

CARBONIC ANHYDRASE IX ASSAY: A PARADIGM SHIFT IN DIAGNOSIS OF MALIGNANT CYSTIC RENAL LESIONS.

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ABSTRACT

Carbonic anhydrase IX Assay: A paradigm shift in diagnosis of malignant cystic renal lesions.

INTRODUCTION AND OBJECTIVES:

Cystic renal neoplasms are always decided radiologically using Bosniak classification system for renal cysts with many times the histopathology being benign in final report. Carbonic anhydrase IX (CA IX), a known marker for solid clear cell renal cell carcinoma (CCRCC) with good sensitivity and specificity, may be useful for differentiating benign versus malignant renal cystic lesions. The purpose of the study was to demonstrate the usefulness of CAIX in these confusing scenarios and to have a new tool for definitive diagnosis in such cases before contemplating radical surgery.

METHODS:

A total of 22 patients were included in the study prospectively. Twelve patients diagnosed with Complex renal cysts-Bosniak type IIF, III, IV on Contrast-Enhanced Computerized Tomography (CECT) Abdomen and Pelvis undergoing partial/radical nephrectomy were analysed. Intra-operatively, the aspirates were collected from complex cystic lesions and they were subjected to western blot analysis. Similarly, aspirates were collected from 10 patients with simple renal cysts undergoing surgery for other indications and their aspirates were also subjected to western blotting to act as a control. Histopathological examination (HPE) of all operated complex cyst patients was done. Immunohistochemical (IHC) staining and scoring for both intensity and extent of positivity was done on the HPE slides. Scoring was done using a composite Score with score <2 was considered negative for CAIX expression while 2-3 was considered moderate and > 3 considered strong resp and CA IX expression detected by western blotting and IHC staining were correlated.

RESULTS:

Majority of the cysts in the study population belongs to Bosniak category III (85%). Diagnostic accuracy of the CA IX assay using Western Blotting was 83.33% and correlated with IHC staining with a negative predictive value 100%. Below table shows the correlation between IHC staining and CA IX expression using wetsern blotting.

CONCLUSIONS:

Positive CA IX assay reflects presence of malignancy and those with negative test can be offered an surveillance thus obviating the need for immediate radical surgery as in this era renal preservation should be the main goal. CAIX should be considered a promising molecular marker to differentiate such complex cystic renal tumors (malignant versus benign cysts) and play role in definitive management.

Introduction

RCC accounts for approx. 2 % of all human cancers. Cystic RCC (4% to 15% of all RCC) however pose a diagnostic challenge, especially for Type IIF, Type III and some IV (Bosniak types).

False positivity of such lesions are in range of 80 % and 60% resp. when evaluated using above criterias. Also, another entity known as Multilocular Cystic Clear Cell Renal cell Carcinoma (MCCRCC) a new variant of CCRCC (good prognosis) is difficult to differentiate from Multicystic nephroma (MCN) on imaging as well as histopathology.

- Multiple diagnostic markers for RCC have been tried to diagnose, immuno-target and prognosticate RCC patients but not a single marker has been validated and new molecules keep evolving. Routinely, a 5- Antibody IHC: CD10, Vimentin, EMA, CK 7, CK 20 are used in combination for difficult cases which again demonstrate lack of a single specific marker for RCC.
- CAIX (a trans-membrane protein, 54/58 kDa), located on Chromosome 9p12-13, positively induced by HIF-1 α (VHL pathway of RCC) has been recently looked upon more intensively. Established data shows CAIX specificity for solid lesions of 90-100% CCRCC, 50% Papillary RCC, <20% Chromophobe RCC. No other benign renal lesions and normal renal parenchyma express this molecule.
- Based on this data, various CAIX protien / mRNA detection techniques have been developed (eg. Western Blotting / ELISA / RT-PCR) for profiling of CAIX expression. Whether same expression profile as shown by solid RCC can be applied to cyst aspirate from Cystic malignant renal lesions remains a dilemma.

Objectives

- To evaluate CAIX expression profile in solid renal lesions in local population and to see whether it correlates with the expression profile already established in literature.
- Applicability of solid CAIX expression profile for diagnosing malignant cystic renal lesions and to differentiates them from other benign cystic renal neoplasm.

Materials

Place : Department of Urology, Amrita institute of medical sciences, Kochi, Kerala.

Study Design: Prospective Study.

Duration of study: 1 year (2013-2014).

Ethical Clearance : Ethical clearance was obtained from The Institute Ethical Review Board.

Estimated sample size: Based on the previous results on accuracy of CA IX expression in solid renal tumours from previous study (i.e. 95-100%) (11), it was found that the number of patients required for studying CA IX expression profile in renal lesions (solid/complex renal cysts) was 20 patients. Total 10 patients, with incidentally detected simple renal cysts (Bosniak type I, II)

(8), undergoing surgery for other indications were included in the study as a control population to compare CA IX expression profile with study population.

