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SYSTEMATIC REVIEW

Carbonic anhydrase IX immunohistochemistry has potential to predict renal cell carcinoma outcomes: A systematic review and meta-analyses

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Abstract

Introduction: Tissue biomarker carbonic anhydrase IX (CAIX) is purported to have prognostic value for renal cell carcinoma (RCC) but contradicting findings from previous studies have also been documented. This study aims to perform a systematic review and meta-analysis on the role of CAIX in RCC disease progression. *Materials and Methods:* Following the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines, online searches of multiple databases were performed to retrieve articles from their inception until December 2017. Inclusion criteria included all English-based original articles of immunohistochemistry (IHC) studies investigating CAIX expression in human RCC tissue. Four articles were finally selected for meta-analysis with a total of 1964 patients. Standard meta-analysis methods were applied to evaluate the role of CAIX in RCC prognosis. The relative risk (RR) and its 95% confidence interval (CI) were recorded for the association between biomarker and prognosis, and data were analysed using MedCalc statistical software. *Results:* The meta-analysis showed that high CAIX expression was associated with low tumour stage (RR 0.90%, 95% CI 0.849-0.969, p= 0.004), low tumour grade (RR 0.835%, 95% CI 0.732-0.983, p= 0.028), absence of nodal involvement (RR 0.814%, 95% CI 0.712-0.931, p= 0.003) and better ECOS-PS index (RR 0.888%, 95% CI 0.818-0.969, p= 0.007). The high tissue CAIX expression in RCC is hence an indication of an early malignancy with a potential to predict favourable disease progression and outcome. *Conclusion:* The measurement of this marker may be beneficial to determine the course of the illness. It is hoped that CAIX can be developed as a specific tissue biomarker for RCC in the near future.

Keywords: carbonic anhydrase IX, CA9, immunohistochemistry, kidney cancer, prognosis, survival

INTRODUCTION

Globally, the frequency of RCC has increased progressively over the last two decades and ranked 3% of all urogenital cancers. In the United States, nearly 65,340 new cases of kidney and renal pelvis cancers are likely to be diagnosed in 2018 and the estimated cancer related death would be 14,970.[1] In the Malaysian population, its incidence is approximately 1.9/100,000.[2]

Currently, the Tumour Node Metastasis (TNM) classification is widely used to predict the outcome of RCC. An advanced TNM stage is related to poor prognosis and survival. Additionally, there are numerous studies that can predict tumour recurrence based on the clinical and histopathological factors. Such factors include Eastern Cooperative Oncology Group Performance Status (ECOS-PS) whose grading depends on the physical activity of the patient; grade '0' means normal activity and grade '5' means death, nephrectomy status, period from nephrectomy to metastatic disease

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or to starting immunotherapy, prior radiotherapy, location and severity of metastatic sites, serum-corrected haemoglobin, calcium, lactate dehydrogenase and blood counts like platelets and lymphocytes.[3-12] In spite of all these factors, patients do have recurrences (with or without metastasis) and many have poor prognosis.

Over a third of patients who undergo surgery for clinically localised lesion develop RCC recurrence either locally or as distant metastasis. These patients are then submitted for adjuvant chemo- or immunotherapy. But the treatment response varies. Development of tissue prognostic biomarkers may therefore help predict which patients are more likely to develop recurrence and who will be more likely to benefit from adjuvant therapy. Such biomarkers can also provide further predictive information, which can help monitor tumour behaviour and response to the treatment. Recently, various biomarkers have been studied with regards to RCC.[13-18] Amongst them, carbonic anhydrase IX (CAIX) has been considered as a promising candidate to be developed as a prognostic marker for RCC. However, data on CAIX had shown some conflicting findings.[19-21]

CAIX is a transmembrane enzyme that catalyses the reversible hydration of carbon dioxide into carbonic acid.[22] This enzyme is induced by hypoxia via the hypoxia-inducible factor-1 alpha (HIF-1α)[23] and plays a vital role in RCC growth and progression.[24] Various studies demonstrated that CAIX is highly expressed in RCC and is absent in adjacent normal kidney tissues.[25] However, CAIX is also expressed in other types of malignancy.[26-28] Although many studies support the presence of CAIX as a potential prognostic marker for RCC[29-31], there are some that disapproved this after adjusting for TNM stage grouping, nuclear grade and ECOS-PS status.[19-21] Due to these conflicting findings, our team has decided to perform a systematic review and meta-analysis in order to determine unequivocally if tissue CAIX expression can be considered a potential marker for RCC outcome.

