Diagnostic Value of Circulating microRNAs in Laryngeal Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

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Abstract

Background : Circulating microRNAs (miRNAs) play an important role in the biological processes of cancers and are promising biomarkers of cancer diagnosis. **Objectives :** To estimate the diagnostic value of microRNAs in laryngeal squamous cell carcinoma (LSCC) patients, comparing with the non-LSCC controls. **Population :** Six eligible LSCC studies including 1,585 LSCC patients with corresponding 2,095 non-LSCC controls from years 2000 to 2017 were analyzed.

Methods : Medline, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, EMBASE, Cochrane Library and Web of Science were searched in this study. We conducted a meta-analysis to identify studies that reported the diagnostic data of miRNAs both in LSCC patients and controls. In addition, we evaluated and compared the diagnostic value of upregulated miRNAs with downregulated miRNAs.

Results : Six studies with corresponding specificity and sensitivity data were included in this study. The pooled sensitivity, specificity and AUC were 0.89 (95% CI: 0.79-0.94), 0.87 (95% CI: 0.77-0.93), and 0.94 (95% CI: 0.92-0.96), respectively. However, the heterogeneities of these studies were quite high, the value of I^2 for the pooled sensitivity, specificity were 96.82% and 97.08%, respectively. Subgroup analysis of upregulated and downregulated miRNAs showed a similar diagnostic value but the heterogeneity remained high. Publication bias was found in Funnel plot of pooled and upregulated miRNAs, while not obvious in downregulated miRNAs.

Conclusions : Circulating miRNAs showed diagnostic significance in laryngeal cancer, however, the results of this meta-analysis revealed significant heterogeneity. Therefore, the diagnostic value of miRNAs in LSCC seems limited.

Background:

Despite the advances of therapy applied for laryngeal cancer (LC), there were still 177.4 million new cases of LC globally during 2018 according to the American Cancer Society, and the survival rate had not improved significantly since the number of deaths caused by LC were 94.8 million over the last year [1, 2]. Laryngeal squamous cell carcinoma (LSCC) is the most common histological type of cancer in larynx, accounting for approximately 85 ~ 90%, and eighty percent of patients are males [3, 4]. The diagnosis of LSCC depends on pathological findings, imaging examination and laryngoscopies, however, no reliable biomarker has been identified to screen or diagnose LSCC[5].

MicroRNAs (miRNAs) are small non-coding, single stranded RNAs containing about 22 nucleotides. They regulate gene expression by influencing the 3'-UTP-binding of target genes at transcription or post-transcription level. Over the past decades, plenty of studies demonstrated that miRNAs played critical roles by controlling the expression of targeted mRNAs; studied the mechanism of miRNAs in affecting the occurrence and development of LSCC; and identified that miRNAs had effects on the biological processes, proliferation, invasion and metastasis in tumors[6].

Koichiro Saito et al. reported that miR-196a was cancer-specific expressed in LSCC[7]. MiR-331-3p, miR-603, miR-1303, miR-660-5p and miR-212-3p were reported to be only detected in the plasma of LSCC[8]. These previous studies demonstrated that circulating miRNAs were capable of becoming potential less-invasive LSCC diagnostic biomarkers, while the comprehensive diagnostic value of miRNAs for LSCC is still unclear.

This systematic review and meta-analysis included all qualified studies, evaluated the differential expression of candidate circulation miRNAs in LSCC and summarized their roles as biomarkers to diagnose LSCC.

Methods:

Protocol and registration

This systematic review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) checklist [9], with PROSPERO registration number of 159381.

Publication Search

Literature sources mainly came from bibliographic databases, including EBM Reviews - Cochrane Central Register of Controlled Trials (September 2019), EBM Reviews - Cochrane Database of Systematic Reviews (2005 to October 9, 2019), Embase (1974 to 2019 October 11), Ovid MEDLINE (R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions (R, 1946 to October 11, 2019) from OVID database; WEB of SCI (All Years); and PUBMED MEDLINE (All Years).

Study Eligibility

The inclusion criteria were as follows: (a) analysis of an association between the miRNA and laryngeal squamous cancer; (b) case-control or cohort study design; (c) pathology-proven laryngeal carcinoma; (d) providing the sensitivity and specificity of diagnostic value, ROC curve and AUC with 95% confidence interval (CI), and (e) providing detailed information about the recruitment of participants, diagnostic protocols, genotyping, statistical analysis and other relevant methodological data.

