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ORIGINAL ARTICLE

Long Non-Coding RNA HOTAIR as Ideal Biomarker for the Diagnosis of Various Carcinomas

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SUMMARY

Background: **HOTAIR is a variety of long non-coding RNA that has been recognized as a predictive factor for most cancers. This meta-analysis examined the complete investigative effectiveness of the level of HOTAIR expression for various cancers.**
Methods: **Research on the diagnostic value of HOTAIR in different carcinomas was acquired by searching the online databases. Twelve studies consisting of 927 cancer cases were chosen in our research. The sensitivity as well as specificity of the involved articles was helpful to establish the summary receiver operator characteristic (SROC) curve in addition to compute the area under the SROC curve (AUC). In addition, a meta-regression test was done to determine the heterogeneity sources among available studies.**
Results: **The combined effect sizes calculated from involved studies were as follows: sensitivity, 0.73; specificity, 0.83; PLR, 4.4; NLR, 0.32; DOR, 14; and an AUC of 0.85. Deeks' funnel plot asymmetry test showed no probable publication bias. The meta-regression analyses signified that the type of ethnicity is the major cause of heterogeneity.**
Conclusions: **The present meta-analysis suggested that elevated HOTAIR can be considered as a relatively accurate marker for cancer diagnosis and can be applied to support the diagnosis of various cancers.**
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KEY WORDS

HOTAIR, cancer, diagnosis, meta-analysis

INTRODUCTION

Despite the progress made in surgery and chemotherapy worldwide, cancer still remains a serious public health problem [1]. In 2018, it was estimated that there would be 18.1 million new and 9.6 million deaths due to cancer [2]. The mortality of most cancers is still high and researchers have begun exploring new biomarkers for the recognition and prognosis of these cancers. Therefore, finding possible biomarkers for primary recognition of cancer is urgently needed. Long noncoding RNAs (lncRNAs) are a collection of RNAs of greater than 200 nucleotides in length [3]. Despite no noteworthy protein-coding capability, lncRNAs have significant parts in controlling gene expression at epigenetic, transcriptional as well as post-transcriptional levels [4]. The

increasing data recommended that lncRNAs play a probable part as biomarkers for the diagnosis and prediction of various tumors [5-7].

HOTAIR, localized on the homeobox C (HOXC) gene cluster of the chromosome, was recognized as a blocker of the HOXD genes when revealed in the beginning [8]. As a spliced as well as polyadenylated transcript with more nucleotides, lncRNA HOTAIR transcribed from the HOXC locus might silence HOXD, by attaching to the polycomb repressive complex2 (PRC2) [9]. Lately, numerous studies described that higher HOTAIR level has been recognized as a probable predictive factor for some types of cancers, including gastrointestinal cancers [10], colorectal cancers [11], esophageal squamous cell carcinomas [12], and breast cancers [13] etc. Some meta-analyses have documented the clinical value of HOTAIR in predicting survival in cancers [14-15], however, few studies on its diagnostic value in cancers and no meta-analysis of its application in cancer diagnosis and detection have been published. In order to confirm the specific diagnostic value of HOTAIR in identification of cancers and providing valid evidence, we performed this meta-analysis using standard statistical methods. Our results might be helpful to understand the medical importance of elevated HOTAIR in the identification of various tumors.

MATERIALS AND METHODS

Literature search

This meta-analysis was done according to the guidelines for diagnostic meta-analysis. PubMed, Embase, Web of Science, Cochrane library, Chinese Biomedical Literature Database (CBM), as well as Chinese National Knowledge Infrastructure (CNKI) were explored up to December 1, 2018 without language limitations. Below mentioned keywords were used to search: (1) HOTAIR or HOX transcript antisense intergenic ribonucleic acid, (2) cancer or carcinoma or neoplasm or tumour or tumor, and (3) sensitivity or specificity or diagnosis or ROC curve. Furthermore, the reference lists of associated reviews were physically glanced over to see if additional articles were overlooked.

Inclusion and exclusion criteria

The following criteria were included: (1) illustrating the diagnostic value of HOTAIR for cancer; (2) using the pathological method (gold standard) to diagnose cancers; (3) defining article population and control sources; (4) providing diagnostic test indexes (sensitivity, specificity, and AUC) or which could be calculated from available data; The exclusion criteria were as follows: (1) repeated/duplicate identical data from database or extra studies; (2) editorials, letters, case reports or reviews.

