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ORIGINAL RESEARCH

Expression and Serum Levels of Mucin 5AC (MUC5AC) as a Biomarker for Cholangiocarcinoma: a Meta-analysis

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Abstract
Aim The potential of biomarkers in detecting early cholangiocarcinoma (CCA) is facilitated by examining CCA-associated proteins from primary studies. One such protein is mucin 5AC (MUC5AC) but inconsistency of reported associations between its expression/serum levels and CCA prompts a meta-analysis to obtain more precise estimates.
Methods A literature search yielded 17 included articles where multiple data in some raised the number of studies to 22. We calculated pooled odds ratios (OR) and 95% confidence intervals from negative and positive readings of MUC5AC levels. Data were subgrouped by ethnicity, detection method, sample source, and cancer type.
Results Outcome in the overall analysis was non-significant but those in the subgroups were. Thus, significant associations ($P < 0.001$) indicating high MUC5AC levels were found in three subgroups: (i) Thai (OR 8.32) and (ii) serum (OR 4.52). Heterogeneity of these two outcomes ($I^2 = 90$–93%) was erased with outlier treatment ($I^2 = 0\%$) which also modulated the pooled effects (OR 2.48–2.59). (iii) Immunoblot (OR 2.61) had low initial heterogeneity ($I^2 = 2\%$). Robustness and significant tests for interaction ($P_{\text{interaction}} = 0.01$–$0.02$) improved MUC5AC associations with CCA in the Thai population.
Conclusions Our pooled effect findings target the biomarker potential of MUC5AC to the Thai population.

Keywords MUC5AC · Biomarker · Meta-analysis · Cholangiocarcinoma

Introduction

Cholangiocarcinoma (CCA) originates from bile duct epithelial cells and is among the most common biliary and hepatic malignancies after hepatocellular carcinoma. Comprising 10 to 25% of all liver cancers [1, 2], CCA is a slow-growing but highly metastatic tumor, often detected at an unresectable stage. This presents poor prognosis [3] with a median survival of approximately 6–9 months [4]. Thus, early detection of CCA underpins the importance of novel biomarkers that enable early diagnosis and help develop effective therapies [5, 6]. Increase in incidence and mortality rates of this lethal cancer [7, 8] highlights the urgency to find more accurate diagnostic and therapeutic strategies for improved survival outcome [9].

Most CCA in humans are mucin-based [10]. Mucins are heavily O-glycosylated proteins where their expression in human genes are cell and tissue specific [11]. Moreover, neo-expressed and overexpressed mucins are clinically important as markers for diagnosis and prognosis of CCA [12, 13]. Two types of mucin, membrane bound (MUC1, MUC3, MUC4, MUC12, MUC13, MUC16) and secreted (MUC2, MUC5AC, MUC5B, MUC6, and MUC7), are classified based on their structure and function [14]. Secreted MUC5AC is a cysteine-

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rich protein encoded by the *MUC5AC* gene found in chromosome 11 (11p15) [15]. MUC5AC overexpression is strongly associated with aggressive tumor development [16, 17]. Primary study evidence suggests MUC5AC as a putative biomarker for CCA [18] and tumor progression [19]. However, these primary study outcomes have been methodologically inconsistent, warranting a meta-analysis to obtain more precise estimates.

Materials and Methods

Literature Search and Article Selection

Using the terms, "mucin5AC" and "cholangiocarcinoma" without language restriction, we searched MEDLINE using PubMed, ScienceDirect, and Google Scholar for publications as of May 13, 2017. References cited in the retrieved publications were screened manually to identify additional eligible articles. We included the articles if they presented MUC5AC data indicating expression levels, staining extent, or concentrations.

Data Extraction and Quality Assessment

Two investigators (NP and VT) independently extracted data and reached consensus on all the items. The following information was obtained from each publication: first author's name, published year, country, detection method, sample source, positive numbers out of the total, cut-off for positivity, sensitivity, and specificity proportions. Methodological quality was examined using Quality Assessment of Diagnostic Accuracy Studies (QUADAS) where each article was either scored as yes (positive), no (unsupported), or unclear (insufficient information) in terms of 14 assessment items [20].

