

Immunohistochemical Expression of Aldehyde Dehydrogenase 1 (ALDH1) in Renal Cell Carcinomas

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Abstract

- Background** Renal cancer is the 13th most common malignancy globally. Cancer stem cells display progenitor cell properties such as self-renewal and ability to re-establish tumors that explain the tumor of origin. Aldehyde dehydrogenase 1 (ALDH1) has been known as a general marker of both normal stem cells and cancer stem cells.
- Objective** To evaluate the immunohistochemical (IHC) expression of cancer stem cell markers ALDH1 in renal cell carcinomas (RCCs).
- Methods** A study was designed that included 70 paraffin blocks of renal cell carcinoma tissues obtained from patients who underwent nephrectomy. The control group included 30 samples of normal renal tissue of autopsy cases from the Forensic Medicine Institute. From each two sections of 5 μ m thickness were taken, one section was stained with Hematoxylin and Eosin (H&E) and the other section was immunohistochemically stained for ALDH1A1 (an isoform of ALDH1 that is expressed in the renal epithelium).
- Results** The difference in the IHC expression of ALDH1 is highly significant between RCC cases and control group ($p < 0.001$). ALDH1 expression was increased in high tumor grade of RCC cases ($p = 0.007$) and also its high expression was noted in advanced tumor stage ($p = 0.002$), and with tumor size > 4 cm ($p = 0.023$). RCC cases with renal vein invasion revealed high expression of ALDH1 ($p = 0.003$), also ALDH1 expression increased in cases with perinephric fat invasion ($p = 0.014$).
- Conclusion** ALDH1 showed higher expression in RCC tissues than normal renal tissues and it is associated with clinicopathological variables (tumor grade, tumor size, tumor stage, renal vein invasion, and perinephric fat invasion). This may reflect the role of ALDH1 in disease progression and poor prognosis of RCC.
- Keywords** Renal cell carcinoma, cancer stem cell marker, ALDH1, immunohistochemistry.
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List of abbreviations: ALDH1 = Aldehyde dehydrogenase 1
CSCs = Cancer stem cells, IHC = Immunohistochemical, RCC = Renal cell carcinoma

Introduction

The National Cancer Institute has defined renal cell carcinoma (RCC) as the most common type of malignant tumors in

the kidney, which arises from lining of the kidney renal tubules ⁽¹⁾.

Renal cancer is the 13th most common malignancy globally ⁽²⁾. Iraqi cancer registry recorded 386 cases in year 2010 with male to female ratio is about 1.4:1 ⁽³⁾. The peak incidence exists in the sixth and seventh decades of life ^(4,5). The histological

classification of RCC is very important, considering the significant effects of the subtypes in the prognosis and treatment of this tumor. The most common histological subtypes are clear cell renal cell carcinomas, papillary renal cell carcinomas, and chromophobe renal cell carcinomas. These three subtypes composed more than 90% of all RCCs ⁽⁶⁾.

Cancer stem cells (CSCs) display progenitor cell properties such as self-renewal, clonogenic ability and multipotency which are responsible for initiation and maintenance of cancer. Renal CSCs may have a significant role in tumor establishing, progression, and recurrence for their resistant to chemo and radio therapy ⁽⁷⁾.

Aldehyde dehydrogenase 1 (ALDH1) has been known as a general marker of both normal stem cells and CSCs. It was reported that ALDH1 expression is associated with nuclear grade of tumor cells in RCC. ALDH1 positive cells have greater ability in tumor development than ALDH1 negative cells and also are resistant to conventional therapies for RCC ⁽⁸⁾.

The objectives of this study was to evaluate the IHC expression of cancer stem cell markers ALDH1A1 in renal cell carcinomas.

Methods

A case control study was designed that included 70 paraffin blocks of RCC tissues obtained from patients who underwent nephrectomy, which were collected from Teaching Laboratory of Al-Imamein Al-kadhimein Medical City, Pathology Departments of Ghazi Al- Harreri Surgical Specialties Teaching Hospital and private laboratories for the period from January 2012 to April 2016. The control group included 30 samples of normal renal tissue of autopsy cases from the Forensic Medicine Institute with their relative consent for the period from December 2015 to February 2016. These specimens were processed and paraffin embedded in the Pathology Department in the Medical College of Al-Nahrain University. So, the total number of samples was 100 cases. The clinicopathological parameters were taken

from patients' admission case sheets and pathology reports.