The exclusion criteria included: (1) non-English papers; (2) case reports, letters, historical reviews; (3) studies performing on animal samples; (4) studies without specific diagnostic data (5) studies without control group of non-LSCC population; (6) studies repeated or overlapping publications with the same

Data Extraction

Data extraction was performed independently by two investigators (DNC and WDP). In case of any disagreement, a consensus was reached by a consultation among all authors. The data we extracted from those included studies were: (1) basic characteristics, including the first author, publication year, country, ethnicity, male ratio, source of control, cancer type, miRNA profiling type, sample type, method of miRNAs detection; (2) diagnostic value, including (i) sensitivity and specificity; (ii) ROC curve; (iii) AUC with 95% CI.

Assess Bias Across Individual Studies:

The qualities of studies, especially the quality of diagnostic accuracy, were assessed independently by 2 reviewers using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria[10]. The QUADAS-2 tool contains 4 key domains: patient selection, index test, reference standard, and flow and timing, which gives a maximum score of 7 (Fig. 5).

Statistics Analysis

All statistical analyses were conducted by using STATA version 15. Diagnoses of LSCC were based on the accuracy of the identified miRNAs. The bivariate meta-analysis models were applied to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). The sensitivity and specificity of all included studies were used to plot a summary receiver operator characteristic (SROC) curve, and the area under the SROC curve (AUC) was calculated in the meta-analysis(Fig. 2C, Fig. 3C, Fig. 4C). Similarity among studies was evaluated by chi square and I^2 statistics to evaluate and substantial heterogeneity was defined if I^2 was above 50%.

Results:

Study selection and characteristics

We obtained 1,497 potential articles in the initial screening, among which, 826 duplicate studies were excluded(Figure 1). Two independent reviewers (DNC and WDP) assessed the remaining 671 studies by screening titles and abstracts, then excluded 658 unrelated articles: 1341 studies were ceRNAs, lncRNAs or other non-microRNAs; 1,714 articles did not involve laryngeal subsite; and 839 studies were systematic reviews, case reports, experimental studies, or commentaries. Subsequently, of the 17 full-text articles evaluated by DNC and WDP, 11 studies were excluded: six of them reported disease management without diagnostic value; two of them reported overall head and neck cancer without certain information for LSCC; three of them reported diagnostic graphs without eligible diagnostic data. Ultimately, six articles with 17 miRNAs reported met the inclusion criteria and included in this meta-analysis.

These included six studies (ranging from years 2000 to 2017) reported 1585 LSCC patients and 2095 controls comprising healthy controls or patients with other diseases (Table 1). Four of the included studies reported a single miRNA (miR-27a[11],miR-21-3p[12]miR-155[13] and miR-21[14], respectively), whereas two studies discussed panels of miRNAs (miR-31miR-141miR-149amiR-182miR-145miR-223miR-let-7a-1miR-133amiR-485-3pmiR-122miR-33[15] and miR-106bmiR-122[16]).

Diagnostic Value Of Circulating miRNAs

The diagnostic value of the 17 miRNAs in peripheral blood circulation of LSCC patients was analyzed as follows (Table 1). Using bivariate meta-analysis models, the pooled sensitivity, specificity and AUC of 17 miRNAs to discriminate LSCC from non-LSCC controls were 0.89 (95% CI: 0.79–0.94), 0.87 (95% CI: 0.77–0.93), and 0.94 (95% CI: 0.92–0.96), respectively. Subgroup analyses of upregulated and downregulated miRNAs are shown in Table 2, which indicated that the sensitivity, specificity and AUC in downregulated miRNAs were 0.89 (95% CI: 0.80–0.94), 0.84 (95% CI: 0.50–0.97), and 0.92 (95% CI: 0.89–0.94), respectively, corresponding to the sensitivity of 0.90 (95% CI: 0.79–0.96), specificity of 0.88 (95% CI: 0.77–0.94), and AUC of 0.95 (95% CI: 0.92–0.96) in upregulated miRNAs, respectively. The pooled results manifested that circulating microRNAs had a high diagnostic accuracy.

Heterogeneity And Subgroup Analysis

The pooled results showed significant heterogeneity in the overall sensitivity ($I^2 = 96.08\%$ for sensitivity, and 96.32% for specificity, $P < 0.001$) (Fig. 2A, Fig. 2B). In order to figure out the possible sources of heterogeneity, we stratified the overall data into subgroups by dysregulated status. Among the six studies, four miRNAs (miR-27a, miR-145, miR-223, miR-133a) were downregulated in LSCC, while twelve miRNAs (miR-21-3p,miR-155miR-21, miR-31miR-141miR-149amiR-182miR-let-7a-1miR-485-3pmiR-122miR-33;miR-106b) were upregulated. The sensitivity, specificity and AUC in downregulated miRNAs were 0.89 (95% CI: 0.80–0.94), 0.84 (95% CI: 0.50–0.97), and 0.92 (95% CI: 0.89–0.94), respectively, and the I^2 for sensitivity and specificity were 85.57% and 95.54%, respectively(Table 2; Fig. 4A, Fig. 4B). Meanwhile the pooled sensitivity, specificity, AUC and I^2 for sensitivity and specificity in upregulated miRNAs were 0.90 (95% CI: 0.79–0.96), 0.88 (95% CI: 0.77–0.94), 0.95 (95% CI: 0.92–0.96), 96.96%, and 97.00%, respectively(Fig. 3A, Fig. 3B).