Data extraction

Two researchers individually studied all involved studies, then recorded the subsequent information: Name of the first author, year of publication, country of origin, ethnicity, sample size, cancer type, specimen type, test method, source of control, sensitivity, specificity, AUC, true positive, false positive, true negative, and false negative.

Quality assessment

Two researchers individually evaluated the quality of incorporated studies by means of the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. The QUADAS-2 tool involves 4 key contents: patient selection, reference standard, index test, flow, and timing. Fourteen questions were used to assess the quality of diagnostic accurateness of the studies, and an answer of "yes," "no" or "unclear" was concluded. Only "yes" can be scored.

Statistical analysis

Statistical analysis was done by the STATA 12.0. The bivariate meta-analysis model was used to compute the relevant parameters, including the pooled sensitivity, specificity, positive likelihood ratios (PLR), negative likelihood ratios (NLR), and diagnostic odds ratio (DOR). Summary receiver operator characteristic (SROC) curves as well as the AUC were performed to assess the overall investigative value of HOTAIR in cancers; in addition, the outcomes were established by a hierarchical summary receiver operating characteristics (HSROC) model. The heterogeneity of non-threshold effects was evaluated by the Cochran's Q and inconsistency index (I^2), with $p < 0.10$ for the Cochran's Q test or $I^2 > 50\%$ designated apparent heterogeneity among the studies. Fagan's nomogram was used to confirm the relationship among the pre-test probability, the post-test probability, and the likelihood ratio. The publication bias was detected by funnel plots, with $p < 0.01$ showing significant publication bias. Lastly, we performed meta-regressions and subgroup analyses to investigate the probable sources of heterogeneity.

RESULTS

Literature search

A total of 590 articles were obtained using the search terms stated in the databases above, and twelve more articles were included based on the established criteria. The detailed screening process was shown in Figure 1. Of all the studies included, two of them proved HOTAIR as a useful biomarker for colorectal cancer [16,17], and gastric cancer [18,19], while one study each proved it as a biomarker for cervical tumor [20], pancreatic tumor [21], bladder cancer [22], breast cancer [23], non-small cell lung cancer [24], laryngeal squamous cell carcinoma [25], esophageal squamous cell carcinoma [26], and glioblastoma multiforme [27].

Table 1. Summary of information of the included studies.

First author	Year	Country	Ethnicity	Cancer	Cases/ controls	Sample type	Method	AUC	Diagnostic power				QUADAS-2
									TP	FP	FN	TN	
Huang	2014	China	Asian	CC	218/218	Tissues	RT-PCR	0.803	132	28	86	190	5
Svoboda	2014	Czech	Caucasian	CRC	73/52	Plasma	RT-PCR	0.87	49	4	24	48	6
Wang	2014	China	Asian	LSCC	52/49	Serum	RT-PCR	0.727	48	21	4	28	6
Zhao	2015	China	Asian	CRC	32/32	Plasma	RT-PCR	0.777	22	3	10	29	4
Xie	2016	China	Asian	PC	55/20	Salivary	RT-PCR	0.88	43	8	12	47	4
Zhang	2016	China	Asian	BC	88/100	Plasma	RT-PCR	0.8	61	7	27	93	5
Chen	2017	China	Asian	GC	65/65	Tissues	RT-PCR	0.709	42	16	23	49	6
Li	2017	China	Asian	NSCLC	105/80	Plasma	RT-PCR	0.806	80	22	25	58	5
Wang	2017	China	Asian	BC	96/96	Tissues	RT-PCR	0.713	64	29	32	67	4
Wang	2017	China	Asian	ESCC	50/20	Serum	RT-PCR	0.793	28	2	22	18	6
Elsayed	2018	Egypt	Caucasian	GC	50/50	Plasma	RT-PCR	0.944	44	8	6	42	5
Tan	2018	USA	Caucasian	GBM	43/40	Serum	RT-PCR	0.913	37	5	6	35	6

Study and patient characteristics

The characteristic of the twelve studies were summarized in Table 1. In the current meta-analysis, these studies assessed the investigative importance of HOTAIR in numerous tumors, which incorporated 927 patients as well as 822 controls comprising para-carcinoma tissue, healthy people, and benign tissue. The articles incorporated were from 2014 to 2018. All studies tested the ex-

