Head & Neck Oncology
Open Access

Original research study

The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis

ZHJ Li[1†], W Gao[1†], WB Lei[2], WK Ho[1], YWJ Chan[1], TS Wong[1*]

Abstract

Background

Laryngeal squamous cell carcinoma (LSCC) is the second most common head and neck squamous cell carcinoma (HNSCC). Although early detection of LSCC is a good prognostic factor for patients, they usually present late and with high recurrence rate. The use of methylated deoxyribonucleic acid (DNA) is considered to be a good surrogate marker of cancer as each cancer type has a specific methylation phenotype, which is distinguishable from the normal counterparts. Although intensive efforts had been made on the clinical use of methylation markers for cancer surveillance, the use of methylated DNA in differentiating LSCC patients remains unclear.

Methods

Pooled sensitivity and specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and the summary estimates of diagnostic odds ratio (DOR) were elucidated from the synthesized data. The diagnostic performance of methylated DNA markers was assessed by summary receiver operating curve (SROC) using random-effects models. Publication bias was examined with the use of Deeks' funnel plot.

Results

Data from 27 studies containing 2262 tissue samples were extracted. Among the 16 methylated DNA markers, which had been applied on LSCC, 6 of the 16 (37.5%) methylated genes were statistically significant ($P < 0.05$) in differentiating cancerous tissues from the normal counterparts. Combined sensitivity and specificity were 0.62 (95% CI: 0.46–0.76) and 0.91 (95% CI: 0.79–0.97), respectively; the pooled PLR and NLR were 7.2 (95% CI: 2.5–20.4) and 0.42 (95% CI: 0.27–0.65), respectively, and DOR was 17 (95% CI: 4–69).

Conclusion

Because aberrant DNA methylation occurs in the early developmental stages of LSCC, methylation markers are good for the detection of visually undetectable cases in LSCC tissues. However, based on the current markers panel, the accuracy of methylated DNA markers is not satisfactory for clinical use. Identification of novel methylated markers with higher sensitivity and specificity is warranted.

* Corresponding author
Email: thiansze@graduate.hku.hk
† These authors contributed equally to this study.

[1] Department of Surgery, The University of Hong Kong, Hong Kong
[2] Laryngeal Department, Hospital of Otorhinolaryngology, Institute of Otorhinolaryngology, The First Affiliated Hospital Sun Yat-sen University, Guangzhou, People's Republic of China

Introduction

The larynx is the second most common site for head and neck cancer development[1]. The larynx could be divided into 3 parts including the glottis, supraglottis, and subglottis, and laryngeal carcinoma usually originates in the glottis and supraglottis[2]. Histologically, most of the laryngeal carcinoma is squamous cell carcinoma (LSCC). Despite the advances in molecular diagnostic and therapeutic modalities in the previous decades, the diagnosis and prognosis of LSCC patients showed no significant improvement. The 5-year survival of patients with LSCC remains unchanged. In the United States, the mortality rate caused by LSCC changed from 2.97 (year 1990) to 2.05 (year 2007) with no significant improvement[3]. Early diagnosis improves the prognosis of patients with carcinoma in this region. Symptoms for LSCC include voice change, hoarseness, swallowing difficulties and dyspnoea. In general, patients perceive the symptoms as innocuous conditions, leading to a delay in diagnosis[4]. Some symptoms such as voice change may occur only in the later stage of disease (e.g. supraglottic carcinoma), resulting in a delay in treatment. Early detection is important because it is the determining factor for curative and function-preserving therapy[5]. However, at present, there is still no molecular marker for use in early diagnosis of the disease.

Deoxyribonucleic acid (DNA) methylation refers to the covalent addition of a methyl group by DNA methyltransferases to the cytosine residue of the CpG islands (around 500 bp to 2000 bp) in gene regulatory regions[6]. In normal cells, tumour suppressor genes are usually unmethylated[7]. The additional methyl group will alter the chromatin structure, which changes the transcriptional rate of the associated genes. Further, the methylated cytosine residue has a higher mutation rate and is linked with the allelic loss observed in human malignancies. Methylated tumour suppressor genes are frequently reported in cancerous tissues and are used to differentiate clinical samples containing preneoplastic and/or tumour cells from the normal counterparts[8]. The methylated DNA is suitable for use as an early cancer biomarker because DNA methylation is an early event in the carcinogenic process and is regarded as an early indicator for cancer development before symptoms arise[9]. At present, methylated

