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" *Immunohistochemical expression of SLP2 in Papillary Thyroid Carcinoma Compared to Follicular adenoma*"

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ABSTRACT:

Background: Thyroid neoplasms are common worldwide as well as in Egypt. The most common follicular neoplasm is follicular adenoma, and the most common thyroid carcinoma is papillary thyroid carcinoma. Stomatin-Like Protein 2 (SLP2) antibody is expressed in various types of thyroid lesions.

Aim: Assessment of immunohistochemical expression of SLP2 in papillary thyroid carcinoma and in follicular adenoma. Also, to assess its expression in PTC with variable demographic and histopathological parameters.

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Subjects and Methods: The current study is a cross-sectional analytical study, executed in the pathology laboratory of Suez Canal University Hospital over 30 samples, 15 blocks are of PTC samples, and 15 blocks are of follicular adenoma, gathered during the period of January 2019 to January 2020.

Results: SLP2 is positively expressed in 86.7% of PTC samples and in 20% of follicular adenoma samples. There is a statistically significant association between positive expression of SLP2 in PTC samples, compared to follicular adenoma samples (p = **0.0007**). There is no statistically significant association between SLP2 expression and variable demographic and histopathological parameters in PTC samples. SLP2 has 86.67% sensitivity, 80% specificity, 81.25% positive predictive value, 85.71% negative predictive ratio and 83.33% diagnostic accuracy respectively. .

Conclusions: SLP2 is a good diagnostic marker of PTC compared to follicular adenoma with higher intensity and proportion of staining in PTC samples.

Keywords: SLP2, PTC, follicular adenoma.

INTRODUCTION

Thyroid neoplasms are common worldwide as well as in Egypt. The most common follicular neoplasm is follicular adenoma and the most common thyroid carcinoma is papillary thyroid carcinoma (PTC) (Sung et al., 2021; Ahn et al., 2022; Limaiem et al., 2022).

The nuclear features of PTC are nuclear elongation, enlargement, overlapping, clearing, irregular nuclear membrane, longitudinal nuclear

grooving and nuclear pseudoinclusions (Bychkov & Jung., 2024; Lebrun & Salmon., 2024).

Some of follicular adenomas may show focal papillary pattern, but the proliferating cells are low columnar to cuboidal cells with small, rounded nuclei showing absence of papillary nuclear features as presence of these features suggests diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (Nikiforov et al., 2016).

For diagnosis of difficult cytological and histopathological samples of PTC an immunohistochemical (IHC) cocktail of markers is adopted which is formed of CK19, HBME1 and Galactin3. Yet, no single IHC marker is sufficient for diagnosis (Vella et al., 2022; Abouelfadl et al., 2024).

SLP2 is a mitochondrial membrane protein, it is encoded by stomatin-homologous gene (Zhou et al., 2019). SLP2 is highly expressed in many tumors as ovarian, pancreatic, colonic and esophageal squamous cell carcinoma (Q. Liu et al., 2020; Wang et al., 2020; Chao et al., 2021; Qin et al., 2023).

SLP2 overexpression leads to phosphorylation inhibition of MAPK, inducing MAPK pathway, promoting PTC pathway and SLP2 overexpression leads to low ROS levels promoting tumorigenesis, angiogenesis and tumor cells migration (Chen et al., 2023).

Some studies have shown overexpression of SLP2 protein in PTC (Yang et al., 2018). However, further studies are needed to assess SLP2 levels of expression in PTC and follicular adenomas to evaluate its diagnostic accuracy and to assess association with other demographic and histopathological parameters.

MATERIAL AND METHODS

Study setting and Study population:

The current study was a cross-sectional analytical study. This study included 30 cases of PTC and follicular adenoma diagnosed during the period of January 2019 to January 2020 at Suez Canal University Hospital. Paraffin blocks were retrieved from archive of pathology laboratory, Suez Canal University Hospitals; 15 blocks are of PTC and 15 blocks are of follicular adenoma. Demographic and histopathological parameters including age at time of operation, gender, T stage, capsular invasion, extrathyroidal extension, lymph node metastasis state and tumor stage were assessed in the PTC group.

Inclusion Criteria:

1- Adequate samples diagnosed as PTC and follicular adenomas.
2- Samples with available histopathological and demographic data.

Exclusion Criteria:

1- Insufficient samples for proper immunohistochemical staining.
2- FNAC cell blocks specimens.

Histopathology and immunohistochemistry procedure:

Sections were cut at 5 µm thickness, stained by hematoxylin and eosin and double blindly reviewed by researchers, classified based on the latest WHO classifications of endocrine neoplasms (Baloch et al., 2022). Staging of the tumor was based on the TNM system according to AJCC, eighth edition (Amin et al., 2017). Assessed for tumor capsular invasion, extrathyroidal extension and lymph nodal metastasis.