Meta-analysis Protocol

Odds ratio (OR) estimates and 95% confidence intervals (CI) were calculated for each test using Review Manager 5.3 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2014). OR estimates were interpreted from the fulcrum of 1 (null association) where less and more than this number indicate low and high levels, respectively. Pooled estimates were obtained using either the fixed [21] (absence of heterogeneity) or random [22] (in its presence) effects models. Heterogeneity between studies was estimated using the χ^2-based Q test [23]. Recognizing the low power of this test [24], significance threshold was set at $P = 0.10$. Sources of heterogeneity were identified with meta-regression [25] and outlier analysis [26]. Outlier treatment has been shown to impact not only on heterogeneity, but on pooled effects as well [27], hence its application on both heterogeneous and significant outcomes. Heterogeneity was quantified with the I^2 statistic which measures the degree of inconsistency among studies [28]. Pooled estimates were subjected to sensitivity analysis which involved omitting one study at a time followed by recalculation to test for robustness of the summary effects. Subgroup analysis, limited to $N \geq 3$, was based on the following: (i) ethnicity where we examined Asians and non-Asians. Among Asians, we examined the Japanese and Thai subgroups; (ii) detection method, where we examined studies that used immunohistochemistry [IHC], enzyme-linked immunosorbent assay [ELISA], and immunoblot [IB]; and (iii) sample source (tissue biopsy, serum). The probability of differential risk associations (low level versus high level) between these subgroups warranted testing for presence of interactions where multiple P values were subjected to the Bonferroni correction. Publication bias was statistically evaluated with Egger's regression asymmetry test [29] and the Begg–Mazumdar correlation 30, which were applied where studies were ≥ 10 [31]. All P values were two-tailed, set a ≤ 0.05 throughout, except in heterogeneity estimation.

Results

Search Results

Figure 1 outlines the study selection process in a flowchart following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [32]. A total of 36 citations during the initial search were followed by a series of

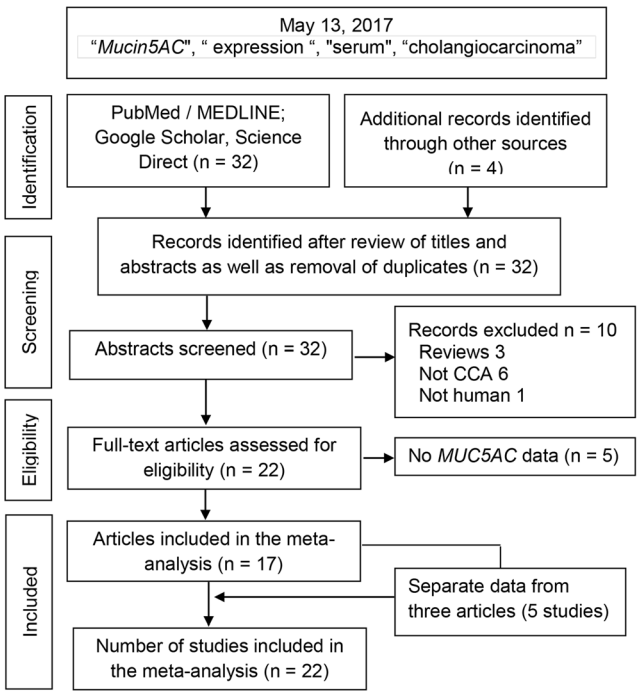

Fig. 1 Study selection process following PRISMA guidelines

omissions that eventually yielded 17 articles for inclusion in the meta-analysis [33–49]. Separate data from three articles 41, 48, 49 placed the included total number of studies to 22 (Table 1).

