Expression of Snail, Insulin-like mRNA-binding Protein3 and Aldehyde Dehydrogenase 1 as Diagnostic Markers in Clear Cell, Papillary, and Chromophobe Variants of Renal Cell Carcinoma

Hanaa A. Atwa♦, MD, Noha F. Elaidy, MD

Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Abstract

Background: Renal cell carcinoma (RCC) is the most prevalent malignancy of kidney. Snail is a zinc-finger transcription factor, associated with advanced tumor stage and poor prognosis of RCC. Insulin-like mRNA-binding protein3 (IMP3) immunopositivity predicts metastatic progression and patients' survival in RCC. Aldehyde dehydrogenase 1 (ALDH1) is a stem cell marker, expressed in many different solid tumors of bladder, pancreas, colon, and kidney.

The present study aimed to assess the diagnostic value of Snail, IMP3, and ALDH1 expression in clear cell, papillary, and chromophobe variants of RCC.

Method: This retrospective study included 50 tissue blocks of RCC cases (35 clear cells, along with 11 papillary and four chromphobe carcinomas). Immunohistochemical staining was evaluated using antibody against Snail, IMP3, and ALDH1 in all the cases.

Results: Snail was observed to be 74.3% in clear cell RCC and 18.2% in papillary, but negatively expressed in all chromophobe carcinomas. IMP3 was expressed in 72.7% of papillary carcinomas and 28.6% of clear cell RCC; it was totally expressed in all chromophobe carcinomas. ALDH1 was expressed in 94.2% of clear cell RCC, 54.6% of papillary carcinomas, and 50% of chromophobe carcinomas. Snail, IMP3, and ALDH1 were found to be associated with advanced tumor stage and high grade; accordingly, they could be considered as poor markers for RCC patients.

Conclusion: ALDH1 is a good sensitive marker for diagnosis of RCC, especially for clear cell type, while IMP3 is a good marker for diagnosis of chromophobe carcinoma and Snail can be used for diagnosis of clear cell carcinoma.

Keywords: Carcinoma, Renal cell, Diagnosis, Immunohistochemistry

♦Corresponding Author:
Hanaa A. Atwa, MD
Department of Pathology, Faculty of Medicine, Zagazig University, Zagazig, Egypt
Email: hanaaatwa@yahoo.com

Introduction

Renal cell carcinoma (RCC) is the most prevalent malignancy of the kidney, which accounts for 3% of all malignant tumors and about 90% of

malignant renal neoplasms.[1]

The classification of RCC is of great importance in the prognosis and treatment. The most common histological subtypes include clear cell RCC (CCRCC), papillary, and chromophobe carcinomas. CCRCC accounts for approximately 70%-80% of all RCC histological subtypes.[2]

Understanding the molecular mechanism involved in RCC carcinogenesis is necessary for identification of predictive response to treatment. Epithelial-mesenchymal transition (EMT) is a biological process through which epithelial cells lose polarity and acquire a mesenchymal phenotype.[3]

Snail is a zinc-finger transcription factor, considered as a promoter of EMT, overexpressed in malignant tumors, where it is correlated with tumor invasion and metastasis.[4] In RCC, Snail expression is associated with advanced tumor stage and poor prognosis. Targeting Snail expression inhibits invasion in vitro, which suggests that Snail plays a role in invasion and metastasis.[5]

Insulin-like growth factor mRNA-binding protein3 (IMP3) is an oncofetal protein that plays a vital role in cell growth. It is expressed negatively or at low levels in normal adult tissues.[6]

IMP3 expression is an independent prognostic factor in various types of human cancers, such as breast, lung, and urinary bladder carcinomas.[7-9] IMP3 overexpression is correlated with aggressive tumor features in these cancers.[10] In RCC, serum and tissue IMP3 levels are correlated with poor survival.[11]