Inclusion criteria: Patients undergoing partial/radical nephrectomy for solid renal lesions and complex cystic lesions (Bosniak type IIF, III, IV) (8), diagnosed and confirmed on imaging (CECT/MRI abdomen and pelvis).

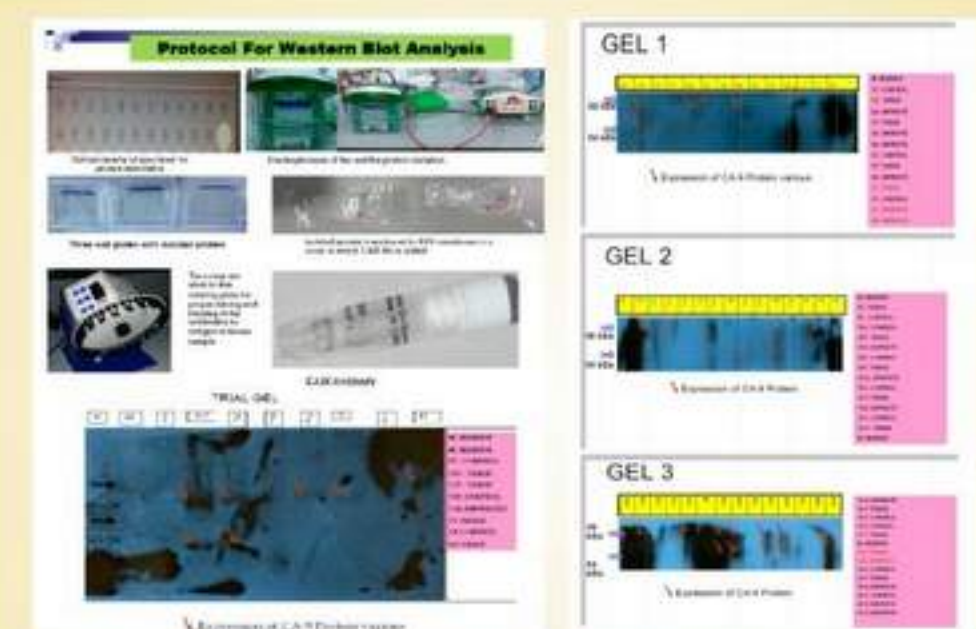
Primary antibody : Mouse Monoclonal antibody (2D3) to Carbonic anhydrase IX (ab107257), IgG1 (Abcam Inc, Kendall Square, 341 Cambridge, MA, USA)

Statistical Analysis: SPSS software version 20., McNemar's ChiSquare test, Mann Whitney U test.

Methodology

- Patients with inclusion criteria's were enrolled into the study after formal informed consent.
- Pre-designed performa were maintained for each patient.
- Histopathology report was obtained for each patient undergoing surgery mentioning AJCC TNM 2010 classification, histologic subtype, regional lymph node involvement, nuclear grade, coagulative tumor necrosis, and sarcomatoid differentiation.
- Total 21 patients underwent nephrectomy. All the tissue samples were taken on bench after retrieval of nephrectomy specimen from patient and preserved in 4 C cold saline till further storage in 20 C Freezer in Department of Nanosciences, Amrita institute of medical sciences till further processing was done. Ten patients with incidentally found simple renal cysts were aspirated and included in the study to act as a control.
- The usual protocol consisted of a 5mm bit of tissue from the tumour region/complex cyst wall, from the normal renal parenchyma and an aspirate in cases of complex cystic lesions. The aspirated fluid was sent as such in sterile bottle for storage.
- All the specimen were later analysed using Western Blot assay analysis and IHC staining for expression levels of CA IX in tissue (tumour and normal renal parenchyma) and cystic fluid aspirates. The protocol for WB Analysis and IHC staining are easily available along with the CAIX antibody analysis kit.
- IHC staining using composite score was done
- Composite scoring criteria for intensity and extent of staining tumour cells

Score	0	1	2	3
Intensity	No staining	Weak	Moderate	Strong
Extent	No staining	<25%	25-50%	>50%



CAIX expression profile in the patients Western blot analysis. Gel plates prepared for analysis of protein band corresponding to CAIX (54/58kDa)