Characteristics of the Studies

Table 1 features characteristics of the included publications, the years of which ranged from 2003 to 2015. Ten articles were from Asia [34–40, 44, 45, 47] and five were non-Asian [33, 41–43, 46]. In terms of detection method, ten [33–37, 39, 42–45], four [38, 39, 41, 46], and two [40, 47] articles used IHC, ELISA, and IB, respectively. As to sample source, ten [33–37, 39, 42–45] and five [38, 40, 41, 46, 47] articles obtained theirs by tissue biopsy and serum, respectively. Subjects in four articles had intrahepatic cholangiocarcinoma (ICC) [35–37, 39] and the rest had CCA. Sample sizes of the studies ranged from 26 to 184 with a combined total of 1858. Sensitivity indicates the proportion of diseased subject with positive test result and specificity determines the proportion of non-diseased subject with negative results [50]. Sensitivity and specificity values of the studies ranged from 12 to 92% and from 34 to 97%, respectively. QUADAS scoring showed the mean and standard deviation of the included studies to be 11.1 ± 1.22, range of 10–14, and median of 11 indicating that the quality of the selected studies was good. The PRISMA checklist was generated to provide detailed description of this meta-analysis (Supplementary Table S1).

Overall and Subgroup Findings

Significance was not observed in our overall finding but was found in the subgroups. Thus, pooled OR (OR 1.51, $P = 0.19$)

Table 1 Characteristics of the included studies examining MUC5AC levels in cholangiocarcinoma (CCA)

A	First author year [reference]	*S*	Country	Detection method	Sample acquisition	Cancer type	Positive numbers/total	Cut-off for positivity	SEN (%)	SPE (%)	QUADAS
1	Abe 2015 [35]	1	Japan	IHC	TB	ICC	13/42	5%	31.0	69.0	12
2	Aishima 2006 [37]	2	Japan	IHC	TB	ICC	40/100	10%	40.0	60.0	10
3	Aishima 2007 [36]	3	Japan	IHC	TB	ICC	40/112	1%	35.7	64.3	13
4	Bamrungphon 2007 [38]	4	Thailand	ELISA	Serum	CCA	103/169	$A_{450\ nm} > 0.07$	71.0	90.0	14
5	Boonla 2005 [39]	5	Thailand	IHC/PCR**	TB	ICC	112/179	NS	62.6	96.9	12
6	Boonla 2003 [40]	6	Thailand	IB	Serum	CCA	111/177	±	62.7	47.1	12
7	Danese 2014* [41]	7	Italy	ELISA	Serum	CCA	36/46	10.5*	80.0	73.1	11
		8			Bile	CCA	10/46	6.25	75.0	73.1	
		9			Serum:bile	CCA	23/46	0.85	92.3	95.0	
8	Guedj 2009 [33]	10	France	IHC	TB	CCA	43/111	20%	38.7	NS	11
9	Lee 2003 [34]	11	Korea	IHC	TB	CCA	46/90	10%	51.1	NS	10
10	Lok 2014 [42]	12	USA	IHC	TB	CCA	5/41	5%	12.2	87.8	12
11	Mall 2010 [43]	13	South Africa	IHC	TB	CCA	12./26	1%	46.2	53.8	11
12	Matull 2008* [48]	14	UK	IHC	Biliary tract	BTC	7/39	5%	17.9	100.0	10
		15		IB	Bile	BTC	27/39	±	78.0	24.1	
		16		IB	Serum	BTC	17/39	±	43.6	96.0	
13	Onoe 2015 [44]	17	Japan	IHC	TB	CCA	121/184	10%	65.8	34.2	10
14	Park 2009 [45]	18	Korea	IHC	TB	CCA	52/85	10%	61.2	38.8	10
15	Ruzzenente 2013 [46]	19	Italy	ELISA	Serum	CCA	15/33	10.5 ng/ml	71.0	94.7	11
16	Silsirivanit 2011* [49]	20	Thailand	IHC	TB	CCA	42/45	1%	93.3	93.5	10
		21		ELISA	Serum	CCA	85/97	$A_{450\ nm} > 0.11$	87.6	89.6	
17	Wongkham 2003 [47]	22	Thailand	IB	Serum	CCA	112/179	1%	62.6	96.9	10

A number of articles, *S* number of studies, *IHC* immunohistochemistry, *ELISA* enzyme linked immunosorbent assay, *PCR* polymerase chain reaction, *IB* immunoblot, *TB* tissue biopsy, *ICC* intrahepatic cholangiocarcinoma, *BTC* biliary tract cancer, *NS* not specified, *ng/mL* nanogram per milliliter, *SEN* sensitivity, *SPE* specificity, *QUADAS* Quality Assessment of Diagnostic Accuracy Studies

