlation between STIP-1 and FOXE-1 Expressions and Outcomes of the Study Patients

Outcome	All (N=36)		STIP-1			P-value	FOXE-1				P-value
			Low (N=20)		High (N=16)		Low (N=22)		High (N=14)		
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	
OS											
Mean (month) (95%CI)	59.7 months (59.4-60.1)		59.9 months (59.9-60.2)		59.4 months (58.7-60.2)		59.9 months (59.6-60.2)		59.4 months (58.7-60.2)		
HR (95%CI)	----		4.825 (1.051 - 22.148)				4.825 (1.051 - 22.148)				
24 month OS (%)	100%		100%		100%	0.014†	100%		100%		0.014†
36 month OS (%)	100%		100%		100%		100%		100%		
48 month OS (%)	100%		100%		100%		100%		100%		
60 month OS (%)	57.9%		75%		0%		75%		0%		
DFS											
Mean (month) (95%CI)	52.9 months (49.3-56.5)		55.3 months (50.2-60.4)		49.9 months (45.1-54.7)		20 (66.7%)		10 (33.3%)		
HR (95%CI)	----		3.241 (1.308 - 8.029)				2.370 (0.975 - 5.761)				
24 month PFS (%)	97%		94.4%		100%	0.006†	95%		100%		0.043†
36 month PFS (%)	93.9%		94.4%		93.3%		90%		100%		
48 month PFS (%)	75.8%		83.3%		66.7%		80%		69.2%		
60 month PFS (%)	29.6%		45.8%		0%		41.3%		0%		

Categorical variables are expressed as number (percentage); † Log rank test; HR, hazards ratio; 95%CI: 95% confidence interval; P <0.05, the level of significance

OS, overall survival; DFS, disease free survival

Table 5. Correlation between the Expression of Both Markers Together in the Study Patients and Their Outcomes

Outcome	All (N=36)		STIP-1/FOXE-1						P-value
			Low/Low (N=20)		High/Low (N=2)		High/High (N=14)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Response to treatment									
SD	1	(2.8%)	1	(5%)	0	(0%)	0	(0%)	
PR	2	(5.6%)	1	(5%)	0	(0%)	1	(7.1%)	0.541§
CR	33	(91.7%)	18	(90%)	2	(100%)	13	(92.9%)	
NR	3	(8.3%)	2	(10%)	0	(0%)	1	(7.1%)	
OAR	33	(91.7%)	18	(90%)	2	(100%)	13	(92.9%)	0.732§
Time to complete remission									
Mean ± SD	14.51	± 4.77	14.94	± 4.19	16.50	± 0.70	13.61	± 5.83	
Median (Range)	13	(4-29)	14	(10-24)	16.50	(16-17)	13	(4-29)	0.387•
Post-ablation TG									
Mean ± SD	23.28	± 126.37	0.98	± 3.07	0.25	± 0.21	58.44	± 202.01	
Median (range)	0.40	(0.1-759)	0.20	(0.10-14)	0.25	(0.1-0.4)	0.50	(0.1-759)	0.097•
Mortality									
Absent	29	(80.6%)	17	(85%)	2	(100%)	10	(71.4%)	0.380§
Present	7	(19.4%)	3	(15%)	0	(0%)	4	(28.6%)	