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

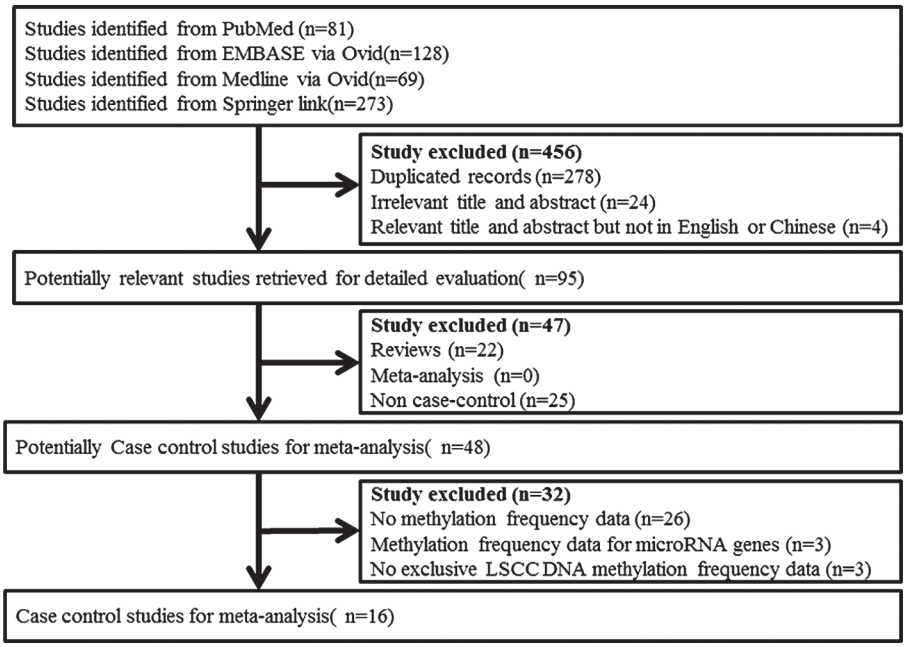

Figure 1: Flow diagram showing the literature search strategy and review process.

DNA is under clinical assay and is moving on to retrospective or longitudinal studies[7,10].

The use of molecular markers in cancer diagnosis has emerged as an effective approach because these markers are sensitive and have a high predictive value. In LSCC, however, there is still no consensus on the impact of molecular markers as early screening tools. Although the diagnostic value of methylated DNA has been extensively studied in other solid tumours and is suggested to be clinically useful in specific cases, its clinical significance in LSCC diagnosis remains unresolved. Therefore, we report an evaluation of the diagnostic accuracy of methylated DNA as biomarkers for LSCC diagnosis.

Methods and materials

Search strategy

A systematic literature search (publications from 1980 to January 2013) was performed independently by two authors (LZH and GW) in PubMed, EMBASE, Medline and Springer link, using 'laryngeal/ larynx/glottis/supraglottic/subglottic', 'cancer/carcinoma' and 'methylation/hypermethylation/hypomethylation/demethylation' as keywords. No restriction was set during this search. Duplicated results, irrelevant articles and publications not in English or Chinese were removed from this study. Papers of non-case-control studies were further excluded. Lastly, articles lacking in gene methylation frequency data or without exclusive methylation frequency and papers involving microRNA methylation in LSCC were also excluded.

Data extraction and quality assessment

Information extracted from the selected papers included first author, publication year, study area, research genes, case populations, test methods and the true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) results. The methodological quality of each study was assessed by Quality Assessment of Diagnostic Accuracy Studies (QUADAS)[11]. The data were extracted and reviewed independently by two authors (LZH and GW).

Data analysis

Mantel-Haenszel odds ratios (ORs) and random-effects model were used to examine the effect size of each individual DNA methylation, and the results were presented in a forest plot. According to these results, only significantly differentially methylated genes were selected for further testing. Pooled estimates on sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) were employed to examine the diagnostic accuracy of methylated genes in LSCC. Pooled estimates of sensitivity and specificity were used to construct the hierarchical summary receiver operating characteristic (SROC) curves. The area under the curve (AUC) was used to measure the summary diagnostic value. The heterogeneity between studies was assessed by the test of inconsistency using I-squared statistic. $I^2 \geq 50\%$ was considered as substantial heterogeneity. Publication bias was detected by the Deeks' funnel plot asymmetry test[12]. A *P* value of less than 0.1 for the slope coefficient was considered as significant asymmetry, which indicated potential publication bias. All analysis was performed with Stata software version 11.0.

Results

Study characteristics and quality

A total of 551 potential relevant papers were identified from PubMed, EMBASE, Medline and Springer link (Figure 1). Among these papers, 278 and 24 articles were excluded due to duplicated identification and irrelevance, respectively. Moreover, 4 papers in the language other than English or Chinese were excluded. Subsequently, 22 reviews and 25 non-case-control studies were excluded from the remaining 95 papers in the second round after detailed evaluation. In the final round, 26 articles without gene methylation frequency data, 3 articles studying microRNA methylation and 3 articles without available

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

Table 1 Study characteristics. Twenty-seven studies from 16 publications were included. Studies 8–19 were reported in the same article

	First author	Year	Country	QUADAS score	Research genes	LSCC	Normal	Methods	TP	FP	FN	TN
1	Yang[13]	2012	China	10	MYCT1	73	73	BSP base-sequencing	59	13	14	60
2	Li[14]	2012	China	9	BRMS1	70	60	MSP	34	0	36	60
3	Hartmann[15]	2011	Poland	10	GNG7	98	8	BSP base-sequencing	42	0	56	8
4	Yang[16]	2011	China	10	RASSF1A	50	15	MSP	31	0	19	15
5	Wang[17]	2011	China	10	CHD5	65	65	BSP base-sequencing	39	9	26	56
6	Tawfik[18]	2010	Egypt	10	HMLH1	26	49	MSP	6	10	20	39
7	He[19]	2010	China	10	SPARC	41	9	MSP	23	1	18	8
8	Paluszczak[20]	2010	Poland	9	RARbeta	41	40	MSP	24	17	17	24
9	Paluszczak[20]	2010	Poland	9	RARbeta	41	40	MSP	24	19	17	21
10	Paluszczak[20]	2010	Poland	9	RASSF1A	41	40	MSP	13	9	28	32
11	Paluszczak[20]	2010	Poland	9	RASSF1A	41	40	MSP	13	9	28	32
12	Paluszczak[20]	2010	Poland	9	GSTP1	41	40	MSP	2	1	39	40
13	Paluszczak[20]	2010	Poland	9	GSTP1	41	40	MSP	2	2	39	39
14	Paluszczak[20]	2010	Poland	9	MGMT	41	40	MSP	22	15	19	26
15	Paluszczak[20]	2010	Poland	9	MGMT	41	40	MSP	22	15	19	25
16	Paluszczak[20]	2010	Poland	9	DAPK	41	40	MSP	31	32	10	9
17	Paluszczak[20]	2010	Poland	9	DAPK	41	40	MSP	31	32	10	9
18	Paluszczak[20]	2010	Poland	9	FHIT	34	40	MSP	9	8	25	26
19	Paluszczak[20]	2010	Poland	9	FHIT	34	40	MSP	9	7	25	27
20	He[21]	2010	China	10	CHFR	50	15	MSP	11	0	39	15
21	Brieger[22]	2010	Germany	9	HIC1	5	3	MSP	4	3	1	0
22	Tang[23]	2010	China	10	RUNX3	40	29	MSP	38	0	2	29
23	Xu[24]	2006	China	10	RASSF1A	48	48	MSP	34	11	14	37
24	Zhang[25]	2006	China	10	MGMT	46	51	MSP	16	0	30	51
25	Kong[26]	2005	China	10	DAPK	58	63	MSP	39	6	19	57
26	Yin[27]	2005	China	10	FHIT	41	41	MSP	10	0	31	41
27	Bai[28]	2000	China	10	p16	32	32	MSP	6	0	26	32