*Three studies provided separate data

**Quantitative data were unseparated

Table 2 Summary of effects of MUC5AC levels on cholangiocarcinoma (CCA)

		Test of association			Test of heterogeneity		
	N	OR	95% CI	P^a	P^b	I^2 (%)	AM
Overall	22	1.51	0.81–2.83	0.19	< 0.0001	94	R
Subgroup by ethnicity							
Japanese	4	0.58	0.14–2.38	0.45	< 0.0001	96	R
Thai	6	**8.32**	**3.10–22.36**	**< 0.0001**	< 0.0001	93	R
Thai post-outlier	3	**2.48**	**1.88–3.28**	**< 0.0001**	0.67	0	F
Non-Asian	10	0.73	0.25–2.10	0.56	< 0.0001	90	R
Subgroup by sample acquisition							
Tissue biopsy	11	1.05	0.43–2.59	0.92	< 0.0001	95	R
Bile/bile tract	3	0.55	0.12–2.59	0.45	0.0002	84	R
Serum	7	**4.52**	**1.88–10.85**	**0.0007**	< 0.0001	90	R
Serum post-outlier	4	**2.59**	**1.97–3.40**	**< 0.0001**	0.45	0	F
Subgroup by detection							
IHC	12	1.05	0.44–2.50	0.92	< 0.0001	94	R
ELISA	6	2.11	0.45–10.01	0.35	< 0.0001	96	R
IB	4	**2.61**	**1.86–3.67**	**< 0.0001**	0.38	2	F

Only the initially heterogeneous and significant pooled effects were subjected to outlier treatment

Values in bold indicate significant association

IHC immunohistochemistry, *ELISA* enzyme-linked immunosorbent assay, *IB* immunoblot, *N* number of studies, *OR* odds ratio, *CI* confidence interval, P^a *P* value for association, P^b *P* value for heterogeneity, *AM* analysis model, *R* random effects, *F* fixed effects

in the overall analysis (Table 2 and Fig. 2) contrasted with those in the following subgroups ($P < 0.001$): (i) Thai (OR 8.32), (ii) serum (OR 4.52), and (iii) IB (OR 2.61). Outlier treatment on the Thai and serum subgroups ($I^2 = 90$–94%) erased heterogeneity ($I^2 = 0\%$), retained high significance ($P < 0.0001$), and modulated the pooled ORs (OR 2.48–2.59). Furthermore, the wide pre-outlier 95% CIs (1.88–22.36) were narrowed considerably (1.88–3.40) in the post-outlier outcomes (Table 2). Contrasting pooled effects between the subgroups were subjected to statistical tests for interaction where post-Bonferroni values ($P_{interaction} = 0.01$–0.02) improved MUC5AC associations in the Thai population (Table 3). We applied meta-regression analysis to the overall outcome and found the ethnic subgroup ($P = 0.02$) as contributor to heterogeneity, but not sample size, sample acquisition, or detection method ($P = 0.32$–0.80).