MATERIALS AND METHODS

A systematic search of novel articles regarding the prognosis of CAIX for RCC was directed on four databases. The following keywords were included: CAIX, CA9, carbonic anhydrase IX, renal cell carcinoma, kidney cancer or carcinoma, prognosis and survival. Following PRISMA guidelines, different combinations of these keywords were used in PubMed, Medline, Web of science and World cat databases (Appendix 1). Inclusion criteria focused on all original articles of CAIX expression in tissue immunohistochemistry (IHC) conducted on humans and published in English language. Articles with incomplete data, duplicates, book chapters, genetic study and non-English articles were excluded.[32] When several articles were published by the same author on similar patient cohort, the latest article was included for this study[19]. Two different researchers (SPS and IS) extracted the data independently. Each incorporated the essential parts of the study such as name of researcher, publication year, sample size, tumour stage, tumour grade, CAIX expression cut-off value, survival outcomes and quality assessment. Any discrepancy between researchers' data was discussed with the team and a consensus was made.

Quality Assessment

The quality of each individual article was assessed independently by two researchers using Newcastle-Ottawa quality assessment scale (NOS) for cohort study (Appendix-2). This assessment included three main categories: selection of cohort, comparability of cohort, and ascertainment of outcome. The scale is comprised of eight components and a star was awarded for high quality component within the selection and outcome criteria. Two stars were awarded for comparability criteria. Articles with maximum number of stars were selected as they reflect better methodological quality. Any discrepancy in the assessment of these articles were discussed and finalized among the researchers.

Statistical Analysis

Meta-analysis was done using MedCalc software (Mariakerke, Belgium). Patients who had low CAIX expression were categorized as control group, while patients with high CAIX expression were categorized as the intervention group. Odds ratio (OR) and 95% confidence intervals (CI) were stated for binary outcome parameters. Heterogeneity among studies was tested by the χ^2 test and I^2 metrics. A random effect model was used when the data being analysed had significant heterogeneity. The fixed effect model, however, was applied for homogenous studies. $p < 0.05$ was considered statistically significant.

RESULTS

Systematic review results

Endnote software (X7) was used to extract the articles. Initial search yielded the following numbers: Pubmed (681), Medline (191), Web of science (881) and World catalogue (151). A total of 1904 articles were recorded. Upon instruction, this software scrutinized the articles and excluded 1242 articles which were duplicates. Articles (662) were then screened for titles and abstracts and only 47 were qualified for final assessment (Fig. 1). Each article was then assessed following NOS and more articles were excluded for the following reasons: two articles were excluded because they were genetic studies;[33,34] twelve articles were excluded because they were review articles[35-46]; one was a meta-analysis;[32] four articles were studying blood/ serum levels of CAIX;[47-50] one was a cell study;[51] two studies had incomplete data/ is a press report;[44,52] one study was excluded as it showed comparative study between primary antibody and commercially available antibody[53]; five studies focused on multiple biomarkers[30,31,54-56]; two articles were non-RCC related;[36,57] eight were treatment based studies;[37,58-64] one was current issues based on tissue research[65] and one study focused on HIF-1α expression.[66] Two articles matched our inclusion criteria, but were rejected due to non-standardised cut-off point for the assessment of CAIX expression.[21,67] Finally, one article had to be rejected as some basic data was not documented as per the requirement.[68] In the end, four articles qualified for the final meta- analyses as listed in Table 1.[19,34,69,70]