From each block, two sections of 5µm thickness were taken, one section was stained with Hematoxylin and Eosin for the histopathological diagnosis revision and the other section was deparafinized and dehydrated. Antigen target retrieval solution (DAKO, Denmark) (ready to use) (pH 6.0, 20 minutes in microwave) was used. ALDH1A1 (an isoform of ALDH1 that is expressed in the renal epithelium) was used in this study. Monoclonal ALDH1A1 rabbit antibodies, clone (EP1933Y) (Abcam, United Kingdom) (dilution 1:100) were incubated overnight. After that sections were treated with ab80436 – EXPOSE Mouse and Rabbit Specific Streptavidin and Di-aminobenzidine chromogen Detection immunohistochemical (IHC) Kit (Abcam, United Kingdom), and counterstained with hematoxylin. Technical negative control was obtained by omission of primary antibody.

Interpretation of ALDH1 IHC staining and quality control

Brown membranous and/or cytoplasmic staining pattern of epithelial cells even if staining was focal in tumor cell ⁽⁹⁾. Positive control is the human liver tissue. Technical negative control was obtained by omission of primary antibody.

The results of IHC expressions of ALDH1 were scored semi-quantitative through assessing both staining intensity and percentage of stained cells (staining ratio) concerning the total number of cells and as following ⁽¹⁰⁾:

Staining intensity

Score 0: negative staining intensity,
Score 1: weak staining intensity,
Score 2: moderate staining intensity,
Score 3: severe staining intensity.

Proportion of positive cells

Score 0: negative,
Score 1 positive in <25%,
Score 2 positive in 25-50%,

Score 3 positive in 51-75%,
Score 4 positive >75%.

Then the two scores were multiplied for each case, and the expressions were graded as:
Score 0: was negative,
Score 1-4: was low expression grade,
Score 5-12: was high expression grade

Statistical analysis

The statistical analysis of this case control study was performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Numerical data were described as mean and standard error. Independent t-test was used for comparison between groups. While, categorical data were described as count and percentage, and Chi-square test was used to estimate the association between variables. The lower level of accepted statistical significant difference is below 0.05.

Results

According to ALDH1 grading score, 36 (51.43%) cases were considered high expression grade, and 34 (48.57%) cases were considered low expression grade. All control cases had shown low expression grade. The difference in the IHC expression of ALDH1 is highly significant between RCC cases and control group ($p < 0.001$) (Table 1).

ALDH1 expression displayed non-statistical significant association with age, sex, and histopathological types of RCC ($p > 0.05$) (Table 2).

High expression grade of ALDH1 was recorded in 24 out of 47 cases of clear cell type (Figure 1), 5 out of 10 cases of papillary type (Figure 2), 3 out of 5 cases of sarcomatoid type (Figure 3), 2 out of 5 cases of chromophobe type, 1 out of 2 cases of granular type, and in the only one case of collecting duct RCC (Figure 4).

High expression grade of ALDH1 recorded high percentage in tumors with high nuclear grade and advanced stage, tumors that measure more than 4 cm, and in RCC cases that have renal vein invasion and/or perinephric fat invasion. So that expression of ALDH1 showed significant association with tumor grade, tumor size, tumor stage, renal vein invasion, perinephric fat invasion of RCC cases with p value ($p = 0.007$), ($p = 0.023$), ($p = 0.002$), ($p = 0.003$), ($p = 0.014$) respectively (Table 2).

Discussion

In the present study, high expression of ALDH1 marker was recorded in 51.43% of RCC cases, which is higher than in normal tissue of control group that is parallel to Wang et al. ⁽¹⁰⁾ study in which, high expression was shown in 55.8% of RCC cases. A Turkish study done by Ozbek et al. ⁽¹¹⁾ recorded higher expression of ALDH1 in cancerous tissue than in normal tissue.