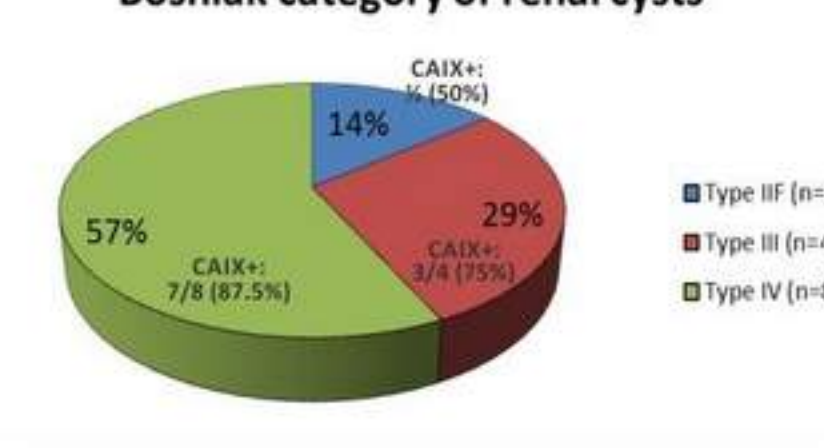
Results

Tumour Characteristics in study population

Tumour Characteristics	Number of cases n (%)
Tumour size (cm) (n=21)	6.74 \pm 3.21 (1-14 cm)
(AJCC TNM staging 7 th ed., 2010) (n=19 cases)	
T1	10 (52.61)
T2	2 (10.5)
T3	6 (31.5)
T4	1 (5.2)
Fuhrman Nuclear Grade (n=19 RCC cases)	
I	2 (10.52)
II	11 (57.8)
III	6 (31.57)
IV	0 (0)
Rhabdoid differentiation	1(4.7)
Infected Cysts	2(0.1)

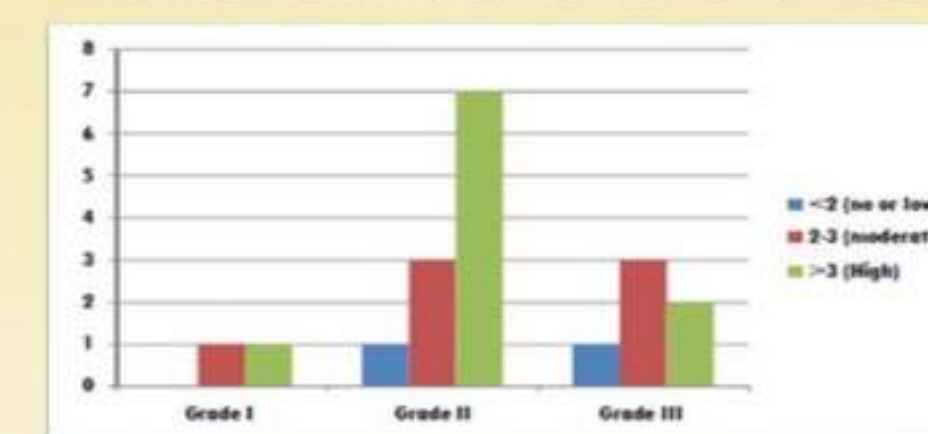
Tumour Type	N	Composite Score (Intensity + Extent)			IHC staining with CAIX Expression (%)	Western Blot Analysis CAIX expression n (%)
		<2 (No/Low)	2-3 (Moderate)	>3 (High)		
CCRCC	14	1	3	10	100	93
Papillary RCC	2		2		100	50
Chromophobe RCC	1		1		100	100
CCRCC Rhabdoid variant	1			1	100	100
Infected Cyst	2	2			0	50
MCCRCC	1			1	100	100
Normal Parenchyma	21	21			0	0

Bosniak category of renal cysts

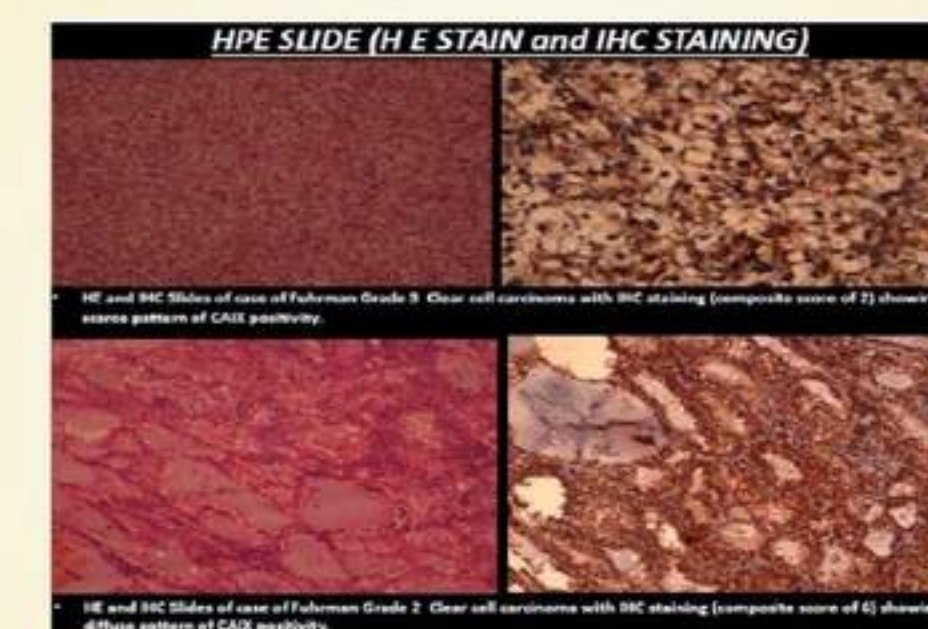


CAIX expression profile in complex cystic renal lesion in the study (Diagnostic Accuracy 83.3%)