LSCC: laryngeal squamous cell carcinoma; MSP: methylation-specific PCR; TP: true positive; FP: false positive; FN: false negative; TN: true negative; QUADAS: quality assessment for studies of diagnostic accuracy (maximum score: 14).

methylation frequency in LSCC were excluded. Finally, 27 case-control studies reporting methylation of 16 individual genes were selected for meta-analysis (Table 1). The quality of selected articles was evaluated using quality assessment for studies of diagnostic accuracy (QUADAS). However, 13 of the 16 articles were considered as high quality with a score of 10, while the other 3 had a score of 9 (Table 1).

Genes differentially methylated between LSCC tumour tissue and normal control

Significant differentially methylated genes were detected in 6 of the 16 individual genes (Table 2). All 6 genes

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

Table 2 Differentially methylated genes in LSCC tumour tissues compared with the normal counterparts

Research genes	Studies	Study ID	No. in the figure	Overall OR [95% CI]
RASSF1A	4	Yang2011[16]	①	3.93 [1.236, 12.516]
		Paluszczak2010[20]	②	
		Paluszczak2010[20]	③	
		Xu2006[24]	④	
RUNX3	1	Tang2010[23]	⑧	908.6 [42.01, 20000]
BRMS1	1	Li2012[14]	⑤	114.37 [6.80, 1922.38]
MYCT1	1	Yang2012[13]	⑦	19.45 [8.43, 44.88]
CHD5	1	Wang2011[17]	⑥	9.33 [3.94, 22.08]
SPARC	1	He2010[19]	⑨	10.2 [1.169, 89.388]

OR: odd ratio; 95% CI: 95% confidence intervals.

Table 3 The diagnostic accuracy of methylated DNA in LSCC. Pooled diagnostic accuracy

	LSCC vs. normal control
No. of studies	9
Combined sensitivity [95% CI]	0.62 [0.46, 0.76]
Combined specificity [95% CI]	0.91 [0.79, 0.97]
PLR [95% CI]	7.2 [2.5, 20.4]
NLR [95% CI]	0.42 [0.27, 0.65]
DOR [95% CI]	17 [4, 69]
AUC [95% CI]	0.86 [0.82, 0.88]

OR: odds ratio; 95% CI: 95% confidence intervals; PLR: Positive likelihood ratio; NLR: negative likelihood ratio; DOR: diagnostic odds ratio; AUC: area under the curve.

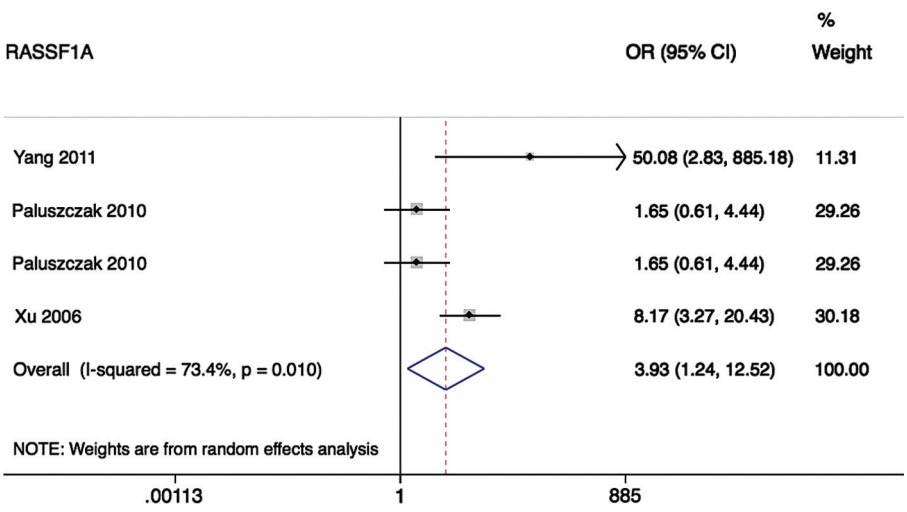

Figure 2: Forest plot of methylated RASSF1A gene in LSCC.

(RASSF1A, RUNX3, BRMS1, MYCT1, CHD5 and SPARC) showed a significantly higher methylation in the LSCC tumour tissue compared with that in the normal control. The 95% CI of their OR did not overlap the no effect value. Methylated RASSF1A was reported by multiples studies (Figure 2), and methylated RUNX3 demonstrated the largest effect size.