Outcome	All (N=36)		STIP-1/FOXE-1						P-value
			Low/Low (N=20)		High/Low (N=2)		High/High (N=14)		
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Relapse									
Absent	12	(33.3%)	9	(45%)	0	(0%)	3	(21.4%)	0.102§
Present	21	(58.3%)	9	(45%)	2	(100%)	10	(71.4%)	
Events									
No	12	(33.3%)	9	(45%)	0	(0%)	3	(21.4%)	
LR	18	(50%)	8	(40%)	2	(0%)	8	(57.1%)	0.306§
DM	3	(8.3%)	2	(10%)	0	(0%)	1	(7.1%)	
LR + DM	1	(2.8%)	0	(0%)	0	(0%)	1	(7.1%)	
LRR									
Absent	14	(38.9%)	10	(50%)	0	(0%)	4	(28.6%)	0.558§
Present	19	(52.8%)	8	(40%)	2	(100%)	9	(64.3%)	
Tumor bed recurrence									
Absent	23	(63.9%)	13	(65%)	2	(100%)	8	(57.1%)	0.971§
Present	10	(27.8%)	5	(25%)	0	(0%)	5	(35.7%)	
Neck node recurrence									
Absent	23	(63.9%)	17	(85%)	0	(0%)	6	(42.9%)	0.202§
Present	10	(27.8%)	1	(5%)	2	(100%)	7	(50%)	
Mediastinal recurrence									
Absent	32	(88.9%)	18	(90%)	2	(100%)	12	(85.7%)	0.916§
Present	1	(2.8%)	0	(0%)	0	(0%)	1	(7.1%)	
Distant metastasis									
Absent	31	(86.1%)	18	(90%)	2	(100%)	11	(78.6%)	0.226§
Present	4	(11.1%)	2	(10%)	0	(0%)	2	(14.3%)	
OS									
Mean (month) (95%CI)	**59.7 months (59.4-60.1)**		**59.9 months (59.6-60.1)**		**60 months**		**59.4 months (58.7-60.2)**		**0.380§**
24 month OS (%)	100%		100%		100%		100%		
36 month OS (%)	100%		100%		100%		100%		
48 month OS (%)	100%		100%		100%		100%		---
60 month OS (%)	57.9%		75%		100%		0%		
DFS									
Mean (month) (95%CI)	**52.9 months (49.3-56.5)**		**55.3 months (50.2-60.4)**		**41.5 months (18.9-64)**		**51.2 months (46.6-55.7)**		**0.006†**
24 month PFS (%)	97%		94.4%		100%		100%		
36 month PFS (%)	93.9%		94.4%		50%		100%		
48 month PFS (%)	75.8%		83.3%		50%		69.2%		0.558§
60 month PFS (%)	29.6%		45.8%		0%		0%		

Categorical variables are expressed as number (percentage); † Log rank test; HR, hazards ratio; 95%CI: 95%, confidence interval; P <0.05, the level of significance

OS, overall survival; LR, local recurrence; DM, distant metastasis; DFS, disease free survival

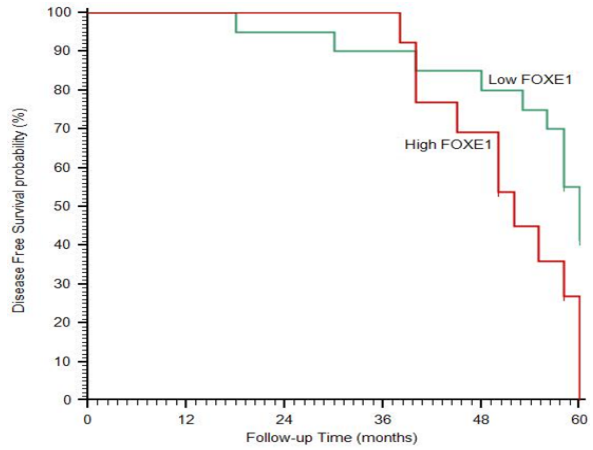


Fig 3 A

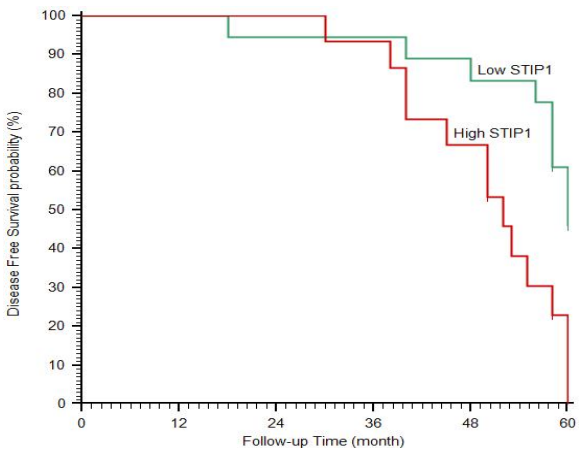
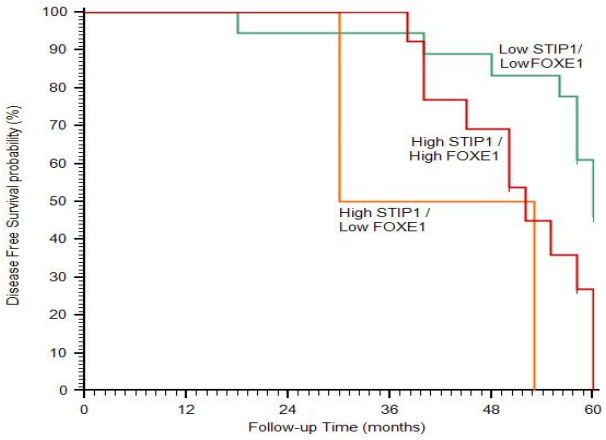