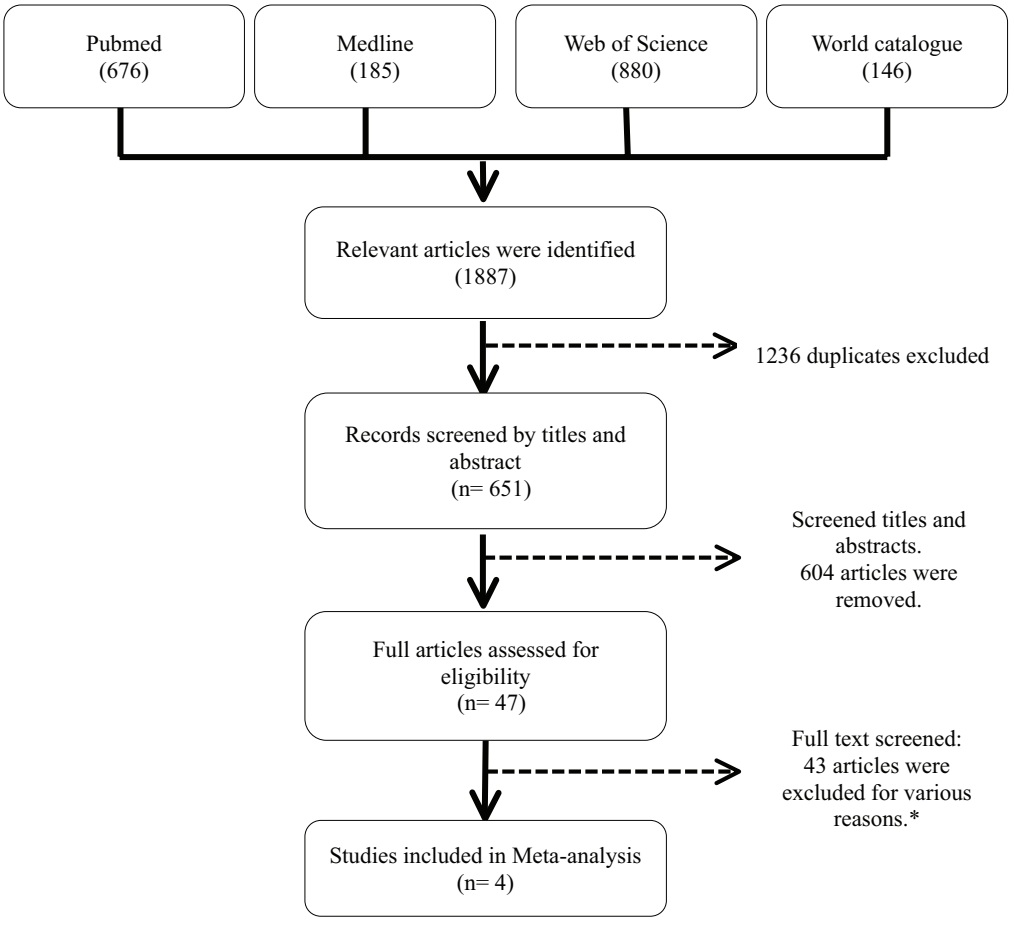

FIG. 1: Flow chart of study selection according to PRISMA criteria from inception until December 2017. * The 43 articles excluded from this systematic review include: 2 genetic studies, 12 review articles, 1 meta-analysis, 4 blood / serum-based articles, 1 cell line study, 2 incomplete data / press report, 1 comparative study between primary and commercially available antibody, 5 multiple biomarker studies, 2 non-RCC studies, 8 treatment based studies, 1 tissue based study, 1 study focused on HIF-1α expression, 2 studies used a nonstandardized cut-off point and 1 study had incomplete data.

TABLE 1: Articles selected for meta-analysis

Author	Year	Country	Sample size	T-stage	Grade	Cut-off value	Survival outcome	NOS
Bui *et al.*	2003	USA	321	114/39/150/18	38/151/110/22	85%	DSS	7
Chamie *et al.*	2015	USA	813	N/A	N/A	85%	DFS, OS	8
Patard *et al.*	2008	France and USA	100	29/20/48/3	2/37/41/20	85%	DSS	7
Zhang *et al.*	2013	USA	730	N/A	N/A	85%	DSS	7

T-stage refers to tumour stage, DSS refers to disease specific survival, DFS refers to disease free survival, OS refers to overall survival, N/A refers to not applicable NOS refers to Assessment stars based on Newcastle- Ottawa scale.

The four selected studies were conducted in France and the United States of America (USA). The total numbers of patients included in these studies were 1964. Of the four studies, disease specific survival (DSS) was reported in 3 studies[19,34,69] whereby disease-free survival and overall survival (OS) was evaluated by Chamie *et al*. (2015).[70] The characteristics of the included studies are listed in Table 1 whereas details of their NOS quality assessment are listed in Appendix 3.

Meta-analysis results

In accordance with the inclusion criteria, four articles were selected for meta-analysis. Heterogeneity between the articles was analysed using χ^2 test and I^2 metrics. High and low CAIX expressions were measured with respect to tumour stage, grade, ECOS-PS and lymph node involvement (Table 2 and Table 3).