Other sections were cut at 4 µm thickness from each block, then automated staining of SLP2 Mouse purified monoclonal IgG antibody of catalogue number sc-376181, at a dilution of 1/50 for 60 minutes based on the protocol of company data sheet (Santa Cruz biotechnology, Inc), using Venata GX device, by Ventana CC2 retrieval buffer (low ph.) and Ventana detection kit. Positive control of cervical tissue and negative control by exclusion of antibody were used.

Evaluation of SLP IHC staining:

Immunohistochemically stained slides were examined using microscope (Olympus CX23) and independent double blinded evaluation by at least two researchers was done for expression of SLP2 for both its proportion and intensity as negative, moderate and strong staining. Slides have been interpreted as positive for SLP2 when showing adequate moderate to strong positive cytoplasmic staining of more than 10 % of stained cells (Yang et al., 2018).

Statistical analysis:

Descriptive analysis and calculation of percentage of different studied groups were done. Association between SLP2 expression and different histopathological and demographic parameters in PTC group were assessed using appropriate statistical tests according to types of variables. Data illustration using tables, figures and charts was done. p-value was considered as significant when ≤ 0.05. Statistical analysis was done using the statistics software SPSS (Version 25) for windows 10.

Ethical considerations:

The study protocol was reviewed and accepted by the (IRB/IEC) institutional review board and institutional research ethics committee of PSU prior to initiation (ERN: MED (1/6/2023) s.no (95) PTH 904_001).

Funding source: Covered by the researchers.

Conflict of interest: Authors declared absence of conflict of interest related to the research.

Data Availability: All raw data supporting findings are available upon request from the corresponding author (Ahmed A. Elmetwally).

RESULTS

Histopathological and demographic characteristics:

This study was done on 30 samples; 15 samples are of PTC and 15 samples are of follicular adenoma. The demographic data of these samples are listed in **(Table 1)**.

The mean age of PTC samples was 44.8 ±13.56 and the mean age of follicular adenoma samples was 35.067 ±9.498. There was a statistically significant association between older age cases and diagnosis as PTC, compared to follicular adenoma (p= **0.0306).** Yet, regarding classification of age of the cases, into more or less than 55 years, there was no statistically significant association between patients' age and PTC, compared to follicular adenoma **(Table 1).**

There was no statistically significant association between the gender of the cases diagnosed as PTC, compared to cases of follicular adenoma **(Table 1)**.

The rest of histopathological parameters as capsular invasion, extra thyroid extension, T stage, lymph nodal invasion status and stage were limited to PTC samples **(Table 3)**.

SLP2 IHC expression:

Regarding PTC, the cases stained positive for SLP2 were 13 out of 15 (86.7%) of PTC samples were positive for SLP2. Eight of them showed strong intensity and 5 showed moderate intensity. The mean proportion of stained cells was 53 ± 35.597 **(Table 2 and figure 1)**.

Regarding follicular adenoma, the cases stained positive for SLP2 were 3 out of 15 (20%). One of them showed strong intensity and 2 showed moderate intensity. The mean proportion of stained cells was 14 ± 29.548 **(Table 2 and figure 1)**.

There was a statistically significant association between positivity as well as intensity of expression of SLP2 and PTC, compared to follicular adenoma (p =**0.0007 and 0.0007) (Table 2 and Figure 2)**. Also, There was a statistically significant association between higher proportions of stained SLP2 cells in PTC cases, compared to follicular adenoma cases (p =**0.0029) (Table 2)**.

Association between SLP2 expression and different demographic and histopathological characteristics in PTC group and follicular adenoma group:

There were no statistically significant association between results of SLP2 expression and variable demographic and histopathological parameters in cases of PTC (p >0.05) **(Table 3)** or demographic parameters in cases of follicular adenoma (p >0.05) **(Table 4)**.

Diagnostic Performance of SLP-2 for discrimination between PTC samples and follicular adenoma samples:

The calculated diagnostic performance of SLP2 in PTC group and follicular adenoma groups showed a diagnostic accuracy of SLP2 to differentiate PTC samples by 83.33%. The calculated SLP2 sensitivity was 86.67%, specificity was 80%, positive predictive value was 81.25% and negative predictive ratio was 85.71% **(Table 5)**.