Diagnostic accuracy of DNA methylation in LSCC

The 6 genes were reported in 9 studies from 8 articles including 469 tumour tissue samples and 379 normal control tissue samples (Table 3). Methylation markers had a pooled sensitivity of 0.62 (0.46–0.76), ranging from 0.32 to 0.95, and a pooled specificity of 0.91 (0.79–0.97), ranging from 0.77 to 1 (Figure 3). Heterogeneity between studies was observed in both sensitivity (Q-test = 68.71, $P < 0.01$, $I^2 = 88.36$) and specificity (Q-test = 31.56, $P < 0.01$, $I^2 = 74.65$) tests. Pooled PLR and pooled NLR were 7.2 (95% CI: 2.5–20.4) and 0.42 (95% CI: 0.27–0.65), respectively. We also generated the Fagan nomogram using PLR and NLR (Figure 4). With PLR and NLR of 7.2 and 0.42, the post-test probability increased to 64% from a given pre-test probability of 20% when the index test was positive, and dropped to 9% when the index test was negative. DOR of the methylation markers was 17 [4, 69]. Figure 5 showed the summary receiver operating characteristic (SROC) curve, with the summary operating point as 0.62 of sensitivity and 0.91 of specificity. The area under the curve (AUC) was 0.86. The summary likelihood matrix point was in the right lower quadrant (PLR <10 and NLR >0.1), indicating that the methylated DNA markers were not useful predicators for LSCC (Figure 6).

Publication bias

The selected studies were assessed based on the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) items and scoring guideline[12].

Competing interests: none declared. All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Head & Neck Oncology
Open Access

Original research study

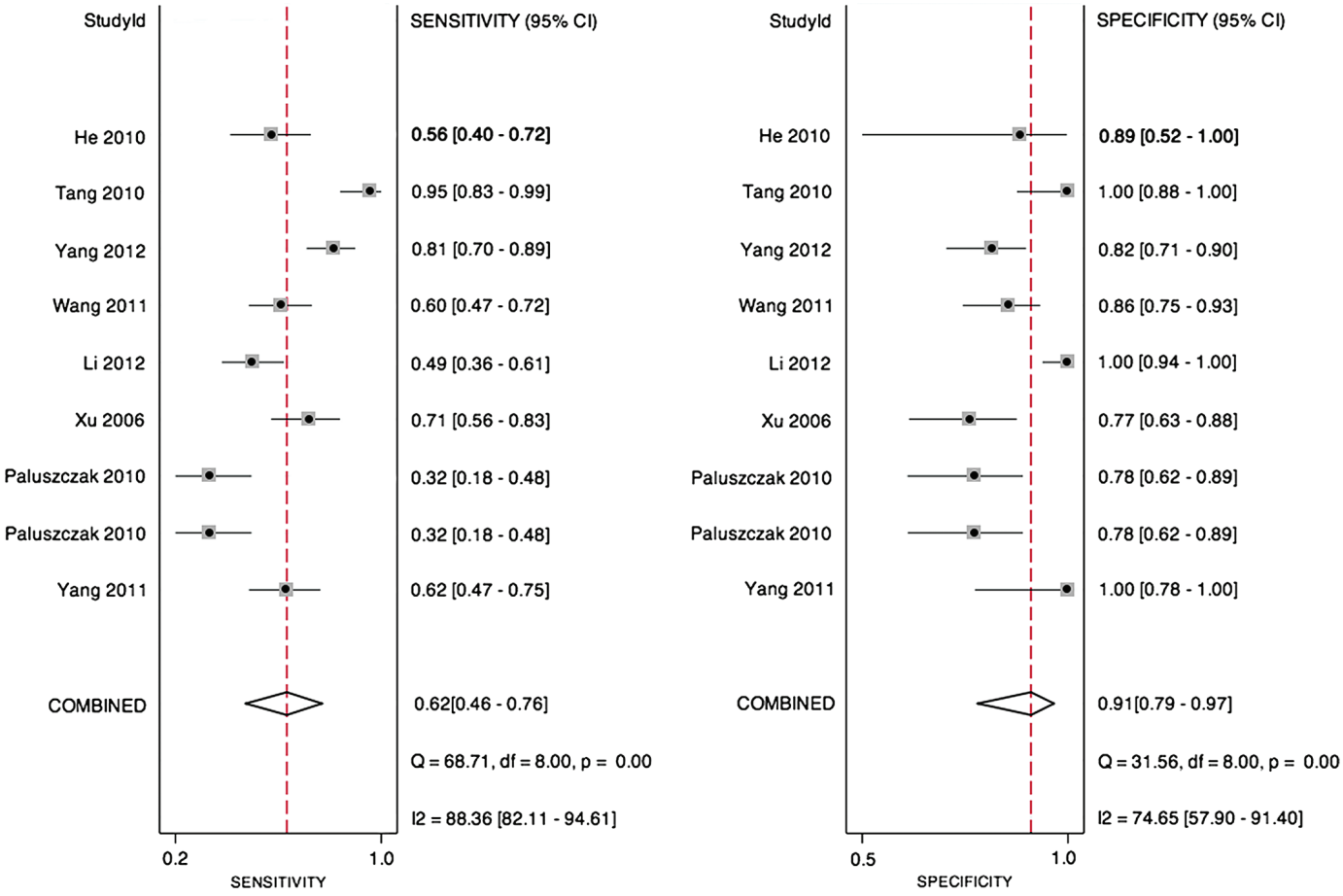

Figure 3: Forest plot of the pooled sensitivity, specificity, PLR, NLR and DOR. PLR: positive likelihood ratio; NLR: negative likelihood ratio; DOR: diagnostic odds ratio.