Tumour stage

CAIX expression was measured against tumour stages. Stage 1 (T1) & stage 2 (T2) were grouped as low stage and stage 3 (T3) & stage 4 (T4) were grouped as high stage. Out of the three articles analysed, Patard *et al*. (2008) and Zhang *et al*. (2013)[19,34] demonstrated that high CAIX expression (>85%) is linked to low tumour stage (Table 1) and vice versa. Overall analysis also documented that high CAIX expression was associated with lower tumour stage with the 95% CI of 0.849 - 0.969 (Fig. 2).

Tumour grade

Analysis of CAIX expression was measured against tumour grades. Grade 1 (G1) & grade 2 (G2) were grouped as low grade whereas grade 3 (G3) & grade 4 (G4) were considered high grade. Out of the three articles analysed, Patard *et al*. (2008) and Zhang *et al*. (2013)[19,34] showed that high CAIX expression (>85%) is linked to low tumour grades. The overall random effect also indicated that high CAIX expression was associated with low tumour grade with a 95% CI of 0.732 - 0.983 (Fig. 2).

Lymph node involvement

Involvement of the lymph nodes was also studied in the four articles[19,34,69,70] and the analysis of CAIX expression was done against absence of lymph node involvement (N = 0) or presence of lymph node involvement (N ≥ 1). In general, high CAIX expression was associated with absence of nodal involvement with 95% CI 0.712 - 0.931 (Fig. 2).

Eastern Cooperative Oncology Group Performance Status (ECOS-PS)

Analysis of the ECOS-PS was made on three articles.[19,34,69] CAIX expression was measured against normal physical activity, (ECOS-PS = 0) versus reduced physical activity, (ECOS-PS > 0). The overall fixed effect showed that high CAIX expression (>85%) was associated with ECOS-PS < 0 with a 95% CI 0.818 - 0.969 (Fig. 2).

DISCUSSION

The key finding for this meta-analysis is that high tissue CAIX expression is associated with favourable disease outcome i.e., lower tumour stage and grade, absence of lymph node metastasis and overall good performance status. These findings are in keeping with the main hypothesis of this study, which states that CAIX is a useful prognostic and survival marker for RCC. The development of such a marker

TABLE 2: Meta-analysis of CAIX expression and RCC outcome

RCC characteristic	Study	Intervention	Control	Relative risk	95% CI	z	P
TUMOUR STAGE	Bui *et al*. (2003)	132/168	123/153	0.977	0.875 to 1.092		
	Patard *et al*. (2008)	34/51	44/49	0.742	0.598 to 0.921		
	Zhang *et al*. (2013)	181/250	386/480	0.900	0.824 to 0.983		
	Total (fixed effects)	347/469	553/682	0.907	0.849 to 0.969	-2.900*	0.004
	Total (random effects)	347/469	553/682	0.895	0.796 to 1.005	-1.872	0.061
LYMPH NODES INVOLVEMENT	Bui *et al*. (2003)	23/37	228/278	0.758	0.586 to 0.980		
	Zhang *et al*. (2013)	42/56	546/702	0.964	0.825 to 1.127		
	Patard *et al*. (2008)	8/12	41/48	0.780	0.514 to 1.184		
	Chamie *et al*. (2015)	31/568	24/245	0.557	0.334 to 0.929		
	Total (fixed effects)	104/673	839/1273	0.814	0.712 to 0.931	-3.008*	0.003
	Total (random effects)	104/673	839/1273	0.801	0.638 to 1.006	-1.911	0.056
TUMOUR GRADE	Bui *et al*. (2003)	104/132	151/189	0.986	0.880 to 1.105		
	Patard *et al*. (2008)	86/122	70/78	0.785	0.685 to 0.901		
	Zhang *et al*. (2013)	237/348	330/382	0.788	0.726 to 0.856		
	Total (fixed effects)	427/602	551/649	0.835	0.786 to 0.887	-5.879	<0.001
	Total (random effects)	427/602	551/649	0.848	0.732 to 0.983	-2.193*	0.028
ECOS-PS	Bui *et al*. (2003)	153/204	100/115	0.863	0.776 to 0.959		
	Zhang *et al*. (2013)	48/69	519/661	0.886	0.754 to 1.041		
	Patard *et al*. (2008)	35/45	43/55	0.995	0.807 to 1.227		
	Total (fixed effects)	236/318	662/831	0.891	0.818 to 0.969	-2.688*	0.007
	Total (random effects)	236/318	662/831	0.888	0.818 to 0.963	-2.863*	0.004

*Indicates significant result, $P < 0.05$, 95% Confidence interval <1 is significant. z shows value for 95% CI; Total fixed effect refers to Homogenous studies and Random effect to Heterogeneous studies.