Table 1. IHC expression of ALDH1 in RCC cases and control group according to expression grade

	Score		Study groups		Total
			Control	RCC cases	
ALDH1 grade	Low (1-4)	Count	30	34	64
		%	100%	48.57%	64.00%
	High (5-12)	Count	0	36	36
		%	0.00%	51.43%	36.00%
Total		Count	30	70	100
		%	100%	100%	100%
p value			<0.001**		

** High statistically significant ($P < 0.001$)

Table 2. ALDH1 expressions in RCC cases

Parameter		Total N=70	(ALDH1A1 expression)		P value
			Low N=34	High N=36	
Sex	Female	26	11	15	0.644 ^{NS}
	Male	44	23	21	
Age group (years)	<50	23	11	12	0.999 ^{NS}
	≥50	47	23	24	
Tumor grade	Grade 2	50	30	20	0.007*
	Grade 3	14	2	12	
	Grade 4	6	2	4	
TNM staging of tumor	Stage-I	27	20	7	0.002*
	Stage-II	17	8	9	
	Stage-III	23	6	17	
	Stage-IV	3	0	3	
Histopathological types of RCC	Clear cell	47	23	24	0.928 ^{NS}
	Papillary	10	5	5	
	Chromophobe	5	3	2	
	Sarcomatoid	5	2	3	
	Granular RCC	2	1	1	
Tumor size	Collecting duct	1	0	1	0.023*
	≤ 4cm	13	10	3	
Renal vein invasion	> 4cm	57	24	33	0.003*
	Absent	62	34	28	
Perinephric fat invasion	Present	8	0	8	0.014*
	Absent	41	25	16	
	Present	29	9	20	

NS: non-statistical significance ($p>0.05$), *statistically significant

Furthermore, all apparently normal renal tissue samples in the present study showed low expression of ALDH1 marker, while in Wang et al. ⁽¹⁰⁾ study 79.3% of samples exhibited low expression while about 21.7% showed high expression.

This variance may be due to technical differences; such as the type of antibody, different methodology of IHC, and may be in the mode of selecting samples because the present study used autopsy samples, while Wang et al. study used biopsy samples from areas adjacent to non-tumor tissues that may

lead to higher detection of enzymes and antibodies.

Ma et al. ⁽¹²⁾ revealed that ALDH1 enzymatic activity was recognized as a marker of both normal stem cells and CSCs. Furthermore, it was observed that ALDH1 distribution patterns in normal tissues were dissimilar, and were classified into three types: tissues with absent or limited expression, tissue with relatively weak expression, and tissue with high expression which is not used as a CSC marker ⁽¹³⁾.