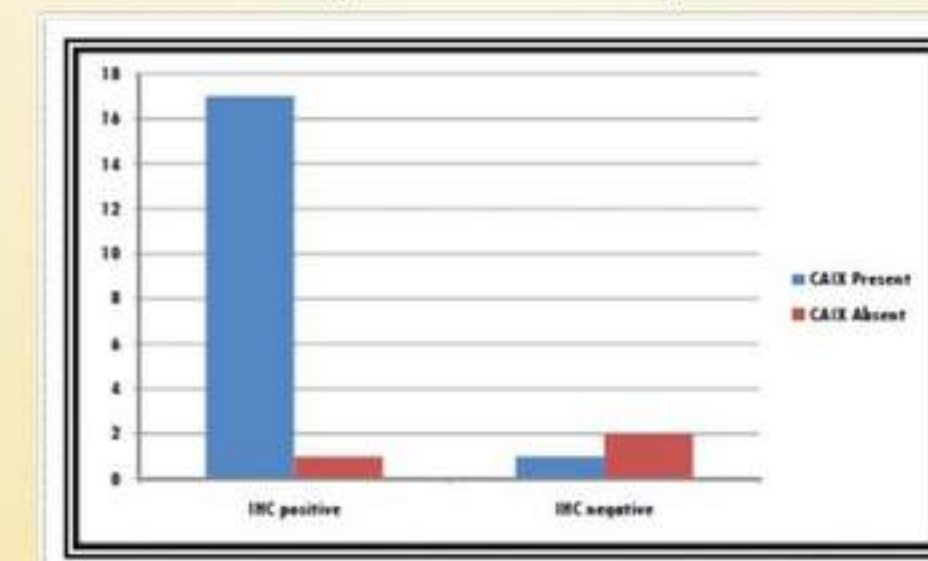
CORRELATION OF CAIX VS. TUMOUR GRADE



CAIX EXPRESSION PROFILE AS DETERMINED USING IHC STAINING IN RELATION TO FUHRMAN GRADE



CORRELATION OF CAIX IHC AND WESTERN BLOT ANALYSIS (C - Index 90.47 %)



Discussion

- This study is based on theory of pathologic changes in cyst wall leads to alteration in metabolic composition of cystic fluid which is in equilibrium with intracellular environment of lining malignant epithelium (Hazelton et al, Wu H et al)
 - Similarly, CAIX is present in the cystic fluid of malignant cysts due to secretion from its trans-membrane location.
 - Various biopsy techniques such as FNAB is unreliable in cystic lesion due to a large sampling error due to septas and unrepresentative tissue.
 - So, Analysis of the aspirate seems a reliable alternative to diagnose malignancy.
- In our study,

- All solid RCC, expressed CAIX protein by WB tissue analysis: 100% specificity
- In case of cystic malignancy, approx 83.6 4% demonstrated CAIX expression in WB aspirate analysis.
- Additionally none of the benign cysts nor normal renal parenchyma had CAIX expression which correlated with the study results of Li G et al who demonstrated an accuracy 88% of CAIX expression to diagnose cystic RCCs.

False positivity

- One case of Infected cysts with pyelonephritis
- Cross reactivity of CAIX rabbit monoclonal antibody to B-tubulin (aspirate overdilution)
- Burnt out RCC with necrosis, with no evidence of clear cell on IHC staining
- Inflammation causing tissue ischemia upregulation of HIF-1-alfa subs. CAIX expression.

False Negativity

- One each in CCRCC and PRCC case resp.
- Protein denaturation due improper storage technique
- Sampling error (although rare)
- Technical error

Conclusion

CAIX expression of cystic lesion can be used as surrogate for diagnosing cystic malignant renal lesions and converse is also true, so that surgical exploration in this confusing categories can be avoided.

Limitations

- Selective availability of Antibody
- Small sample size of study
- Stringent specimen preservation method required

References

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- Hazelton D, Nicosia RF, Nicosia SV. Vascular endothelial growth factor levels in ovarian cyst fluid correlate with malignancy. Clin Cancer Res. 1999; 5: p. 823-9.