According to QUADAS, publication score between 7 and 10 are qualified publication for meta-analysis. All selected studies had QUADAS of more than 8. Figure 7 showed the Deeks' funnel plot asymmetry test[12] based on the above 9 studies. The *P*-value was found to be 0.98, which was not statistically significant and indicated no potential publication bias.

Discussion

Methylated DNA is considered to be a surrogate biomarker as it could elaborate the indicative signals derived from cancer cells. It is present in the cancer-containing specimen as DNA methylation is usually de novo and aberrant to the cancer cells. To the best of our knowledge, there is still no comprehensive evaluation on the diagnostic accuracy of methylation markers in the LSCC. Although there are numerous studies describing the potential use of methylated DNA as biomarkers in LSCC diagnosis, the diagnostic accuracy of these epigenetic markers in the clinical setting remains unclear. Hence, we performed a comprehensive review on the use of methylated DNA markers in detecting carcinoma arising from the laryngeal region.

In LSCC, most of the studies indicated that the detection rate of methylated DNA markers was significantly higher in comparison with the normal counterparts. Of the 16 articles, 15 reported methylation of single methylated genes and 1 reported 5 methylated genes. Taken together, the data of 16 methylated genes reported in 27 case-control studies were extracted. Methylation-specific PCR and bisulphite sequencing were used to detect the methylated gene in the 27 studies on 2262 tissue samples (including 1221 LSCC tissues and 1041 normal controls). Of the 16 genes, 37.5% (6/16) were found to be significantly different between the tumour tissues and the normal counterparts. Methylation-specific PCR was used to detect RASSF1A, RUNX3, BRMS1, MYCT1 and SPARC; bisulphite-sequencing was used to examine CHD5 methylation. Although real-time quantitative technology (e.g. MethyLight) and high-throughput screening platform (e.g. methylation microarray) are available for the detection of methylation, these

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

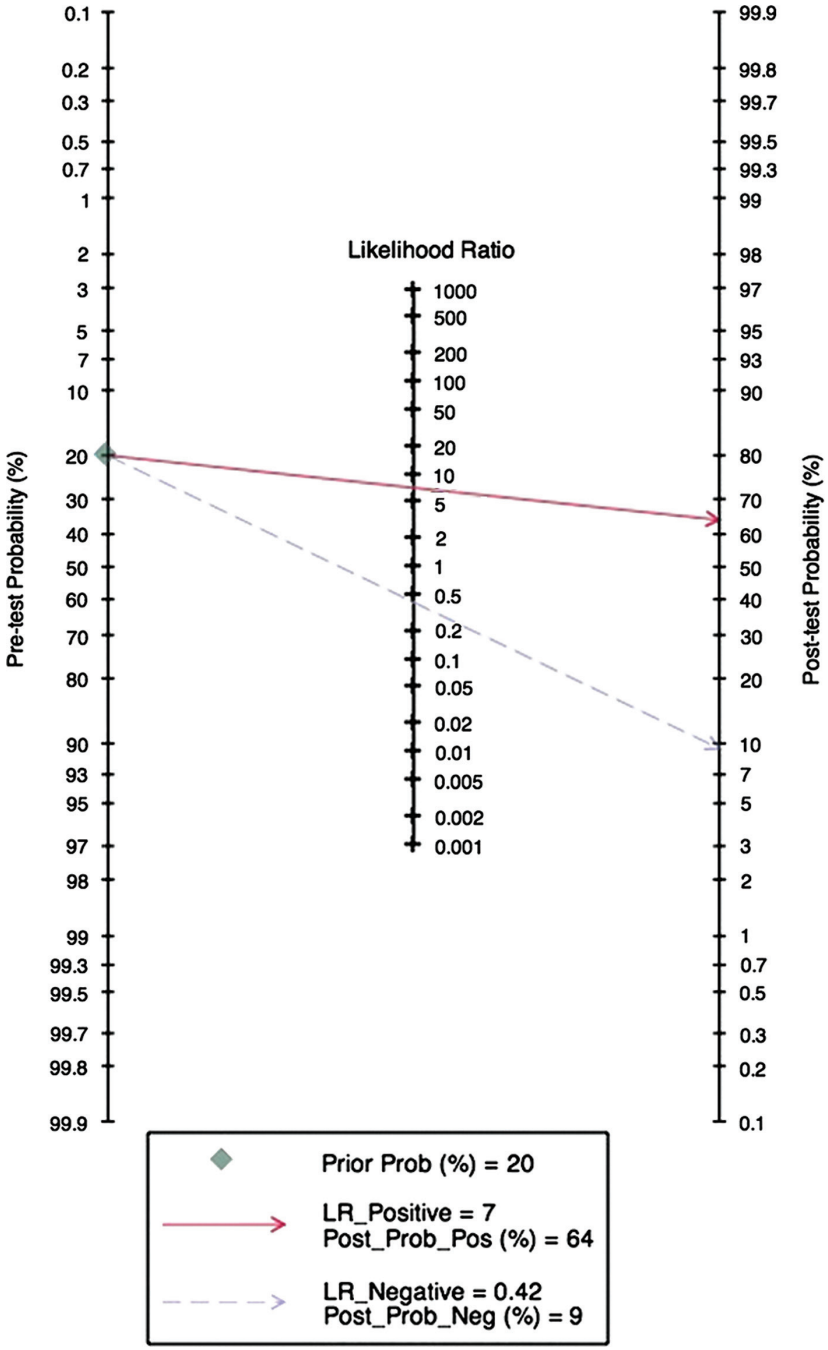

Figure 4: Fagan nomogram. The red line indicates the probability change after positive index test, while the dash line indicates the probability change after negative index test. LR positive: positive likelihood ratio, LR negative: negative likelihood ratio.

are not used in LSCC. Methylated RUNX3 demonstrated the highest odd ratio among the 6 methylated genes. However, it was only reported in a single study with 40 cases. In comparison, methylated RASSF1A was reported in 4 studies on 159 cases. Further, only the qualitative examination method was used with no quantity data.