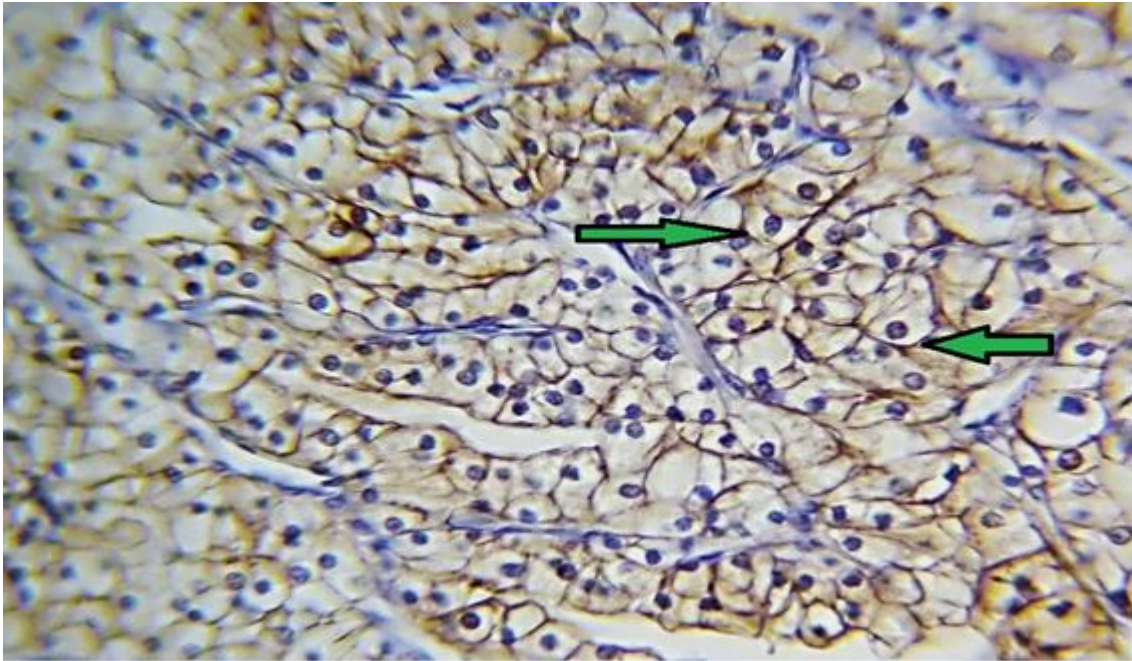


Figure 1. Clear cell RCC (grade 2, stage III) showing high IHC expression (score 6) of ALDH1A1 with brown membranous staining (arrows)(40X)

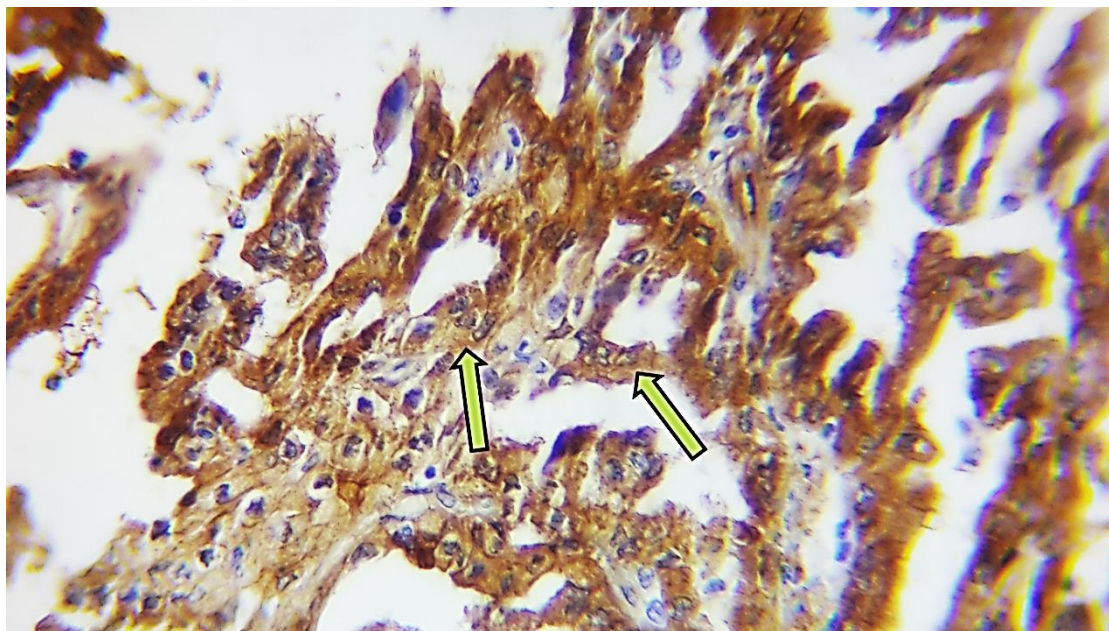


Figure 2. Papillary type RCC (grade 3, stage III) showing high IHC expression grade (score 6) of ALDH1A1 with brown membranous and cytoplasmic staining (arrow) (40X)