Aldehyde dehydrogenase 1 (ALDH1) is a general marker of both normal stem cells and cancer stem cells (CSCs). CSCs are particular cells with self-renewal and multipotency properties. Renal CSCs have a significant role in tumor growth, progression, recurrence, and resistance to chemo- and radiotherapy.[12] This stem cell marker is expressed in a number of different solid tumors of bladder,[13] pancreas,[14] colon,[15] and kidney.[16]

Materials and Methods

The present retrospective study included 50 formalin-fixed, paraffin-embedded tissue blocks of RCC cases (35 clear cell carcinomas, 11 papillary renal carcinomas, and four chromophobe carcinomas). They were retrieved from the archives of the Pathology Department, Faculty of Medicine, Zagazig University, from 2014 to 2019, using immunohistochemical antibodies against Snail, IMP3, and ALDH1.

Complete clinicopathological data, including age, sex, tumor size, histological subtype, Fuhrman grade, stage, lymph node, and distant metastasis of the cases were examined through a retrospective examination of the patients' files. Grading of RCC was based on WHO 2010 published criteria.

The study was approved by the Local Research Ethics Committee of Zagazig University hospital and patients' consents were obtained.

Immunohistochemical staining

Streptavidine-biotin technique was utilized for immune histochemical staining with primary
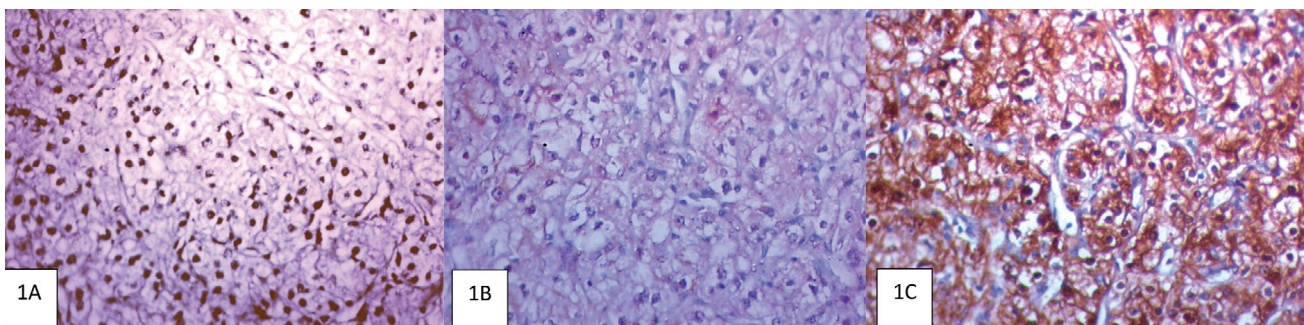
Figure 1. A) Clear cell renal carcinoma showing moderate immune expression of Snail (ABC, DAB, ×100); B) Clear cell renal carcinoma showing immune expression of IMP3 (ABC, DAB, ×200); C) Clear cell renal carcinoma showing strong immune expression of ALDH1 (ABC, DAB, ×200).
IMP3: Insulin-like mRNA-binding protein 3; ALDH1: Aldehyde dehydogenase 1

antibody against Snail (Dako, NBP1-80022, 1:100 dilution), IMP3 (Dako, Clone: 69.1, dilution1:100), and ALDH1 (Abcam, clone: EP1933Y, dilution 1:200).

To evaluate Snail expression, we considered 0 as no stained cells, 1 as <25%, 2 as 25%-50%, 3 as 51%-75%, and 4 as >75% nuclear staining. The intensity was scored as the following: 0 (absent), 1 (low), 2 (moderate), 3 (high).[4] The positive control for Snail was kidney tissue.

According to the percentages of the positive cytoplasmic staining areas of tumor cells, the evaluation of IMP3 staining was as follows: 0 indicated no staining; 1+ indicated <10%; 2+ indicated 10%–70%; 3+ indicated >70%.[10] Fetal liver obtained from aborted fetus was used as the positive control for IMP3.