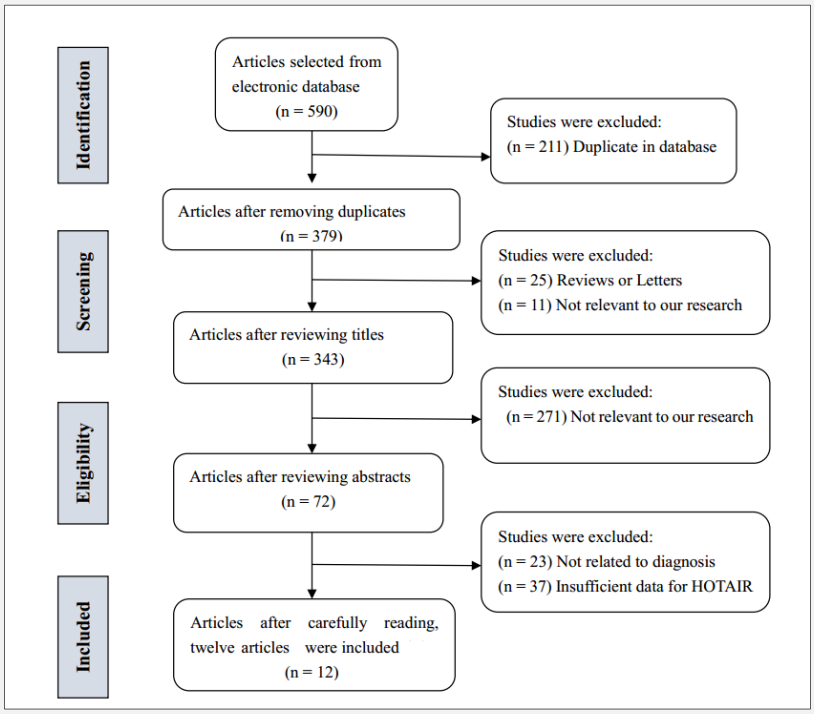

Figure 1. Flow diagram of selection process for included studies.

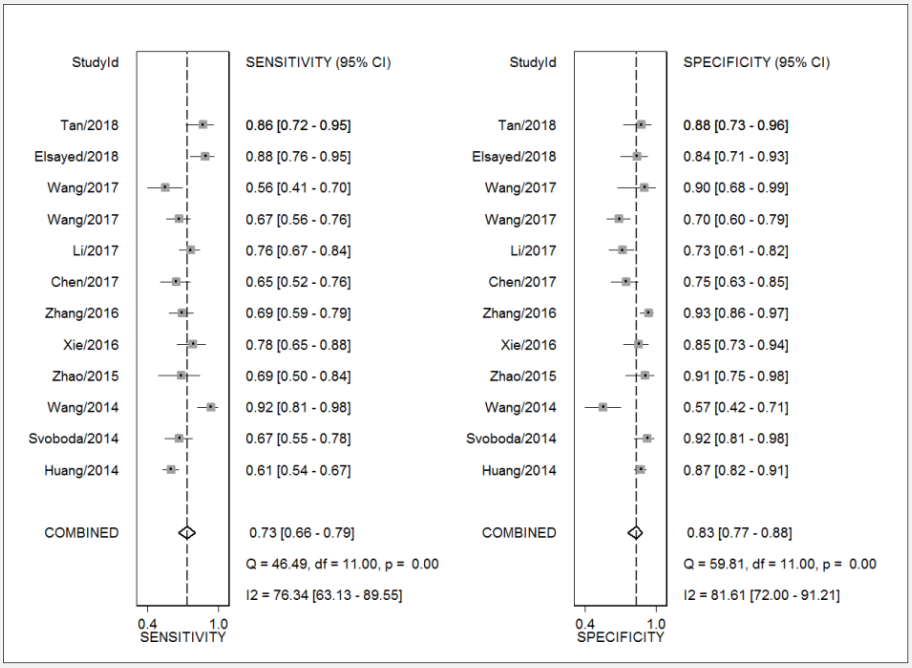

Figure 2. Forest plots of sensitivity and specificity analyses for all studies.