Fig 3 B



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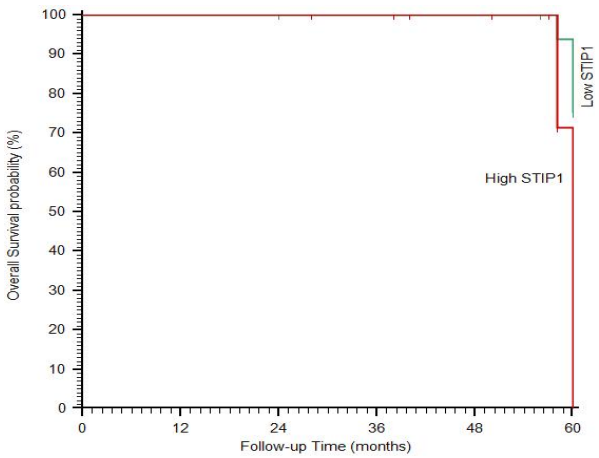

Fig 3 D

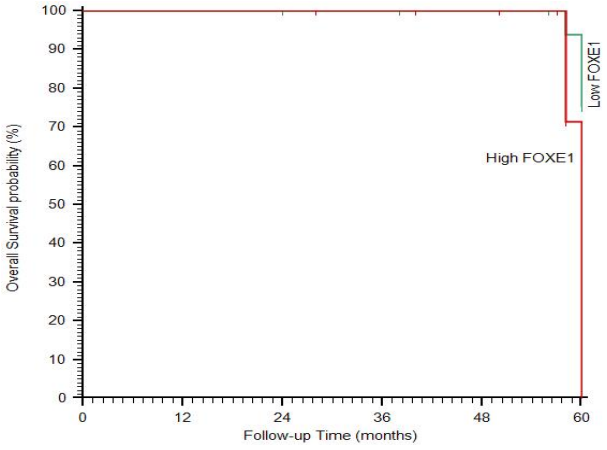

Fig 3 E

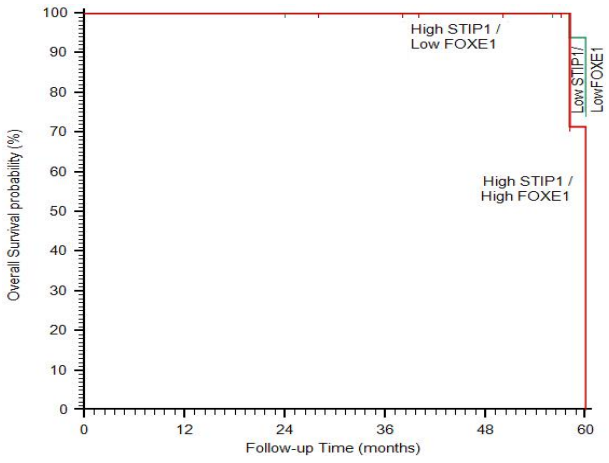

Fig 3 F

Figure 3. Kaplan-Meier survival plots; A, B, and C; DFS, disease free survival; A, Stratified by *FOXE*-1 expression; B, Stratified by *STIP-1* expression; C, Stratified by *STIP-1*+ *FOXE-1* expressions (as low/low, high/low, and high/high) D, E, and F; Overall survival; D, Stratified by *FOXE-1* expression; E, Stratified by *STIP-1* expression; F Stratified by *STIP-1*+*FOXE-1* expressions (as low/low, high/low, and high/high)

The five-year overall survival (OS) rate of the current study patients was 57.9% for all of the study patients, 0 for patients with high *STIP-1*+ *FOXE-1*expression, and 75% in low *STIP-1* + *FOXE-1* expression (P <0.014).

The five-year distant metastases free survival (DMFS) rate was 29.6% for all of the current study patients, 0 for patients with high *STIP-1*+ *FOXE-1* expression, 45.8% for the ones with low *STIP-1* expression (P=0.006), and 41.3% for patients with low *FOXE-1*expression (P=0.043).

The expression of FOXE-1 was significantly correlated with worse DMFS rate and worse five-year OS rate (P <0.001).

Expressions of both of the current study markers were significantly correlated with each other (P <0.001).