We carried out the evaluation of ALDH1 based on the proportion of positive cells, as follows: score 0: negative, score 1: positive in <25%, score 2: positive in 25%-50%, score 3: positive in 51%-75%, score 4: positive in >75%. According to the intensity of staining, score 0 was considered as negative staining intensity, score 1 as weak staining intensity, score 2 as moderate staining intensity, and score 3 as severe staining intensity.[17] The positive control of ALDH1 is the human liver tissue.

Negative control for all the markers was obtained by omission of the primary antibody.

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described with their means and standard deviations. Categorical variables were described using their absolute frequencies. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used in parametric tests. Independent sample t-test was used to compare the means of the two groups. Categorical data were compared via the Chi-square (χ^2) test. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the data were calculated. The level of statistical significance was set at 5% ($P < 0.05$).

Table 1. Clinicopathological features of the studied patients

	N=50	%
Age (years)		
<60 years	22	44
≥60 years	28	56
Gender		
Male	32	64
Female	18	36
Tumor size		
>4 and ≤7	15	30
>7 and ≤10	30	60
>10	5	10
Histological types		
Clear	35	70
Papillary	11	22
Chromophobe	4	8
Stage		
1	9	18
2	19	38
3	16	32
4	6	12
Fuhrman grade		
Low	34	68
High	16	32
Fat tissue invasion		
Absent	28	56
Present	22	44
Renal vein invasion		
Absent	34	68
Present	16	32
Snail		
Negative	22	44%
Positive	28	56%
IMP3		
Negative	28	56%
Positive	22	44%
ALDH1		
Negative	9	18%
Positive	41	82%

IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1

Results

Clinicopathological results

Herein, 18 cases were women and 32 cases were men. The majority of the cases were over the age of 60 (56%). Renal vein invasion was observed in 44% of the cases (Table 1).

Immunohistochemical results

Snail was observed in 74.3% of CCRCC and 18.2% of papillary carcinomas, and it was negatively expressed in all chromophobe carcinomas (Figure1a) (Tables 2- 6).

There was a statistically significant relation

Table 2. Relation between Snail, IMP3, and ALDH1 levels in the studied patients and disease-specific characteristics

	Total	Snail			IMP3			ALDH1		
		Negative N=22(%)	Positive N=28(%)	P#	Negative N=28 (%)	Positive N=22(%)	P#	Negative N=9(%)	Positive N=41(%)	P#
Age group										
<60 years	22	9 (40.9)	13 (59.1)	0.696	15 (68.2)	7 (31.8)	0.124	5 (22.7)	17 (77.3)	0.481
≥60 years	28	13 (46.4)	15 (53.6)		13 (46.4)	15 (53.6)		4 (14.3)	24 (85.7)	
Sex										
Male	32	14 (43.8)	18 (56.2)	0.962	16 (50)	16 (50)	0.254	6 (18.8)	26 (81.2)	>0.999
Female	18	8 (44.4)	10 (55.6)		12 (66.7)	6 (33.3)		3 (16.7)	15 (66.7)	
Pathology										
CCC	35	9 (25.7)	26 (74.3)	<0.001	25 (71.4)	10 (28.6)	0.002	2 (5.8)	33 (94.2)	0.003
Papillary	11	9 (81.8)	2 (18.2)		3 (27.3)	8 (72.7)		5 (45.5)	6 (54.5)	
Chromophobe	4	4 (100)	0 (0)		0 (0)	4 (100)		2 (50)	2 (50)	
Size										
4-<7 cm	15	15 (100)	0 (0)	<0.001	13 (86.7)	2 (20)	0.003	8 (53.3)	7 (46.7)	<0.001
7 - <10 cm	30	7 (23.3)	23 (76.7)		14 (30)	16 (70)		1 (3.3)	29 (96.7)	
≥10 cm	5	0 (0)	5 (100)		1 (20)	4 (80)		0 (0)	5 (100)	
Stage										
1	9	8 (66.7)	1 (33.3)	<0.001	9 (100)	0 (0)	<0.001	7 (44.4)	2 (55.6)	<0.001
2	19	13 (10.5)	6 (90.5)		13 (47.4)	6 (52.6)		1 (5.3)	18 (94.7)	
3	16	1 (0)	15 (100)		6 (0)	10 (100)		1 (0)	15 (100)	
4	6	0 (20)	6 (100)		0 (0)	6 (100)		0 (0)	6 (100)	
Grading										
Low grade	34	18 (52.9)	16 (47.1)	0.042	23 (67.6)	11 (32.3)	0.031	9 (26.5)	25 (73.5)	0.043
High grade	16	4 (22.2)	14 (77.8)		5 (31.3)	11 (68.7)		0 (0)	16 (100)	
Fat invasion										
Absent	28	17 (60.7)	11 (39.3)	0.01	20 (71.4)	8 (28.6)	0.021	9 (32.1)	19 (68.9)	0.003
Present	22	5 (22.7)	17 (77.3)		8 (36.4)	14 (63.6)		0 (0)	22 (100)	
Renal Vein invasion										
Negative	34	19 (55.9)	15 (44.1)	0.017	24 (70.6)	10 (29.4)	0.005	9 (26.5)	25 (73.5)	0.043
Positive	16	3 (18.8)	13 (81.2)		4 (25)	14 (75)		0 (0)	16 (100)	