TABLE 3: Test of heterogeneity between different analyses

Test of Heterogeneity	Tumour stage	Tumour grade	Nodal involvement	ECOS-PS
Q	5.0698	10.8573	6.9685	1.4259
DF	2	2	3	2
Significance level (*P*)	0.0793	0.0044	0.0729	0.4902
I^2	60.55%	81.58 %	56.95 %	0.00
95% CI for I^2	0.00 – 88.76	42.83 – 94.06	0.00 – 85.72	0.00 – 95.29%

Q – Statistic based on Chi-square test, I^2- scores heterogeneity between 0% -100%, 95% Confidence Interval < 1 is significant.

is important in this disease because in general, patients with a more favourable outcome also tend to respond well to vigorous chemo- and immunotherapy and vice-versa. At the moment, predicting which patient may benefit from a more aggressive treatment is only aided by the TNM classification. Hence, the addition of measuring tissue CAIX levels, in which high expression is associated with promising disease outcome, can further assist in clinical decision making.

This study is not the first meta-analysis to review on CAIX expression in RCC. Zhao *et al*. (2014) had also previously documented that low CAIX expression is associated with advanced disease progression and poor RCC outcome[32], which is in keeping with the findings of the current study. In particular, Zhao demonstrated that low CAIX expression are linked to higher tumour grade with lymph node and distant metastasis leading to poor disease specific

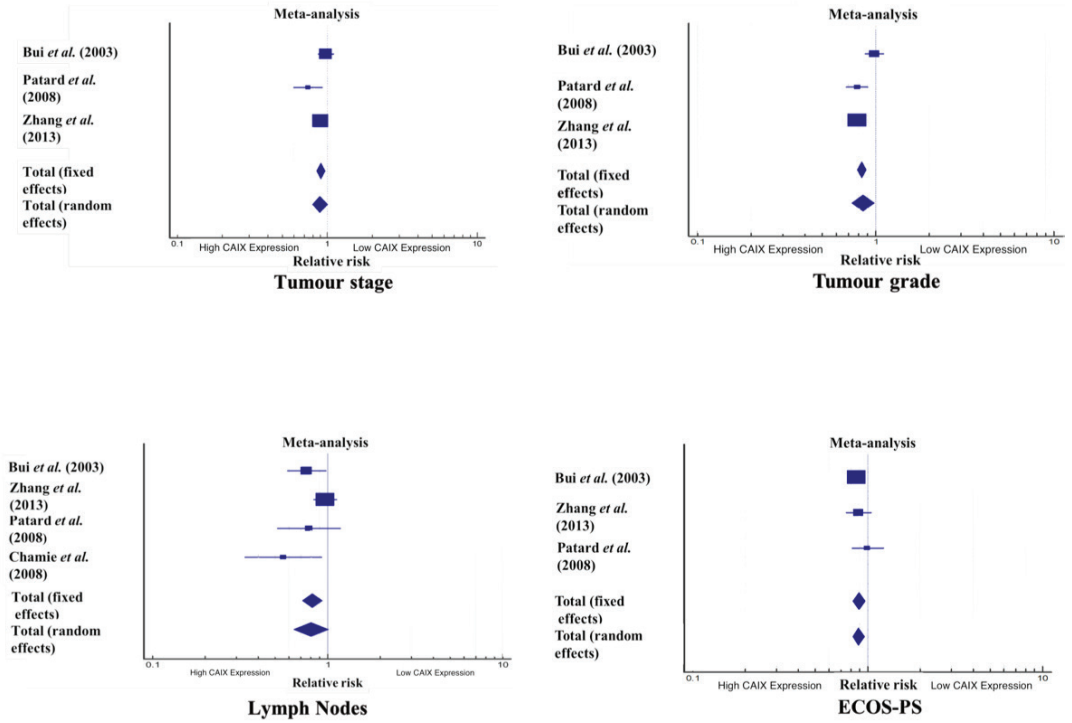

FIG. 2: Meta-analysis of CAIX expression with respect to Tumour Stage, Grades, Lymph nodes and Eastern Cooperative Oncology Group Performance Status (ECOS PS). Overall analysis shows that high CAIX expression is associated with lower tumour stage, lower tumour grade, absence of lymph node involvement and low ECOS PS (PS < 0) indicating localised tumour.