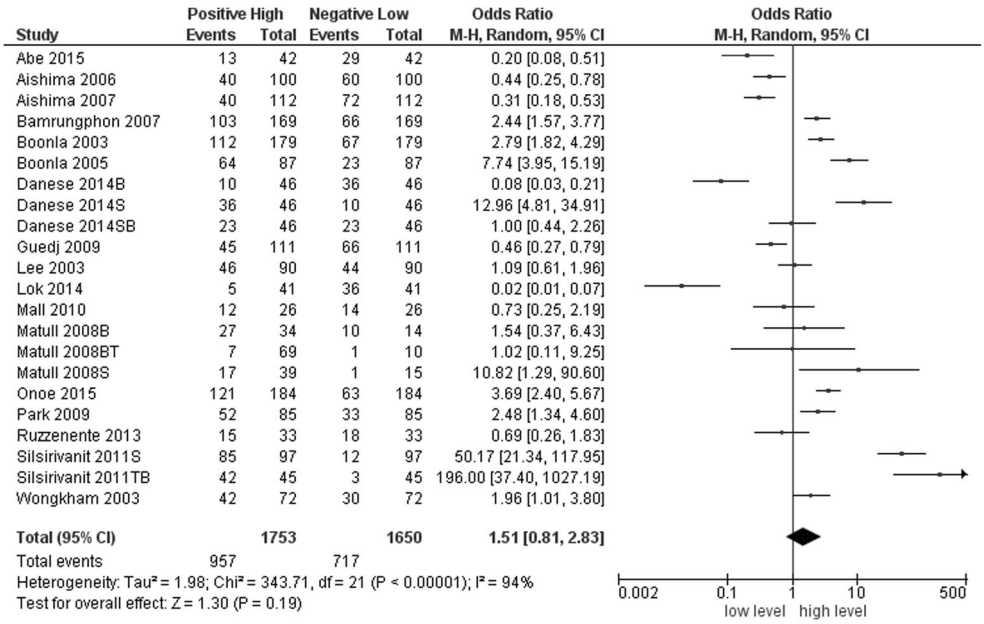

Fig. 2 Overall findings

Table 3 *P* values among the subgroup studies with contrasting pooled ORs resulting from tests of interaction and subjected to the Bonferroni correction

Contrasts				*P* values	
a		b	OR^a v OR^b	Uncorrected	Corrected
Japanese	v	Thai	0.58 v **8.32**	**0.003**	**0.02**
Japanese	v	Thai post-outlier	0.58 v **2.48**	**0.05**	0.30
Non-Asian	v	Thai	0.73 v **8.32**	**0.001**	**0.01**
Non-Asian	v	Thai post-outlier	0.73 v **2.48**	**0.029**	0.17
Bile	v	Serum	0.45 v **4.52**	**0.019**	0.11
Bile	v	Serum post-outlier	0.45 v **2.59**	**0.05**	0.30

Values in bold indicate significance

v versus, OR^b odds ratio for column B with significant associations (Table 2)

Sensitivity Analysis and Publication Bias

Six of the 10 (60%) comparisons were robust indicating stability of the outcomes where significance of the Thai, serum, and IB subgroups was improved (Table 4). Table 5 shows no evidence of publication bias (Egger's regression asymmetry $P = 0.55$–0.89, Begg–Mazumdar correlation $P = 0.25$–0.89).

Discussion

Absence of significance in the overall result confers the main findings to their presence in the subgroups. Here, Thai and serum effects show up to eightfold high level of MUC5AC. While power of the significant associations in these subgroups was improved with tests of interactions, these outcomes were heterogeneous with wide CIs. Applying outlier treatment to address these caveats yielded three effects: (i) homogenized the collection of studies, (ii) induced better precision, and (iii) moderated the pooled effects to 2.6-fold. Along with robustness, these meta-analytical features provide good evidence to render MUC5AC as a potential biomarker for CCA. Two primary studies found a correlation between high expression of MUC5AC and poor survival but were not statistically significant [39, 45]. Nevertheless, several study-specific findings consider MUC5AC to be a useful marker in CCA [35, 40–42, 46, 47, 49].

A recent meta-analysis [51] examined the biomarker potential of serum MUC5AC in CCA using parameters that include area under the curve from six studies [38, 41, 46–49]. By contrast, our approach was based on (±) readings of MUC5AC levels from 22 studies. Sources of heterogeneity from significant outcomes were examined in our study but not in theirs. They mention not performing meta-regression which we do in our study. While their findings do not suggest that serum MUC5AC be used to screen for CCA, we show its biomarker potential benefitting the Thai population. In terms of Thai findings, they invoke parochiality, while ours form the crux of the message suggesting their utility in this population. In sum, the differential methodologies in these two meta-analyses could be contextually seen as complementary with the common endpoint of confirming CCA diagnosis.