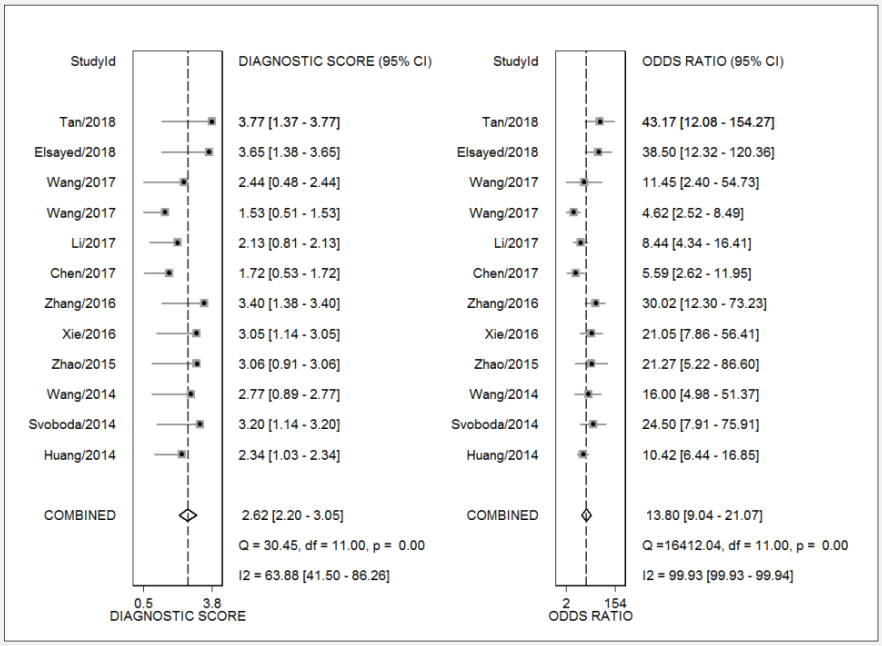

Figure 3. Forest plots of the pooled diagnostic odds ratio for HOTAIR in the diagnosis of cancer.

CI - confidence interval, Q - Cochran's Q value, DF - degrees of freedom, I^2 - inconsistency index.

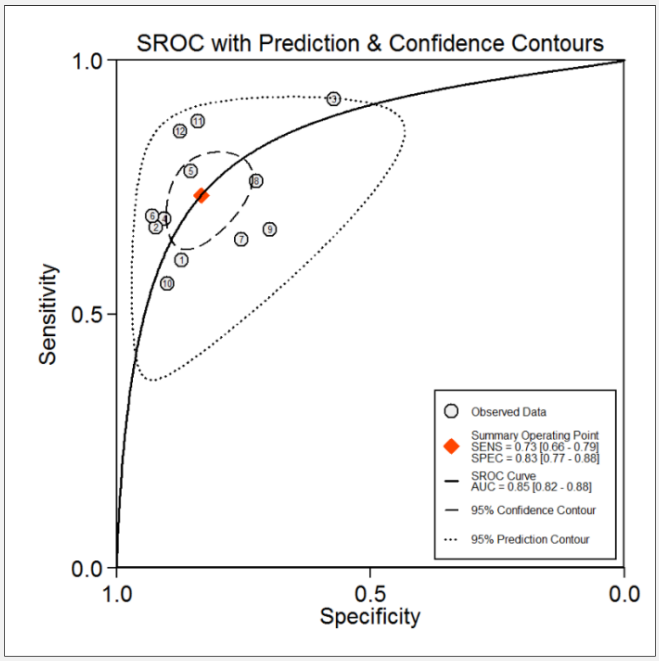

Figure 4. Summary receiver operator characteristic (SROC) curve with pooled estimates of sensitivity, specificity, and area under the curve (AUC).

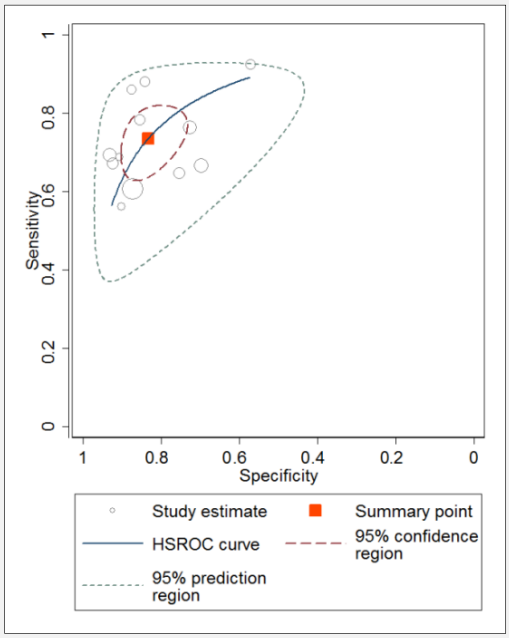

Figure 5. HSROC curve for HOTAIR in the diagnosis of cancer.