*P<0.05 is statistically significant; #Chi square test; ** P ≤ 0.001 is statistically and highly significant; IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1

between Snail expression or tumor stage high grade (*P* < 0.001 and 0.42). Snail was positively correlated with fat and renal vein invasion (*P* = 0.01 and 0.017). Snail sensitivity was higher in CCRCC than that in papillary or chromophobe carcinomas (74.3%, 18.2%, and 0%, respectively).

IMP3 was expressed in 72.7% of papillary carcinomas and 28.6% of CCRCC, and it was totally expressed in all chromophobe carcinomas (Figures1b, 3a, and 3b). IMP3 was positively correlated with advanced stage, high grade, and large size (*P* < 0.001, 0.31, and 0.003). There is a statistically significant correlation between IMP3 and fat or renal vein invasion (*P* = 0.021
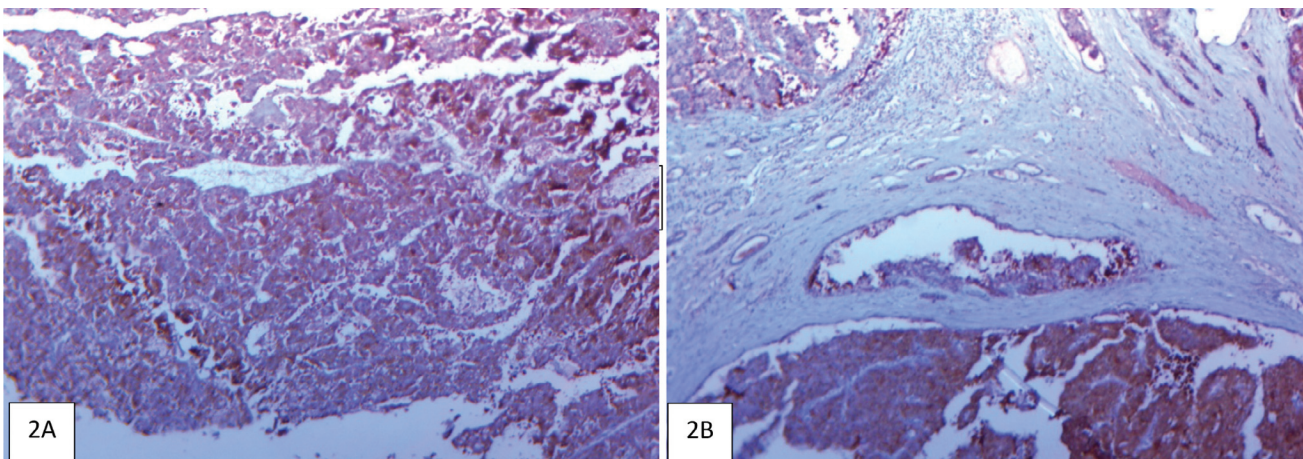
Figure 2. A) Papillary renal cell carcinoma showing positive strong immune staining for ALDH1 (ABC, DAB, ×100); B) Papillary renal cell carcinoma with vascular invasion showing positive strong immune staining for ALDH1 (ABC, DAB, ×100).
ALDH1:Aldehyde dehydrogenase 1