MUC5AC is a gel-forming mucin expressed in both gastric foveolar cells, the mechanism of which has been hypothesized to lower tumor cell adhesion, facilitating metastasis [46]. Thus associated with aggressive tumor development [45], the consequence is reduction in reactivity which is correlated with reduced survival [52]. This cascade of aggression mechanisms is exacerbated by aberrant expression of mucin which is key in protecting tumor cells from host immune response [53]. Aberrant expression of MUC5AC has been reported in pre-

Table 4 Sensitivity analysis for all comparisons to determine robustness of outcomes

	Overall	Ethnicity		Sample acquisition		Detection	
	Robust	Japanese	Robust	Tissue biopsy	39, 44, 45, 49	IHC	44, 45, 49
		Thai	Robust*	Bile/Biliary tract	41	ELISA	Robust
		Thai post-outlier	Robust*	Serum	Robust*	IB	Robust*
		Non-Asian	42	Serum post-outlier	Robust*		
A	0		1		5		3
B	1		2		1		2

A number of references that contributed to instability, *B* number of robust comparisons, *IHC* immunohistochemistry, *ELISA* enzyme-linked immunosorbent assay, *IB* immunoblot

*Indicates significant associations (see Table 2)

Table 5 Publication bias tests in MUC5AC findings

		Egger's regression		Begg–Mazumdar correlation	
	N	Intercept	*P*	Kendall's τ	*P*
Overall	22	−0.35	0.89	0.02	0.89
Non-Asian	10	1.96	0.55	0.29	0.25
Tissue biopsy	11	−1.10	0.83	0.05	0.82
IHC	12	−2.05	0.66	0.03	0.89

IHC immunohistochemistry, *N* number of studies

neoplastic lesions and in carcinomas arising from intrahepatic and extrahepatic bile ducts [39]. In particular, MUC5AC is aberrantly expressed in CCA tissues [13, 53] and its increased synthesis is associated with unfavorable outcomes [45]. Our findings of high MUC5AC levels among Thais may find a functional explanation in chronic inflammation caused by primary sclerosing cholangitis, a precursor of CCA. While development of CCA is found to be hastened with chronic inflammation [54], the molecular mechanism of *Opisthorchis viverrini*-stimulated MUC5AC is unclear. However, Sawanyawisuth et al. [54] showed in experimental *O. viverrini*-infected hamster that MUC5AC was stimulated and detected.

Interpreting our meta-analysis results warrants awareness of its strengths and limitations. Strengths include the following: (i) subgroup (post-outlier Thai and serum, IB) outcomes are significant, homogeneous ($I^2 = 0\%$), and non-heterogeneous ($I^2 = 2\%$); (ii) associations in the Thai subgroup are improved with significant interaction outcomes; and (iii) all significant outcomes were robust, indicating the stability of these findings. On the other hand, limitations of our study include the following: (i) survival rate data were non-uniform where 1 [35, 46], 3 [35, 46], and 5 years [36, 44] were inconsistently reported. (ii) Different measurement parameters of MUC5AC levels, IHC profiles, sources of data, and varying cut-offs for positivity may have contributed to the heterogeneity of outcomes, of which most significant findings were (iii) resulting losses of heterogeneity from outlier analysis were obtained at the expense of statistical power.

Conclusion

Our meta-analysis findings indicate that MUC5AC performs well in diagnosing CCA among Thais. However, the single biomarker approach is clearly inadequate for cancer diagnosis, warranting a panel of biomarkers to make an impact [55, 56]. Given the biomarker potential of MUC5AC from this study, it may well contribute to the panel approach in diagnosing CCA as it may increase sensitivity. Reports showed that MUC5AC is useful for diagnosis and prognosis for CCA [6, 47, 48]. MUC5AC-expressed CCA has poor prognosis when compared to non-MUC5AC-expressed CCA for treatment [45, 57]. At present, however, MUC5AC is not potentially targetable as an anti-CCA drug.

Still, MUC5AC may still be useful in related cancers (gastrointestinal, hepatobiliary, pancreatic). For example, non-detection of MUC5AC in hepatocellular carcinoma [57] excludes this cancer from CCA-diagnosis. Furthermore, utility of MUC5AC in concert with physical examination, ultrasonography, and other imaging instruments (magnetic resonance imaging, computed tomography scan) may help screen other cancers from CCA. However, histopathology of tissue or needle biopsies as standard diagnostic method may still be required for definite diagnosis of cancer. Further studies regarding interaction of MUC5AC with other markers and variables may help better understand the role of MUC5AC in CCA.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interest.

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