Although the significant heterogeneity was not observed between the subgroups according to Fig. 3B and Fig. 4B, the meta-regression was performed with some potential predictor variables: ethnicity (Asian vs. others), sample size (≥ 200 vs. <200) and miRNA profiling (single- vs. multiple-miRNAs assay). The statistical differences existed in ethnicity and sample size, indicating partly origin of heterogeneity.

Risk Of Public Bias Within Studies

The publication bias of the included studies was checked by Deeks' funnel plot test, which indicate that public bias exists in pooled studies and unregulated subgroup, while no significant public bias was observed in downregulated study (Figure S1, Figure S2, Figure S3).

Discussion:

Although larynx is part of the head and neck, the biological characteristics of laryngeal cancer are much different from cancer of the oral cavity and pharynx[17]. HPV or EBV infection would indicate a suspected oropharyngeal or nasopharyngeal cancer origin. P53 and EGFR are normally expressed in HNSCC according to recent studies, however, the mechanism in laryngeal cancer remains occult[17], and no specific biomarkers was identified to be related to laryngeal cancer diagnosis or screening[20].

Current gold diagnostic standard for LSCC patients is invasive pathologic biopsies, accompanying with CT, MRI, or PET-CT[5], and studies focusing on the diagnostic value of non-invasive marker like miRNAs have increased in recent years[21]. It was reported that miRNAs dysregulations were associated with many diseases, such as cancers[23], cardiovascular conditions, even mental disease[22]. There were increasing studies reporting associations between miRNAs and head and neck cancer, especially laryngeal carcinoma. Different miRNAs have potential capacity in serving as new diagnostic, therapeutic and prognostic biomarkers.

In this meta-analysis, we recruited six studies with seventeen reported circulating miRNAs as diagnostic markers for 1585 LSCC patients in comparison with 2095 non-LSCC controls. Among these studies, two miRNAs (miR-21[12, 14] and miR-122[15, 16]) were mentioned more than once, and two studies discussed panels of miRNAs (miR-31, miR-141, miR-149, miR-182, miR-145, miR-223, miR-let-7a-1, miR-133a, miR-485-3p, miR-122, miR-33[15] and miR-106b, miR-122[16]). All of these miRNAs were identified to have great dysregulated effects between LSCC and controls.

In this meta-analysis, the specificity and sensitivity of miR-31, miR-33 and miR-let-7 were extraordinarily high (up to 100%), indicating a high diagnostic efficiency. Yang et al.[27] confirmed that overexpression of miR-31 suppressed LSCC cell growth, cell cycle and cell invasion, besides, they demonstrated that miR-31 was downregulated in LSCC tumors and correlated with advanced cancer stage. Although no explicit mechanism has been confirmed between miR-33 or miR-let-7 in LSCC, miR-33 was reported to mediate proto-oncogene PIM3 repression [28]; as a key regulator of tumor metastasis, miRNA let-7a led to the enrichment of its target gene HMGA2 in tongue squamous cell carcinoma (TSCC) tissues and cell lines [29]. Additionally, the expression levels of miRNA-21, miRNA-145, miRNA-218 [30, 31] were related to the degree of differentiation, lymph node metastasis and TNM staging, which would affect the prognosis of LSCC. Therefore, dysregulation of miRNAs plays a role in various signaling pathways or cell cycles, affecting the occurrence and development of laryngeal cancer.

In this meta-analysis, high efficacy of diagnostic value of miRNAs was pooled while significant heterogeneity was observed ($I^2 = 96.08\%$ for sensitivity, and 96.32% for specificity, $P < 0.001$). The possible explanations for the heterogeneity were: first, the ethnicity (Asia vs. others) and sample size ($n \geq 200$ vs. $n < 200$) might induce the heterogeneity according to the results of meta-regression; second, various miRNAs might be applicable to diverse tumors type or stages on account of the different mechanism of each miRNA; third, no exact cutoff value had been uniformed to determine the dysregulation of miRNAs in LSCC. Moreover, publication bias existed in the pooled and the upregulated studies, but was not obvious in the downregulated subgroup. This study has limitations. On one hand, we did not perform a meta-analysis for any exact miRNA due to the limited number of original studies. On the other hand, since the main source of heterogeneity could not be found in subgroup analysis and meta-regression, the potential discriminative ability of miRNAs as circulating biomarkers for LSCC diagnosis needs to be verified in future.