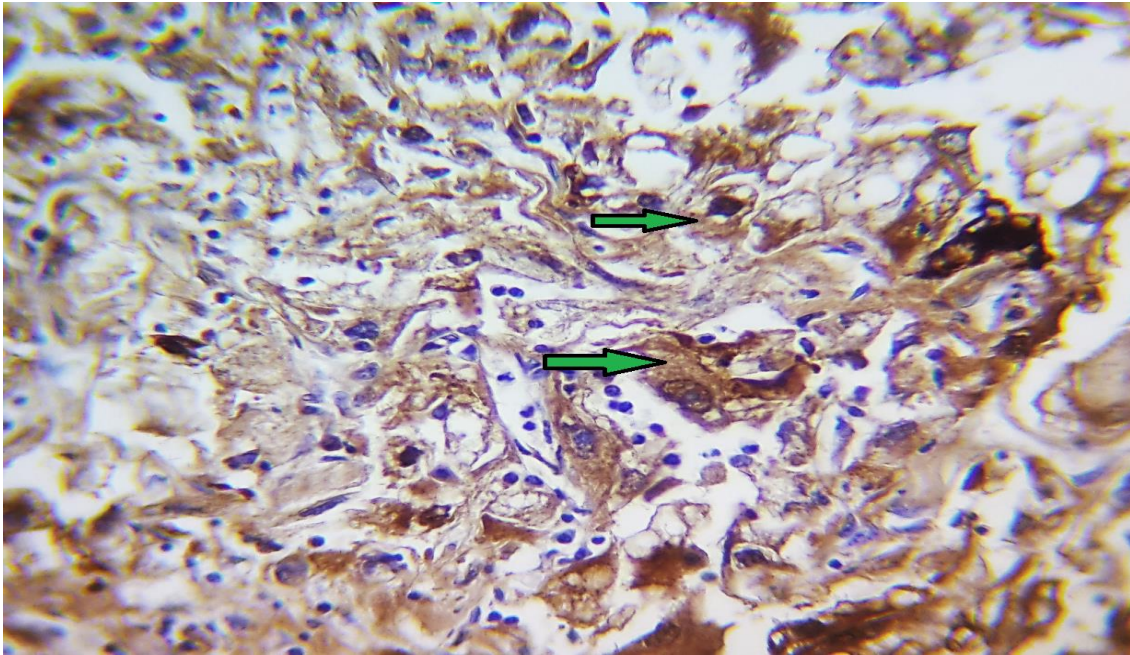


Figure 3. Sarcomatoid type RCC (grade 3, stage III) showing (score 12) ALDH1A1 IHC brown membranous and cytoplasmic staining (arrows) (40X)