DISCUSSION

Papillary thyroid carcinoma and follicular adenomas are the most common lesions affecting thyroid in Egyptian people and internationally (Sung et al., 2021; Ahn et al., 2022; Limaiem et al., 2022). Our study has been conducted to assess SLP2 expression in cases of papillary thyroid carcinoma and follicular adenoma. Also, assessment of association between the expression of SLP2 and variable demographic and histopathological parameters in PTC and demographic variability of follicular adenoma samples have been performed.

SLP2 is a mitochondrial membrane protein (Zhou et al., 2019). It also shows cytoplasmic staining in thyroid lesions (Yang et al., 2018). SLP2 overexpression leads to phosphorylation inhibition of MAPK, inducing MAPK pathway, promoting PTC pathway and SLP2 overexpression leads to low ROS levels promoting tumorigenesis, angiogenesis and tumor cells migration (Chen et al., 2023).

This study has been conducted on 30 samples, 15 samples of PTC and 15 samples of follicular adenomas. In our study SLP2 antibody showed exclusive cytoplasmic staining. This pattern of expression of SLP2

expression is similar to its expression in other studies (Yang et al., 2018; Attia et al., 2024).

Thirteen cases out of 15 cases of PTC showed SLP2 positive expression, representing 86.7%. There are many studies that showed a similar level of expression (80-90%) in cases of PTC. On the other hand, 3 cases of follicular adenomas out of 15 (20%) were positive for SLP2, the difference is statistically significant (p =**0.0007)**. Similar levels of expression and significance were found in other researches, mostly due to adopting a similar method of interpretation of positivity (Bartolome et al., 2016; Yang et al., 2018).

In a study conducted by Yang et al. over 59 surgical specimens, it showed a relatively close result as 83.1% of cases of PTC and 20.7% of follicular adenomas were considered SLP2 positive with a p-value <0.05 (Yang et al., 2018).

In Bartolome et al. (2016) study including 210 cases of benign and malignant thyroid lesions, 65% of PTC tumors were positive for SLP2, all cases of follicular adenoma were negative for SLP2. This variation in levels of expression is due to different producers of primary antibody and different methods of evaluation in their study. The cutoff point for positivity was more than 40%, which is much higher than our study, which is more than 10%. This explains the low levels of sensitivity and higher specificity.

Our study showed calculated sensitivity of 86.67%, specificity of 80%, positive predictive value of 81.25%, negative predictive ratio of 85.71% and diagnostic accuracy of 83.33%. These results are close to the study of Yang et al. (2018).

In our study there is no statistically significant association between all demographic and histopathological parameters and either cases of PTC or follicular adenoma. Many studies have shown similar results (Yang et al., 2018; Attia et al., 2024). This may be due to similar method for interpretation of positivity of SLP2. On the other hand, Bartolome et al. (2016) stated that there is a statistically significant association with positive SLP2 expression and larger tumor size, extrathyroidal extension, presence of lymph nodal metastasis and higher stage in PTC cases, which is most likely due to different primary antibody provider and different method of interpretation of positivity.

Table (1): Comparison between samples of PTC and follicular adenoma samples according to demographic data

		PTC (n = 15)		**Follicular adenoma (n = 15)**		**P**
Age	**Mean and SD**	44.8 ±13.56		35.067 ±9.498		**0.0306**
		No.	%	No.	%	
	Less than 55	13	86.7	14	93.33	1
	55 or more	2	13.3	1	6.67	
Gender	**Male**	0	0	3	20	0.224
	Female	15	100	12	80	

FET: Fisher Exact test p: p value for comparing between the two studied groups

*: Statistically significant at $p \leq 0.05$

Table (2): Comparison between PTC and follicular adenoma according to SLP2 staining

SLP2		PTC (n = 15)		Follicular adenoma (n = 15)		p
		No.	%	No.	%	
Intensity	Negative/faint	2	13.333	12	80	0.0007
	Moderate	5	33.333	2	13.333	
	Strong	8	53.333	1	6.667	
Result	Negative	2	13.3	12	80	0.0007
	Positive	13	86.7	3	20	
Proportion	Min. – Max.	0.0 – 100.0		0.0 – 100.0		P= 0.0029
	Mean ± SD.	53 ± 35.597		14 ± 29.548		

FET: Fisher Exact test **SD: Standard deviation**

p: p value for comparing between the two studied groups

Table (3): Relation between SLP2 results with demographic and histopathological data in PTC group (n = 15)