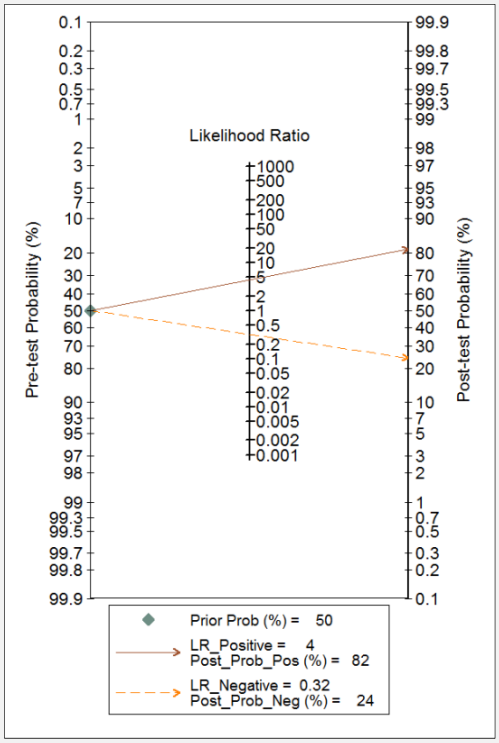

Figure 6. Fagan's nomogram for assessing the post-test probabilities.

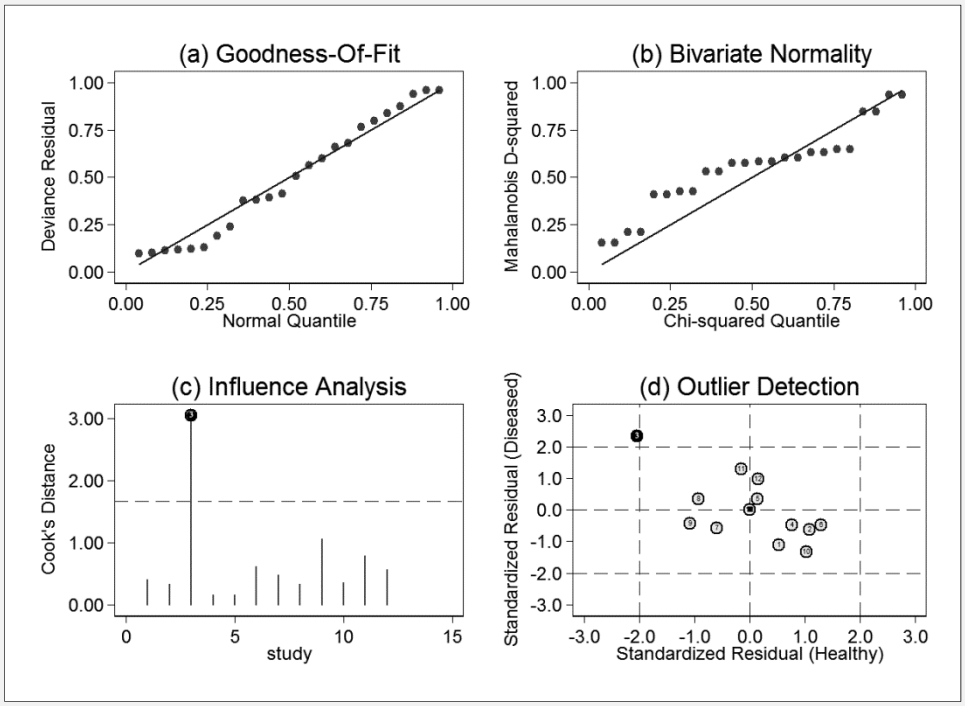

Figure 7. Graphs of sensitivity analyses: (A) goodness-of-fit; (B) bivariate normality; (C) influence analysis, and (D) outlier detection.

The black point represents the outlier, which was excluded.