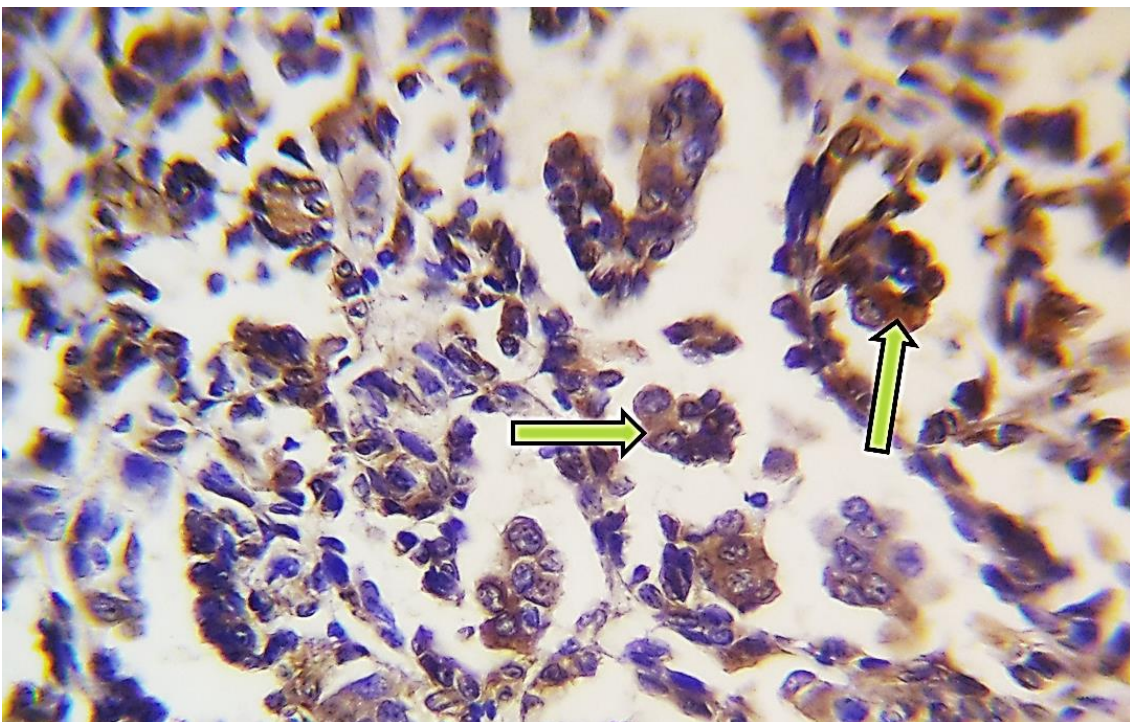


Figure 4. Collecting duct type RCC (grade 2, stage III) showing high IHC expression grade (score 9) of ALDH1A1 with brown membranous and cytoplasmic staining (arrows) (40X).

ALDH1 has been recently introduced as a possibly reliable CSC marker and it has a role in RCC pathophysiology^(9,11). ALDH1 was expressed in non-cancerous and cancerous renal tissues and located in the cytoplasm and cytomembrane⁽¹⁰⁾. Some studies have reported that high expression of ALDH1 was associated with drug resistance due to cellular protection against cytotoxic drugs that exhibited poor prognosis⁽¹²⁾.

The frequency of cases with high expression of ALDH1 in patient <50 years was 52% which is slightly higher than that of patient ≥50 years (51%). These findings are parallel to Wang et al. study⁽¹⁰⁾.

The higher frequency of ALDH1 expression in young age group may be attributed to the intermediate tumor size (4-12 cm), which is associated with poor prognosis and rapid metastasis⁽¹⁴⁾.

In present study, the low expression of ALDH1 was more frequently seen in stage I and II, while cases in stage III and IV were associated with higher frequency of high expression. The frequency of low expression of ALDH1 was increased in low grade (2), while high expression frequency was increased in high grade (3,4). There is a significant relation of ALDH1 expression with tumor stage and with tumor grade that is parallel to Ozbek et al.⁽¹¹⁾ and Wang et al. study. This association may reflect that high expression of ALDH1 is associated with poor prognosis cases⁽¹⁵⁾ with high tumor grade and advanced stage^(16,17). Abourbih et al.⁽⁹⁾ have found discordant results to the current study which may be due to different sample size or method of selection as well as the using of old sample from 1985 to 2006 in which the antigens may be presented at low levels to be detected efficiently by monoclonal antibody.

In the current work, expression of ALDH1 is increased when tumor size is more than 4cm in present study. There is significant relation between ALDH1 expression and tumor size that is parallel to Wang et al.⁽¹⁰⁾ study.

In present study, high expression of ALDH1 was higher in cases with renal vein invasion that is parallel to Wang et al.⁽¹⁰⁾ study.

Tumors with renal vein invasion has worse prognosis and become more aggressive^(18,19) when show higher ALDH1 expression⁽¹⁰⁾.

Current work showed that non-statistical significant relation between the ALDH1 expression and histopathological types of RCC.

The only one case (100%) of collecting duct type in the current research exhibited high ALDH1 expression may be due to this case presented with renal vein invasion. Furthermore, sarcomatoid type recorded high ALDH1 expression (60%) followed by clear, papillary, granular, then chromophobe. This result parallel to literature which stated that sarcomatoid type has worst prognosis followed by clear cell type, papillary and lastly chromophobe type⁽²⁰⁾.

In conclusion, ALDH1 showed higher expression in RCC tissues than normal renal tissues and it is associated with clinicopathological variables (tumor grade, tumor size, tumor stage, renal vein invasion, and perinephric fat invasion). This may reflect the role of ALDH1 in IHC expression could act a necessary role in disease progression and poor prognosis of RCC.

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Authors contributions

Dr. Hassan collected the cases, performed IHC test and analyzed the results. Dr. Qasim helped in study design and supervising the work. Dr. Musa participated collection of cases and revision of histopathological sections.

Conflict of interest

The authors have no conflicts of interest

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