Conclusions:

In this meta-analysis, we recognized that miRNAs have a certain diagnostic role in LSCC, however, the results of this meta-analysis revealed significant heterogeneity, indicating the diagnostic value of miRNA is currently limited.

Abbreviations

microRNAs (miRNAs)

Laryngeal Squamous Cell Carcinoma (LSCC)

Confidence Interval(CI)

Laryngeal Cancer (LC)

Receiver Operating Characteristic(ROC)

Area Under the Curve(AUC)

Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2)

Positive Likelihood Ratio (PLR)

Negative Likelihood Ratio (NLR)

Summary Receiver Operator Characteristic (SROC)

Human Papillomavirus(HPV)

Epstein-Barr virus(EBV)

Epidermal Growth Factor Receptor(EGFR)

Head and Neck Squamous Cell Carcinoma(HNSCC)

Computerized Tomography(CT)

Magnetic Resonance Imaging(MRI)

Tongue Squamous Cell Carcinoma (TSCC)

Declarations

Ethics approval and consent to participate:

Not applicable

Consent for publication:

Not applicable

Availability of data and material:

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests:

The authors declare that they have no competing interests.

Funding:

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Authors' contributions:

JJR and DNC designed the study; DNC and WDP analyzed and interpreted data in including studies; DD and KQ performed quality control of data and algorithms; WJY, YFR and XHY performed the statistical analysis; YS and WY were involved in the preparation of the manuscript; DNC and WDP were major contributor in writing the manuscript; JJR and YZ reviewed the manuscript. All authors read and approved the final manuscript

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Tables

Table 1. Summary of descriptive characters of included studies										
Study	Country	LSCC Patients			Healthy Controls			Type of Sample	MiRNA	Dysregulation
		N	Sex(male/female)	Mean age(SD,range)	N	Sex(male/female)	Mean age(SD,range)			
Bin Zhou 2016	China	107	72/35	45(<60y) 62(>60y)	104	N/A	N/A	Blood	MiR-27a	Down
Enzhu Zhang 2016	China	112	83/29	45(<60y) 67(>60y)	82	N/A	N/A	Blood	MiR-21-3p	Up
Jianling Wang 2016	USA	280	245/35	64(±12.1)	560	488/72	65.1±9.8	Blood	MiR-155	Up
Jingting Wang 2014	China	52	38/14	33(<59y) 19(>59y)	49	N/A	N/A	Blood	MiR-21	Up
WL.Grzelczyk(1) 2019	Poland	66	58/8	62±8.0	100	53/47	65.5±7.5	Blood	MiR-31	Up
WL.Grzelczyk(2) 2019	Poland	66	58/8	62±8.0	100	53/47	65.5±7.5	Blood	MiR-141	Up
WL.Grzelczyk(3) 2019	Poland	66	58/8	62±8.0	101	53/47	65.5±7.5	Blood	MiR-149 a	Up
WL.Grzelczyk(4) 2019	Poland	66	58/8	62±8.0	102	53/47	65.5±7.5	Blood	MiR-182	Up
WL.Grzelczyk(5) 2019	Poland	66	58/8	62±8.0	103	53/47	65.5±7.5	Blood	MiR-145	Down
WL.Grzelczyk(6) 2019	Poland	66	58/8	62±8.0	104	53/47	65.5±7.5	Blood	MiR-223	Down
WL.Grzelczyk(7) 2019	Poland	66	58/8	62±8.0	105	53/47	65.5±7.5	Blood	MiR-let-7a-1	Up
WL.Grzelczyk(8) 2019	Poland	66	58/8	62±8.0	106	53/47	65.5±7.5	Blood	MiR-133a	Down
WL.Grzelczyk(9) 2019	Poland	66	58/8	62±8.0	107	53/47	65.5±7.5	Blood	MiR-485-3p	Up
WL.Grzelczyk(10) 2019	Poland	66	58/8	62±8.0	108	53/47	65.5±7.5	Blood	MiR-122	Up
WL.Grzelczyk(11) 2019	Poland	66	58/8	62±8.0	109	53/47	65.5±7.5	Blood	MiR-33	Up
Yang Meng(1) 2018	China	154	104/50	48.4±12.5	100	68/32	46.9±9.7	Blood	MiR-106b	Up
Yang Meng(2) 2018	China	154	104/50	48.4±12.5	100	68/32	46.9±9.7	Blood	MiR-122	Up