survival with unfavourable progression-free and overall survival.[32] Another meta-analysis done by Simon *et al*. (2016) analysed 147 clinical studies involving 24,523 patients that investigated the prognostic value of this marker in various malignancies except RCC.[36] When RCC was not accounted for, high CAIX expression was found to be associated with an increased risk of locoregional failure, higher disease progression and elevated risk of metastasis irrespective of tumour site or size.[36]

This inverse CAIX relationship is largely contributed by different mechanisms of CAIX upregulation found between RCC and other malignancies. Typically, CAIX is induced by hypoxia upregulation via HIF-1α. Additionally, other mechanisms also regulate CAIX expression via P13K pathway or via unfolded protein response.[36] These pathways further induce expression of CAIX protein at a greater amount. Hence in other types of cancers, high expression indicates CAIX involvement in promoting tumourigenesis and is indicative of a more aggressive tumour behaviour.[36] In this current meta-analysis, we are focusing on IHC analyses of CAIX expression and correlating its level of expression with disease progression and outcome.

The upregulation of CAIX in RCC however, is via an alternative pathway. In clear-cell RCC, 98% of the tumours have a loss of sequence on the short arm of chromosome-3, which harbours Von Hippel-Lindau (VHL) gene. Inactivation of this gene leads to high HIF-1α expression, which along with hypoxia causes the release of CAIX protein into the cell. These proteins catalyse the reversible reaction [$CO_2 + H_2O \rightarrow H_2CO_3$]. Carbon dioxide passively diffuses into extracellular space and converts into bicarbonate, which diffuses back into the cell and reacts with hydrogen causing extracellular acidosis [$CO_2 + H_2O \rightarrow H + HCO_3^-$]. This acidosis facilitates release of growth factors like vascular endothelial growth factor (VEGF) and platelet derived growth factor[71], both of which contribute to tumour growth and promote neovascularisation. These new vessels supply nutrients to tumour cells and eventually lead to subsequent reduction in CAIX expression, indicating an advanced tumour stage and grade. As evidenced by the current meta-analysis, high CAIX expression indicates early disease with localised tumour, hence the more favourable disease outcome.

This meta-analysis reports only on IHC studies that examine CAIX expression in renal cancer. It is important to note that histological analysis is an important inclusion factor in the current meta-analysis. Only studies that utilise the cut-off point introduced by Bui *et al*. (2003) are included in the final meta-analysis (i.e., high CAIX expression is >85% and low is ≤ 85% in the tissue).[69] This cut-off point also represents the rationale for inclusion of only IHC studies since the determination of "high" for serum or mRNA levels for CAIX at present time is still obscure. In addition to CAIX cut-off point, this study also excludes another article for using imaging software to analyse the strength of IHC expression by counting the pixel of brown colour in the tissue.[21] Although the technique is useful, its novelty cannot be compared with the rest of the studies that use a standardised cut-off point. Another limitation for this meta-analysis is the target population. Final data includes only Western population, no representation from other parts of the world was included. This calls for further prospective research on CAIX expression in RCC based on regional populations.

From literature search, there are approximately 1904 studies that investigated the role of CAIX in RCC until December 2017. However, due to stringent inclusion and exclusion criteria, in the end, only four studies were included in this meta-analysis. The findings from the four studies vary with three articles[34,69,70] generally supported possible role of CAIX as prognostic marker while one study disputed this finding.[19] Following meta-analysis, the strength of these three studies was evident in part by their large combined patient pool of 1234 in all aspects of analysis conducted (Table 2). The stringent criteria are important to ensure homogeneity of studies included in this meta-analysis so that a firm conclusion can be formed.

In summary, this meta-analysis showed that high tissue CAIX expression predicts less aggressive, early and localised renal cancers with better disease outcome. It is therefore, trusted that CAIX expression levels in the tumour tissue can be used as a prognostic biomarker for RCC in tandem with TNM classification. CAIX levels (high or low) may also help clinicians predict individual tumour behaviour and potentially help to decide on the next course of action in patient care. Specifically, the findings from this study can be applied in clinical setting using biopsied RCC sample whereby determination of CAIX expression in the tissue can predict the course of disease in a particular patient.

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