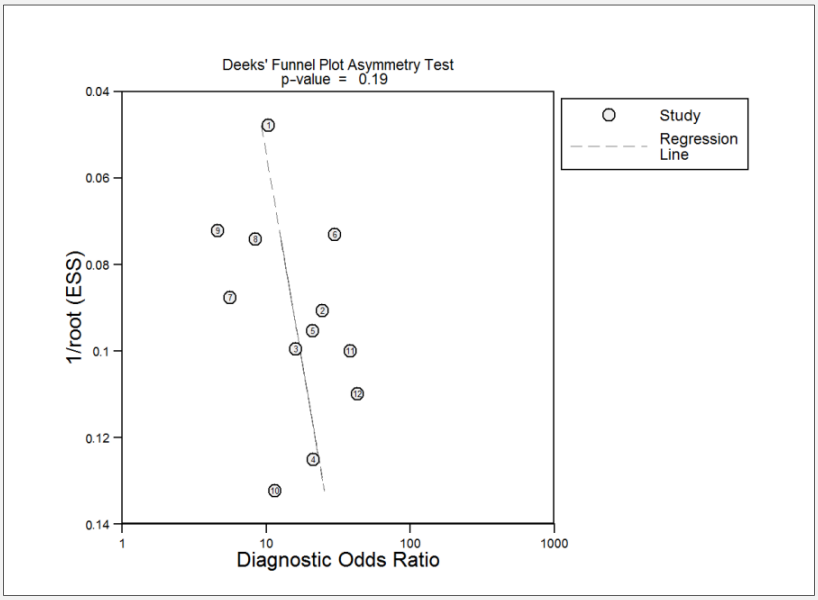

Figure 8. Graph of Deeks' funnel plot asymmetry test.

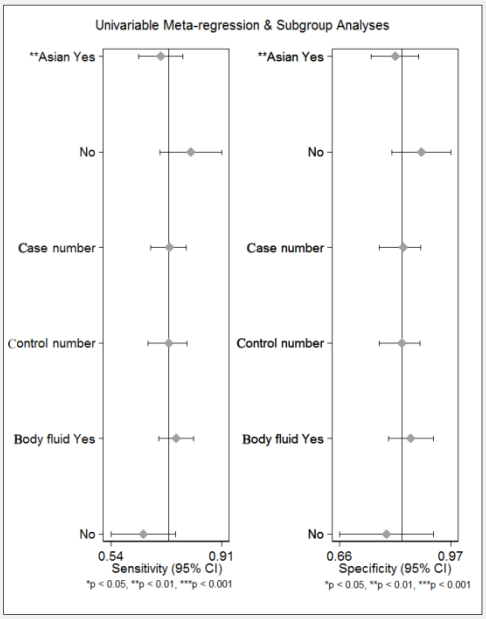

Figure 9. Univariate meta-regression and subgroup analysis on ethnicity for sensitivity and specificity.

Factors with asterisk are potential sources of heterogeneity.

pression of HOTAIR using the quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) method. The quality evaluations for all studies were confirmed by the QUADAS-2 tool and the scores were shown in Table 1. All the included studies were of adequate to great quality with QUADAS-2 scores between 4 and 6.

Diagnostic accuracy of HOTAIR in tumors

Forest plots of data from the included studies on the sensitivity as well as specificity of HOTAIR in diagnosing numerous tumors were presented in Figure 2. Because obvious heterogeneity among studies was found in sensitivity as well as specificity data (I^2 = 76.34% and I^2 = 81.61%, respectively). A random-effects model was engaged to calculate the pool effect. The estimated results from the twelve studies are shown in Figure 3. The equivalent general SROC curve was presented in Figure 4, with an AUC of 0.85, which specifies that HOTAIR is a marker with high accuracy in the diagnosis of several cancers. The HSROC curve of the twelve studies was in accordance with the outcomes from the bivariate model in Figure 5. The value of beta that specified the irregularity of the curve was 0.19, the Z was 0.51, and the p-value was 0.607, which expressed that the HSROC was proportioned. The value of lambda that represented the accuracy of the diagnostic tests was 2.58, which expressed that HOTAIR had great accurateness in distinguishing tumor from control.

Moreover, to evaluate the medical efficacy of HOTAIR, Fagan's nomogram was performed to describe the association between HOTAIR assay results and the probability of cancer. As presented in Figure 6, when HOTAIR was detected in all people with a pre-test possibility of tumor of 50%, a positive outcome enhanced the post-test possibility of tumor to 82%, whereas a negative outcome lowered the post-test possibility to 24%. The outcomes specified that HOTAIR had a moderate accuracy for differentiating patients with tumor from all people.