Theoretically, an ideal biomarker should be both sensitive and specific. It should also have a high positive and negative predictive value with 100% accuracy to differentiate the diseased group from the normal individuals[29]. In practice, the accuracy of cancer biomarkers varies depending on their intrinsic nature, generation mechanisms and extraction methods. To examine the property of methylation markers in LSCC, we first examined pooled sensitivity and specificity. In terms of sensitivity, methylation markers had a high range, probably due to the intrinsic sensitivity difference between the different methylation markers selected by different groups. Cancers had a distinctive methylation phenotype as they have different methylation patterns. Thus, the selection of candidate-methylated genes is important and is the determining factor on the accuracy of the test. The present meta-analysis demonstrated that methylated gene markers had a high pooled specificity (0.91). However, the pooled sensitivity is much lower (0.62) when we employ the methylation markers to examine the LSCC tissues. When we examined the AUC of SROC curve, methylation markers still showed an acceptable diagnostic performance (0.86). However, as indicated in the pooled likelihood matrix, the current studies in the use methylation markers for LSCC diagnosis are neither exclusion nor confirmation. The heterogeneity observed across different studies is mainly caused by the use of different methylation markers as most studies employed the same detection method with QUADAS score over 9. Concerning the potential serological use of methylated DNA markers, all the studies reviewed in the present study examined the laryngeal tissue alone. Attempt to use the cell-free methylated DNA as serological biomarkers in plasma or serum to differentiate

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

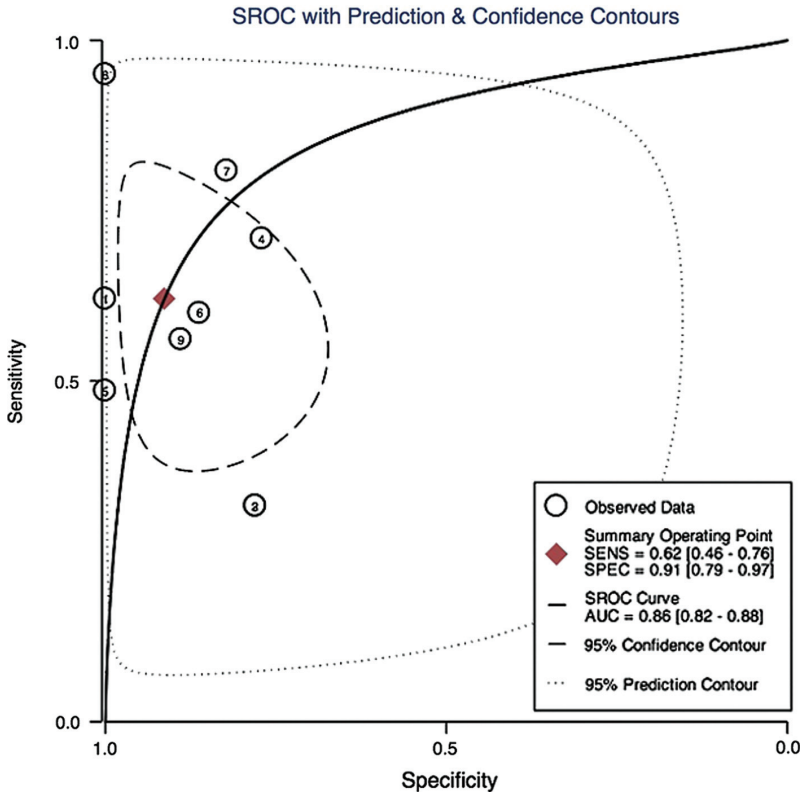

Figure 5: Summary receiver operating characteristic (SORC) curve for individual studies on the diagnostic accuracy of methylated DNA markers. SENS: sensitivity; SPEC: specificity; AUC: area under the curve.