		N	SLP2 Result				p
			Negative (n = 2)		Positive (n = 13)		
			No.	%	No.	%	
Gender	**Male**	**0**	0	0	0	0	$^{FE}p=$ 1.000
	Female	**15**	2	13.3	13	86.7	
Age	**Less than 55**	**13**	2	15.38	11	84.62	$^{FE}p=$ 1.000
	55 or more	**2**	0	0	2	100	
Capsular Invasion	**No**	**13**	2	15.38	11	84.62	$^{FE}p=$ 1.000
	Yes	**2**	0	0	2	100	
Extra-thyroid extension	**No**	**13**	2	15.38	11	84.62	$^{FE}p=$ 1.000
	Yes	**2**	0	0	2	100	
T stage	**T1**	**4**	0	0	4	100	$^{FE}p=$ 0.695
	T2	**3**	0	0	3	100	
	T3	**8**	2	25	6	75	
LN stage	**NX/0**	**14**	2	14.286	12	85.714	$^{FE}p=$ 1.000
	N1	**1**	0	0.0	1	100.0	
Stage	**I**	**15**	2	13.3	13	86.7	$^{FE}p=$ 1.000
	II	**0**	0	0	0	0	

FET: Fisher Exact test

p: p value for Relation SLP2 Result and different parameters

Table (4): Relation between SLP2 Result with demographic data and in follicular adenoma group (n = 15)

		N	SLP2 Result				p
			Negative (n = 12)		Positive (n = 3)		
			No.	%	No.	%	
Gender	Male	3	3	100	0	0	$^{FE}p= 1$
	Female	12	9	75	3	25	
Age	Less than 55	14	11	78.57	3	21.43	$^{FE}p= 1$
	55 or more	1	1	100	0	0	

FET: Fisher Exact test *: Statistically significant at $p \leq 0.05$

p: p value for Relation SLP2 Result and different parameters

Table (5): SLP2 diagnostic performance to discriminate between PTC and follicular adenoma (n = 30)

	Follicular adenoma (n = 15)		**PTC (n = 15)**		**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**P-value**
	No.	**%**	**No.**	**%**						
SLP-2 results										
Negative	12	80	2	13.3	86.67	80	81.25	85.71	83.33	**<0.001***
Positive	3	20	13	86.7						

NPV: Negative predictive value PPV: Positive predictive value

*: Statistically significant at $p \leq 0.05$

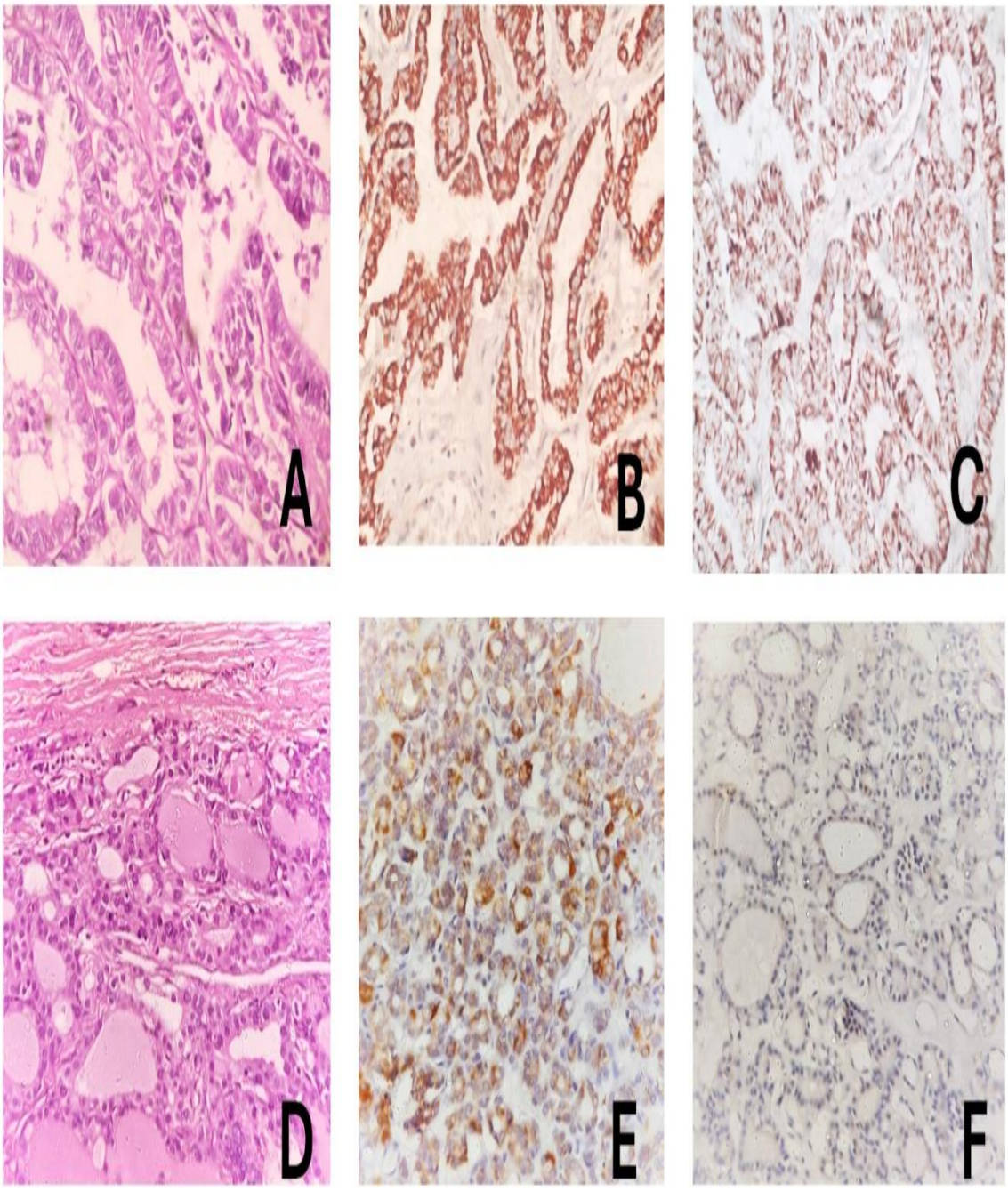
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Figure (1); A. Papillary thyroid carcinoma. Complex papillary architecture with fibrovascular cores, tumor cells are enlarged, oval, optically clear and overlapping (H&E, 400x). B. Strong positive cytoplasmic staining of SLP2 in 100% of tumor cells in a case PTC (SLP2, DAB, hematoxylin, 400x). C. Moderately positive cytoplasmic staining of SLP2 in 70% of tumor cells in a case PTC (SLP2, DAB, hematoxylin, 400x). D. Follicular adenoma. Arranged as micro and macrofollicles, lined by cuboidal cells with small and rounded nuclei and overlying intact capsule (H&E, 400x). E. Moderate cytoplasmic immunohistochemical expression of SLP2 in 50% of tumor cells in a case of follicular adenoma (SLP2, DAB, hematoxylin, 400x). F. Negative immunohistochemical expression of SLP2 in tumor cells in a case of follicular adenoma (SLP2, DAB, hematoxylin, 400x).