Influence analysis and robustness tests

Goodness-of-fit (Figure 7A) as well as bivariate normality (Figure 7B) analyses showed that the bivariate model was reasonably vigorous. Influence analysis (Figure 7C) as well as outlier detection (Figure 7D) showed only 1 outlier. After elimination of outliers, the sensitivity reduced from 0.73 to 0.71, specificity increased from 0.83 to 0.85, PLR increased from 4.4 to 4.7, NLR increased from 0.32 to 0.34, AUC decreased from 0.85 to 0.84, and the DOR showed no change. Deeks' funnel plot asymmetry test indicated no substantial publication bias (p = 0.19; Figure 8). The tests confirmed the strength of the current outcomes in our meta-analysis.

Meta-regression analyses

Meta-regression analyses were done to reveal the probable causes of heterogeneity among included studies and to confirm the outcomes of subgroup analyses. Outcomes are presented in Figure 9. Obviously, the type of ethnicity was the primary cause of heterogeneity ($p < 0.01$). Only three studies were included in the Caucasian group, and therefore, that limited their subgroup analyses.

DISCUSSION

There is a yearly upsurge in the occurrence of tumor and its related mortality rate worldwide [2]. Early diagnosis and timely treatment is very important so as to decrease the cancer deaths. Currently, some tumor markers being used in clinical practice have poor sensitivity and specificity for identifying cancers such as AFP, CEA, CA125, and so on [28-30]. What is more, these markers do not accurately detect the cancers at the initial phase, and diagnosis at the developed stage has very diminutive difference to cancer patients since it is too late for any treatments efficiently. Therefore, it was important to find ideal tumor markers for early diagnosis of cancers.

Mounting data showed that numerous differentially expressed lncRNAs have oncogenic or tumor suppressor roles in carcinogenesis [31-32]. HOTAIR has been recognized as being closely related to the occurrence of various cancers [33]. The diagnostic feature of HOTAIR in cancers has been documented by many single studies [16-27]. The accuracy of a single study is often compromised due to single-center design and limited samples. Therefore, we seek to do a systematic meta-analysis to illustrate the global diagnostic efficacy of HOTAIR in human cancers in the current study.

This current research explains the first meta-analysis that was done to determine the investigative importance of HOTAIR in various cancers. The sensitivity of 0.73, with the specificity of 0.83, as well as with the pooled AUC of 0.85, proved that HOTAIR has great precision in distinguishing tumor patients from controls. The PLRs as well as NLRs also revealed the diagnostic accurateness of HOTAIR. The pooled PLR was 4.4 and the NLR was 0.32 in this analysis. It showed that patients with tumors had a 4.4-fold greater possibility of being HOTAIR-positive compared with control patients, besides 25% of all people were negative. The pooled DOR of 14 (95% CI 9 - 21) suggested that the diagnostic effectiveness of HOTAIR is reliable in cancers. Fagan's nomogram showed that the positive post-test possibility would rise to 82% with a PLR of 4 while a pre-test possibility of 50% was specified, and the negative post-test possibility would decrease to 24% with a NLR of 0.32 in the meantime. The above results validate HOTAIR in cancer diagnosis. To determine the probable cause of heterogeneity in this meta-analysis, we accomplished a regression analysis. The results implicate the type of ethnicity as the main source of heterogeneity. But the subgroup analyses could not be performed due to only three studies in the Caucasian group.

However, our study had numerous limitations: First of all, the quantity of incorporated articles is restricted. Some usable studies might have been overlooked throughout the screening process although we searched all main literature databases. Secondly, the large proportion of Chinese population may cause ethnicity bias. Also, the types of cancer included in this analysis were limited plus the sample size of each cancer is inadequate, which might lead to the irregularity of outcomes. Lastly, only studies available in English or Chinese were incorporated. Hence, it is possible that studies available in additional languages might have been neglected.

CONCLUSION

In summary, the results from our meta-analysis suggest that elevated HOTAIR appeared to be a relatively accurate diagnostic marker for patients with cancer and can be applied to support diagnosis of various cancers. In addition, meta-regression analyses implied that the value of HOTAIR for cancers shows some differences between Asian and Caucasian. However, this conclusion must be confirmed through comprehensive and large-scale studies in future.

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Not applicable.

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Declaration of Interest:

All authors declare that they have no competing interests.

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