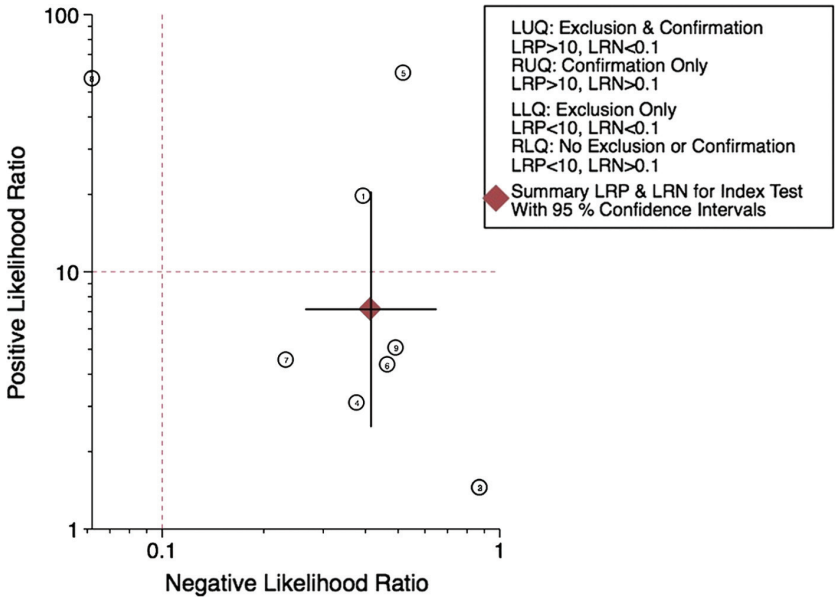

Figure 6: Likelihood ratio scatter plot matrix showing the association of methylated DNA markers with laryngeal carcinoma. The error bar shows the 95% confidence intervals. LRP: positive likelihood ratio, LRN: negative likelihood ratio, LUQ: left upper quadrant, RUQ: right upper quadrant, LLQ: left lower quadrant, RLQ: right lower quadrant.

LSCC from normal individuals are not yet reported. Reports on the use of methylated DNA on other body fluids are also absent. Thus, we still have no information on the implication of methylated DNA as non-invasive biomarkers in LSCC management.

Given the low sensitivity, methylation markers are not recommended for use in population screening due to the costs involved. However, the high specificity of methylation markers makes it a good tool for detecting the visually undetectable cancer cells in the biopsies samples obtained from the high-risk group as aberrant DNA methylation is an early event in cancer tumourigenesis. All reported methylated markers in LSCC had been reported in other human malignancies. Detection of the methylation markers in samples other than the laryngeal tissues (such as saliva, plasma and serum of the suspicious cases) could only reflect the potential risk of cancer and provide no information on the potential anatomical sites for further examination. Because only a few of the available methylation markers are evaluated in LSCC cases, the sensitivity and specificity of methylation markers could be improved when new candidate gene are evolved. To select suitable methylation markers for LSCC detection, it is important to identify the methylation patterns which are specific to LSCC and provide characteristic clinical information about the disease[30]. In conclusion, our results demonstrated that methylated DNA is good to differentiate cancerous laryngeal tissues from the normal counterparts based on its high specificity. However, at the present stages, the use of it in clinical setting is not recommended based on the limited data available. Further studies with the use of high-throughput technologies are warranted to explore novel and effective methylation markers for use in the diagnosis of LSCC.

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

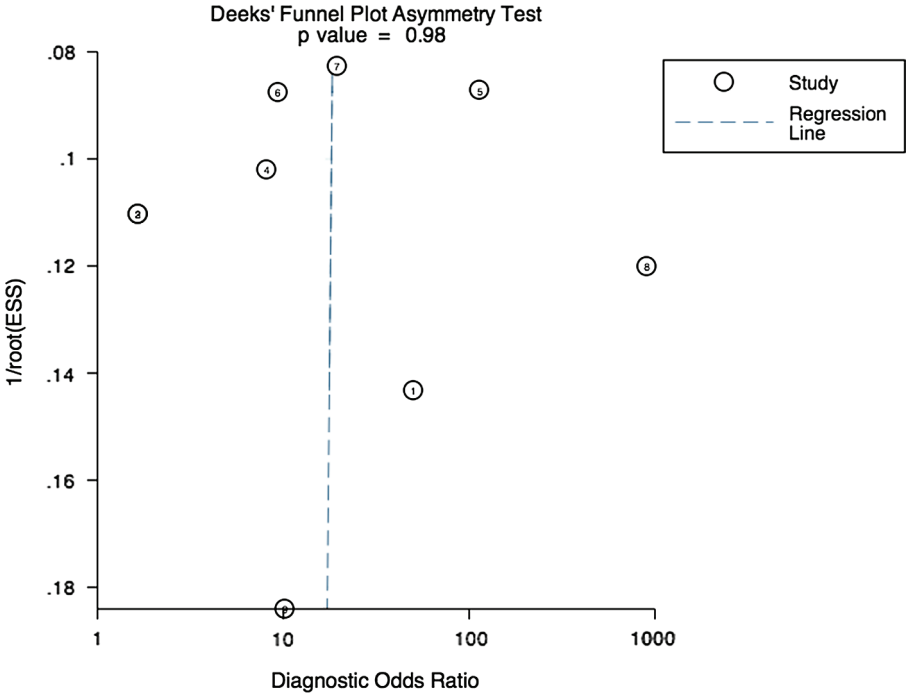

Figure 7: Deeks' funnel plot with superimposed regression line.