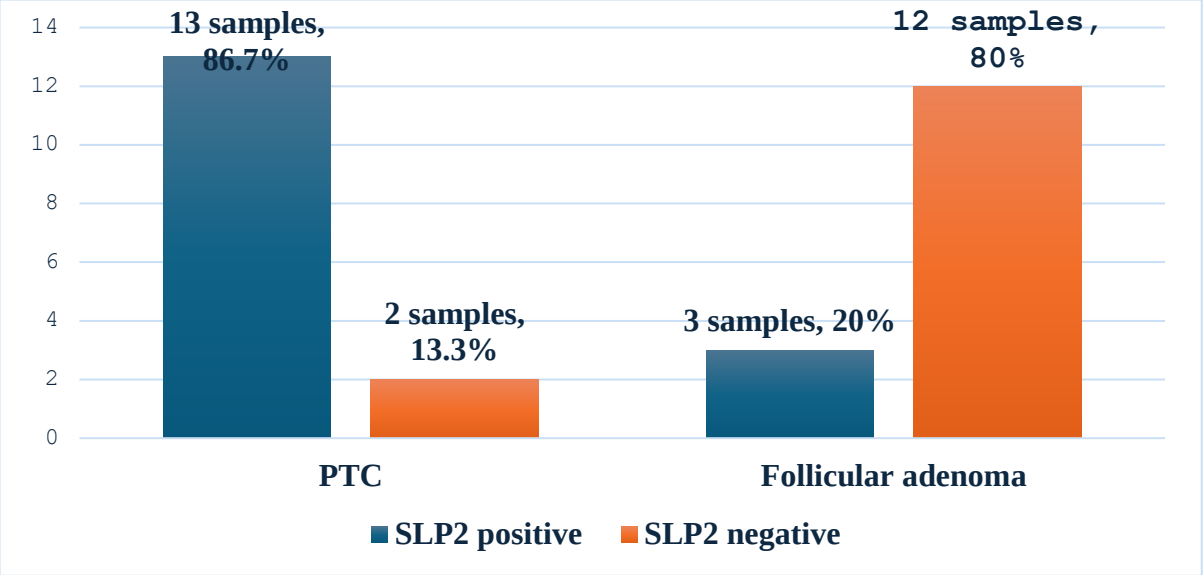

Figure (2): Distribution of PTC and follicular adenoma according to SLP2 expression results.

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