Abbreviations: N=numbers; LSCC= Laryngeal squamous cell carcinoma; miRNA = microRNAs; CI = confidence interval

Table2. Subgroup analyses of dysregulation up or down of included studies											
Subgroup	LSCC	Healthy	Total	TP	FP	FN	TN	Sensitivity(95%CI)	Specificity(95%CI)	PLR	NLR
Dysregulation : up											
Enzhu Zhang,2016	112	112	194	97	18	15	64	0.87 [0.79-0.92]	0.78 [0.68-0.86]	3.945	0.169
Jianling Wang,2016	280	280	840	164	171	116	389	0.59 [0.53-0.64]	0.69 [0.65-0.73]	1.915	0.599
Jingting Wang,2014	52	52	101	36	9	16	40	0.69 [0.55-0.81]	0.82 [0.68-0.91]	3.761	0.377
WL.Grzecznyk(1) 2019	66	66	166	66	1	0	99	1.00 [0.95-1.00]	0.99 [0.82-0.95]	100.000	0.000
WL.Grzecznyk(2) 2019	66	66	166	61	13	5	87	0.92 [0.83-0.97]	0.87 [0.79-0.93]	7.077	0.092
WL.Grzecznyk(3) 2019	66	66	166	38	9	28	91	0.58 [0.45-0.70]	0.91 [0.84-0.96]	6.444	0.462
WL.Grzecznyk(4) 2019	66	66	166	57	25	9	75	0.87 [0.76-0.94]	0.75 [0.65-0.83]	3.480	0.173
WL.Grzecznyk(7) 2019	66	66	166	65	0	1	100	0.98 [0.92-1.00]	1.00 [0.96-1.00]	1.000	0.020
WL.Grzecznyk(9) 2019	66	66	166	65	19	1	81	0.98 [0.92-1.00]	0.81 [0.72-0.88]	5.158	0.025
WL.Grzecznyk(10) 2019	66	66	166	59	6	7	94	0.89 [0.79-0.96]	0.94 [0.87-0.98]	14.833	0.117
WL.Grzecznyk(11) 2019	66	66	166	66	0	0	100	1.00 [0.95-1.00]	1.000 [0.96-1.00]	1.000	0.000
Yang Meng(1),2018	154	154	254	112	38	42	62	0.73 [0.65-0.80]	0.62 [0.52-0.72]	1.921	0.435
Yang Meng(2),2018	154	154	254	103	26	51	74	0.67 [0.59-0.74]	0.74 [0.64-0.82]	2.577	0.446
Dysregulation : down											
Bin Zhou ,2016	107	104	211	92	15	15	89	0.86 [0.78-0.92]	0.86 [0.77-0.92]	5.972	0.164
WL.Grzecznyk(5) 2019	66	100	166	62	1	4	99	0.94 [0.85-0.98]	0.99 [0.95-1.00]	94.000	0.061
WL.Grzecznyk(6) 2019	66	100	166	50	12	16	18	0.76 [0.64-0.85]	0.88 [0.80-0.94]	6.333	0.273
WL.Grzecznyk(8) 2019	66	100	166	62	44	4	56	0.94 [0.85-0.98]	0.56 [0.46-0.66]	2.136	0.107

Abbreviations: LSCC= Laryngeal squamous cell carcinoma; Healthy= Healthy contro; TP= True Positive; FP= False Positive; FN= False Negative; TN= True Negative; PLR= Positive Likelihood Ratio; NLR= Negative Likelihood Ratio

Figures

Figure 1

Flow chart for literature search and study selection

Figure 2

Diagnostic meta-analysis for all included studies. (A) Forest plot of sensitivity (SEN), specificity (SPE) and 95% CI. (B) Galbraith figure of heterogeneity analysis. (C) SROC with Prediction & Confidence Contours

Figure 3

Diagnostic meta-analysis for upregulated miRNAs studies. (A) Forest plot of sensitivity (SEN), specificity (SPE) and 95% CI. (B) Galbraith figure of heterogeneity analysis. (C) SROC with Prediction & Confidence Contours

Figure 4

Diagnostic meta-analysis for downregulated miRNAs studies. (A) Forest plot of sensitivity (SEN), specificity (SPE) and 95% CI. (B) Galbraith figure of heterogeneity analysis. (C) SROC with Prediction & Confidence Contours

Figure 5
Investigators' quality assessment for included studies using the QUADAS-2 assessment. (A) Graph; (B) Summary.

Supplementary Files

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