References

1. Jiao J, Qin Z, Li S, Liu H, Lu Z. Potential role of Notch1 signaling pathway in laryngeal squamous cell carcinoma cell line Hep-2 involving proliferation inhibition, cell cycle arrest, cell apoptosis, and cell migration. Oncol Rep. 2009 Oct;22(4):815–23.
2. Almadori G, Bussu F, Cadoni G, Galli J, Paludetti G, Maurizi M. Molecular markers in laryngeal squamous cell carcinoma: towards an integrated clinicobiological approach. Eur J Cancer. 2005 Mar;41(5): 683–93.
3. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin. 2011 Jul–Aug;61(4):212–36.
4. Brouha XD, Tromp DM, de Leeuw JR, Hordijk GJ, Winnubst JA. Laryngeal cancer patients: analysis of patient delay at different tumor stages. Head Neck. 2005 Apr;27(4):289–95.
5. Kraft M, Betz CS, Leunig A, Arens C. Value of fluorescence endoscopy for the early diagnosis of laryngeal cancer and its precursor lesions. Head Neck. 2011 Jul; 33(7):941–8.
6. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science. 2001 Aug;293(5532):1068–70.
7. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer. 2003 Apr;3(4):253–66.
8. Sepulveda AR, Jones D, Ogino S, Samowitz W, Gulley ML, Edwards R, et al. CpG methylation analysis—current status of clinical assays and potential applications in molecular diagnostics: a report of the Association for Molecular Pathology. J Mol Diagn. 2009 Jul;11(4): 266–78.
9. Laird PW. Oncogenic mechanisms mediated by DNA methylation. Mol Med Today. 1997 May;3(5):223–9.
10. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 001 Jul;93(14):1054–61.
11. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol. 2003 Nov;3:25.
12. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol. 2005 Sep; 58(9):882–93.
13. Yang M, Li W, Liu YY, Fu S, Qiu GB, Sun KL, et al. Promoter hypermethylation-induced transcriptional down-regulation of the gene MYCT1 in laryngeal squamous cell carcinoma. BMC Cancer. 2012 Jun; 6(12):219.
14. Li X, Wu Y, Sun Y, Huang M, Ma D, Xu J, et al. The study of expression of BRMS1 gene protein and the expression of BRMS1 gene promotor area methylation in supraglottic laryngeal carcinoma and its clinical significance. J Clin Otorhinolaryngol Head and Neck Surg. 2012 Aug;26(15):701–3.
15. Hartmann S, Szaumkessel M, Salaverria I, Simon R, Sauter G, Kiwerska K, et al. Loss of protein expression and recurrent DNA hypermethylation of the GNG7 gene in squamous cell carcinoma of the head and neck. J Appl Genet. 2012 May;53(2): 167–74.
16. Yang J, Ji WY, Qu YR, He LX, Zhao XD, Jin MZ. DNA methylation and histone modification relate to RASSF1A gene deletion in laryngeal carcinoma tissues. Chin J Otorhinolaryngol Head and Neck Surg. 2011 Apr;46(4):308–12.
17. Wang J, Chen H, Fu S, Xu ZM, Sun KL, Fu WN. The involvement of CHD5 hypermethylation in laryngeal squamous cell carcinoma. Oral Oncol. 2011 Jul;47(7): 601–8.
18. Tawfik HM, El-Maqsoud NM, Hak BH, El-Sherbiny YM. Head and neck squamous cell carcinoma: mismatch repair immunohistochemistry and promoter hypermethylation of hMLH1 gene. Am J Otolaryngol. 2011 Nov–Dec;32(6):528–36.
19. He Q, Wei J, Zhang J, Jiang H, Wang S, Zhou X, et al. Aberrant methylation of secreted protein, acidic and rich in cysteine in human laryngeal and hypopharyngeal carcinoma. Oncol Lett. 2011 Jul;2(4):725–9.
20. Paluszczak J, Misiak P, Wierzbicka M, Woźniak A, Baer-Dubowska W. Frequent hypermethylation of DAPK, RARbeta, MGMT, RASSF1A and FHIT in laryngeal squamous cell carcinomas and adjacent normal mucosa. Oral Oncol. 2011 Feb;47(2): 104–7.
21. He L, Ji W, Yang J, Zhao X. Study of mRNA expression level and hypermethylation of CHFR promoter in the laryngeal squamous cell carcinoma tissue. J Clin Otorhinolaryngol Head and Neck Surg. 2010 Aug;24(15):673–7.
22. Brieger J, Pongsapich W, Mann SA, Hedrich J, Fruth K, Pogozelski B, et al.

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Original research study

Demethylation treatment restores hic1 expression and impairs aggressiveness of head and neck squamous cell carcinoma. Oral Oncol. 2010 Sep;46(9): 678–83.
23. Tang J, Bai W, Ji W, Gao H, Liu J. Relationship between hypermethylation of Runx3 gene and the development and metastasis of laryngeal carcinoma. J Clin Otorhinolaryngol Head and Neck Surg. 2010 Sep;24(18):834–7.
24. Xu CB, Teng B, Li CQ, Jin CS. Promoter methylation of RASSF1A gene in laryngeal squamous cell carcinoma and protein expression. Journal of Jilin University Medicine Edition. 2006 Mar; 32(2):326–9.
25. Zhang S, Guo C, Kong W, Liu Z. Promoter hypermethylation of DNA repair gene MGMT in laryngeal squamous cell carcinoma. J Huazhong Univ Sci Technolog Med Sci. 2006;26(1):101–4.
26. Kong WJ, Zhang S, Guo C, Zhang S, Wang Y, Zhang D. Methylation-associated silencing of death-associated protein kinase gene in laryngeal squamous cell cancer. Laryngoscope. 2005 Aug;115(8): 1395–401.
27. Yin D, Dong M. Methylation of promoter and expression of FHIT gene in laryngeal squamous cell carcinoma. J Clin Otorhinolaryngol Head and Neck Surg. 2005 Mar;19(6):253–8.
28. Bai S, Gao H, Liang H, Fei S, Zhang X, Sun K. The study of p16 gene mutation and methylation in laryngeal squamous cell carcinoma. Chin J Otorhinolaryngol. 2000 Aug;35(4):292–4.
29. T. Malati. Tumour markers: An overview. Indian J Clin Biochem. 2007 Sep; 22(2):17–31.
30. Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. Nat Rev Cancer. 2005 Mar; 5(3):223–31.

FOR CITATION PURPOSES: *Li ZHJ, Gao W, Lei WB, Ho WK, Chan YWJ, Wong TS*. The diagnostic value of methylated DNA in laryngeal squamous cell carcinoma: meta-analysis. Head Neck Oncol. 2013 Feb 06;5(2):15.