Discussion

Thyroid cancer that is still differentiated; eg, papillary and follicular increasingly occurs and forms the majority (90%) of all malignant thyroid nodules (13). PTC is the 3rd most common cancer in females (14). PTC had LN metastases in about 40% to 90% of the patients leading to poor outcomes and increased mortality rates of such patients. In older patients, it is suggested that tumors grow more rapidly, distant metastases occur more often, and extra-capsular extension of the primary tumor is more common (15).

The current study proved that *FOXE-1* showed high nuclear expression of 38.9% of PTC, regarding the relationship between *FOXE-1* expression and clinicopathological characteristics, the high expression of *FOXE-1* was associated with larger size of the cancer, multifocality, capsular invasion, extra-thyroidal extension, vascular invasion, LN spread (P <0.001), presence of distant metastases (P =0.005), and stage of the tumor (P = 0.012), that was in agreement with the results of FAN et al., and Somuncu et al ., who reported that *FOX-E-1* regulated several genes transcription; eg, thyrotropin receptor, thyroperoxidase, thyroglobulin, and sodium iodide that had essential roles in thyroid hormones synthesis. In addition to their roles in normal thyroid physiology, there is a strong association between the *FOXE-1* expressions and thyroid cancer; they also stated that the coding poly-alanine expansion in *FOXE-1* may be responsible for the association between *FOXE-1* and PTC. Its overexpression was present in PTC cells suggesting that *FOXE-1* had a much more important role in PTC pathogenesis (16,17). Similar to the current study results, Martinez et al., found that the expression of nuclear *FOXE-1* in PTC was related to poor pathological criteria and subsequently poor outcome (18). Bychkov et al., explained the current study results that revealed that the nuclear FOXE-1 accumulation in PTC cells was associated with cancer aggressiveness such as capsular invasion and multifocality. Overexpression of FOXE-1 could be a novel prognostic biomarker with new therapeutic targets in PTC (19).

However, Mond et al., reported that somatic mutations of *FOXE-1* in PTC and inactivating mutations of *FOXE-1* were uncommon events in thyroid cancers, but contributed to PTC carcinogenesis, then, dedifferentiation in associations with other oncogenic signals (20). Also He et al. found that *FOXE-1* was not expressed in PTC tumors, which was due to the absence of a unique mechanism that could explain the functioning of its chromosomal-locus in thyroid cancers (21).

Similar to the current study results in PTC, Sugimachi et al., (2016) showed that *FOX-E-1* expression can be a useful prognostic agent in many cancers such as colorectal carcinoma and non-small cell carcinoma of lung; which was in line with that of the current study indicating that *FOXE-1* expression could be an important prognostic and therapeutic target in cancer (22).

The current study proved that high cytoplasmic expression *STIP-1* was correlated with bad clinic pathological characteristics such as larger size of the tumor, multifocality, capsular invasion, extra-thyroidal extension, vascular invasion, LN spread (P <0.001), presence of distant metastases (P=0.012), and stage of the tumor (P=0.042). Therefore, its expression was associated with worse prognosis. It was in agreement

with the results of Yuan et al., as well as Tsai et al., (2016) where they reported that the high expression of *STIP-1* in cancer was massively related to LN metastasis, cancer size, and TNM stages; therefore, its expression was correlated with poor prognosis and considered it as an essential novel prognostic biomarker for overall survival rate in patients with PTC (11,23).

The *STIP-1* is associated with disappointing prognosis in malignancies of plethora of organs (24-27); it suggests that this biomarker has an anti-apoptotic role and increases cancer cell survival. The effective inhibition of STIP-1-stimulated cancer cell proliferation and migration reached by anti-STIP-1 antibodies indicated that cancer cell secreted STIP1 may be used as a therapeutic target in ovarian, pancreatic, renal, lung, prostate, gastric cancers, and melanoma (28).

In the current study, the expressions of both markers were significantly correlated with each other (P <0.001).

To the authors` best knowledge, no previous researchers studied both *FOXE-1*+ *STIP-1* expression in PTC and researches on both of them in PTC should be continued.

In the current study, the expression of both markers were significantly correlated with each other (P<0.001) and both of them could be used as biomarkers and therapeutic targets for PTC.

Conclusion

The current study results indicated that *FOXE-1*and *STIP-1* were overexpressed and had essential roles in tumor aggression and poor prognosis in patients with PTC. Furthermore, their levels were a predictor of survival for patients with such cancer. Nonetheless, further studies are needed to elucidate the mechanisms by which both markers participate in the development and progression of lung, ovarian, and colorectal cancer, and to clarify whether both of them together could be used as targets for therapeutic approaches.

Conflict of interests

The authors declared no conflict of interest.

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