Table 3. Correlation between Snail, IMP3, and ALDH1 markers among the studied patients

Markers	Snail		IMP3		ALDH1	
	Phi	*P*	Phi	*P*	Phi	*P*
Snail			0.38	0.007	0.529	<0.001
IMP3	0.38	0.007			0.415	0.003
ALDH1	0.529	<0.001	0.415	0.003		

IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1

and 0.00). The sensitivity of IMP3 was high in chromophobe carcinomas (100%) and its specificity was 60.9% higher than that of papillary or clear cell type.

ALDH1 expression was detected in 82% of the RCC cases; this percentage is higher than that in normal kidney, which showed low expression (Figures 1c, 2a, and 2b). ALDH1 was present in 94.2% of clear RCC, 54.6% of papillary carcinomas, and 50% of chromophobe carcinomas. ALDH1 expression was significantly correlated with tumor stage, high grade, and large tumor size >10 cm ($P < 0.001$, 0.031, and 0.003, respectively). All the patients with fat invasion had positive ALDH1 and renal vein; the results were statistically significant at *P*-value of 0.003 and 0.043. The sensitivity of ALDH1 in clear RCC was 94.3% higher than that in papillary or chromophobe carcinomas (54.6% and 50%, respectively).

Discussion

According to our findings, Snail was observed in 74.3% of clear cell CCRCC, while it was negatively expressed in all chromophobe carcinomas. ALDH1 was expressed mainly in CCRCCs; IMP3 was expressed in all the cases of chromophobe carcinoma. Snail, IMP3, and ALDH1 were associated with an advanced tumor stage and high grade. Snail, IMP3, and ALDH1 could be considered as poor prognostic markers for RCC patients.

Snail1 is a zinc-finger transcription factor involved in the regulation of EMT.[18] Positive nuclear Snail immunostaining was observed in 56% of all the studied cases with positive expression in 77.1% of clear cells and 18.2% of papillary carcinomas, whereas it was negatively expressed in all chromophobe carcinomas. Snail staining was not observed in non-neoplastic renal tissues. Snail sensitivity was higher in clear RCC than that in papillary or chromophobe carcinomas (74.3%, 18.2%, and 0%, respectively).

Similar results were reported in another study by Adreiana et al.[4] who reported that Snail immunostaining was in 70 out of 83 (84%) CCRCCs, three of 10 (30%) papillary RCCs, and zero out of four (0%) chromophobe RCCs in their studied cases. Our findings were also in line with those by Cai et al.[19] who observed Snail immunostaining in 82.61% of RCC cases.

High Snail expression in the present study was significantly correlated with a high Fuhrman grade RCC (0.042). In high-grade carcinomas, it was 77.8% with diffuse staining, while focal staining was observed in low-grade carcinomas with only 47.1%. Similar results were documented by Messai et al.[20] who reported that the Snail expression is lower in low-grade carcinomas than that in high-grade ones.

Table 4. Performance of the three markers in diagnosis of CCRCC in the studied cases

		Sensitivity	Specificity	PPV	NPV	Accuracy
Snail	**Value**	71.3%	86.7%	92.9%	59.1%	78%
	95% CI	56.7% – 87.5%	59.5% – 98.3%	77.9% - 98%	44.3% – 72.4%	64% – 88.5%
IMP3	**Value**	28.6%	20%	45.5%	10.7%	26%
	95% CI	14.6% – 46.3%	4.33% – 48.1%	31.8% –59.9%	4.1% – 25.2%	14.6% – 40.3%
ALDH1	**Value**	94.3%	41.2%	76.7%	77.8%	76.9%
	95% CI	80.8% – 99.3%	18.4% – 67.1%	68.7% – 83.2%	44.8% – 93.8%	63.2% – 87.5%

PPV: Positive predictive value; NPV: Negative predictive value; IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1; CCRCC: Clear cell renal carcinoma; CI: Confidence interval

Table 5. Performance of the three markers in diagnosis of chromophobe carcinomas

		Sensitivity	Specificity	PPV	NPV	Accuracy
Snail	Value	0%	52.2%	0%	85.7%	48%
	95% CI	0 – 60.2%	37 – 67.1%		82 – 88.8%	33.7 – 62.6%
IMP3	Value	100%	60.9%	18.2%	100%	64%
	95% CI	39.8 –100%	45.4 – 74.9%	2.22 – 19.2%		49.2– 77.1%
ALDH1	Value	50%	84.8%	22.2%	95.1%	82%
	95% CI	6.8-93.2%	71 .3– 93.7%	8 – 48.5%	87.9–98.1%	68.6 – 91.4%

PPV: Positive predictive value; NPV: Negative predictive value; IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1; CI: Confidence interval

In the current study, the immunohistochemical expression of Snail in RCC was associated with advanced stage, the presence of fat and renal vein invasion, distant metastases, and poor prognosis. The results were in line with those of another study.[21] Liu et al.[22] also confirmed that Snail immunostaining can predict early recurrence of the disease in patients with CCRCC.

IMP3 is expressed in developing human tissues, but are absent in normal adult tissues. Most importantly, IMP3 can be re-expressed in several malignant tumors, such as lungs and gastric, colorectal, and breast carcinomas.

In the present study, IMP3 was expressed in four chromophobe (100%), eight papillary type (72.7%), and 10 (28.6%) clear cell carcinomas. These results are in accordance with those of another study by Eronat et al.[23] Tschirdewahn et al.[11] found that IMP3 concentration was significantly elevated in plasma samples of tumor patients compared with healthy controls, which attributed to poor survival. However, Jiang et al.[24] reported that IMP3 positivity was slightly increased in clear cell carcinomas (23%) compared with papillary (11%) and chromophobe (15%) types.

IMP3 expression was strongly correlated with tumor stage and grade, the presence of fat and renal vein invasion, and distant metastases. The results were in agreement with those of a previous study.[25] Jiang et al.[26] found that IMP3 positive patients were 10 times more likely to develop metastasis compared with those with IMP3 negative tumors.

IMP3 promotes RCC cell migration and invasion, which can be explained by activation of NF-κB pathway.[27]

The sensitivity of IMP3 was high in chromophobe carcinomas (100%) and its specificity was 60.9%, which is similar to the results by another study.[26] Thus, it is a good positive marker for diagnosis of chromophobe carcinoma.

In the current study, ALDH1 expression was
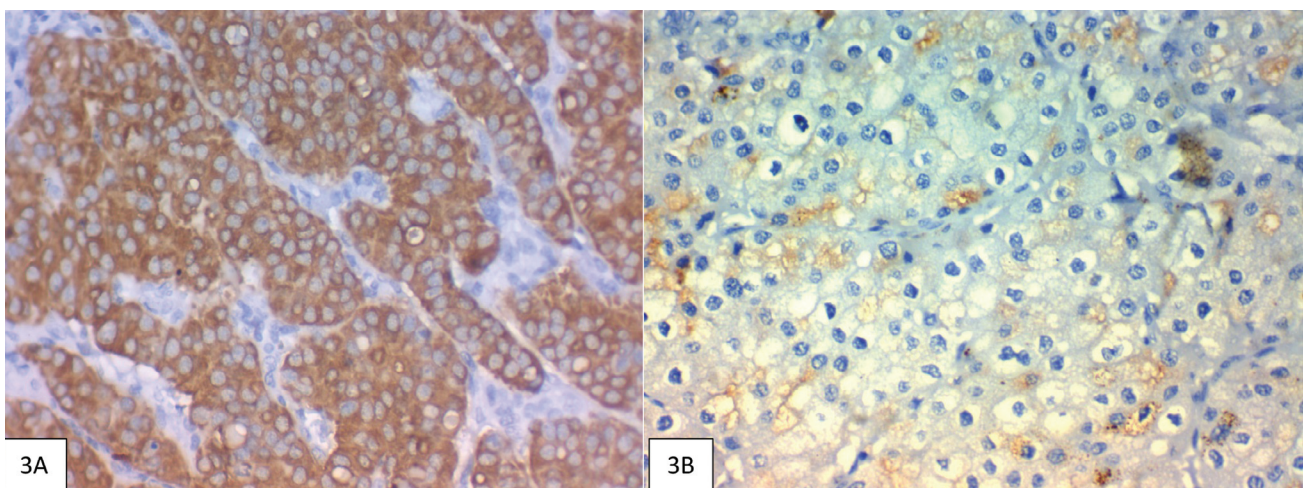
Figure 3. A) Chromophobe carcinoma showing high immune expression of IMP3 (ABC, DAB, ×400); B) Chromophobe carcinoma showing low immune expression of IMP3 (ABC, DAB, ×400).
IMP3: Insulin-like mRNA-binding protein3

Table 6. Performance of the three markers in diagnosis of papillary carcinomas among the studied patients

		Sensitivity	Specificity	PPV	NPV	Accuracy
Snail	**Value**	18.2%	33.3%	7.1%	59.1%	30%
	95% CI	2.3-51.8	19.1 – 50.2%	2.1 – 21.6%	46.1 – 70.9%	17.9 – 44.6%
IMP3	**Value**	72.7%	48.7%	28.6%	86.4%	54%
	95% CI	39-94%	32.4 – 65.2%	19.9 – 39.1%	69.6 – 94.6%	39.3 – 68.2%
ALDH1	**Value**	54.6%	5.13%	14%	28.6%	16%
	95% CI	23.4 – 83.3%	0.6 – 17.3%	8.6 – 21.8%	8.2 – 64.1%	7.17 – 29.1%

PPV: Positive predictive value; NPV: Negative predictive value; IMP3: Insulin-like mRNA-binding protein3; ALDH1: Aldehyde dehydrogenase 1; CI: Confidence interval

found in 33 (94.2%) of CCRCC cases, six (54.5%) of papillary RCC cases, and two (50 %) of chromophobe RCC cases; it is in line with other studies.[17,28-30] This study indicated the high expression of ALDH1 in cases with vascular invasion, which is in accordance with another study.[17]

The sensitivity of ALDH1 in clear RCC was 94.3%, which was higher than that in papillary or chromophobe carcinomas (54.6% and 50%, respectively). Therefore, ALDH1 could be a good positive marker for diagnosis of clear cell carcinoma, especially as confirmed in the study by Ahmed et al.[31]

There was a statistically significant positive correlation between Snail and each of IMP3 and ALDH1. Similarly, there was a statistically significant positive correlation between IMP3 and each of Snail and ALDH1. There was a non-significant relationship between all the three markers in terms of either age or gender.

Sometimes there was an overlap between clear cell, papillary and chromophobe RCC morphological features, which often leads to diagnostic challenges. In this study, immunohistochemical staining with Snail, IMP3, and ALDH1 markers could facilitate the discrimination of these variants. To the best of our knowledge, there were no papers focusing on the combination of these markers in the literature.

Our study has certain limitations due to its retrospective nature; therefore, a prospective study is needed to confirm the obtained results herein. Furthermore, the small number of studied cases, especially papillary and chromophobe variants of RCC, could be considered as a limitation.

Conclusion

ALDH1 is a good sensitive marker for diagnosis of RCC, specifically clear cell type, while IMP3 is a good marker for diagnosis of chromophobe carcinoma and Snail can be conducive to diagnosis of clear cell carcinoma. These markers were also associated with poor clinicopathological parameters; hence, they can be used in the future in order to improve the prognosis of RCC.

Conflict of Interest

None declared.

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