https://bcbn.kaums.ac.ir/
Basic and Clinical Biochemistry and Nutrition. 2024;1(1):10-15
https://doi.org/10.48307/BCBN.2024.190503

Research Article Open Access

Circulating LncRNA HOTAIR as a tumor marker in the serum of patients with esophageal cancer

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Received: 13 January 2024 **Revised:** 13 February 2024 **Accepted:** 14 February 2024 **e-Published:** 18 February 2024

Abstract

Background: Long non-coding RNAs (LncRNAs) have emerged as crucial regulators in the genetic and epigenetic landscape of cancer over the past decade. Among these, HOTAIR stands out as an oncogenic molecule implicated in various cancer types. However, the significance of its serum expression levels and clinical implications in esophageal cancer remain inconclusive.

Objectives: This study aims to investigate the serum expression of HOTAIR and its clinical relevance in patients with esophageal cancer.

Methods: The serum levels of HOTAIR were analyzed in 47 patients with esophageal cancer and 50 healthy controls using quantitative real-time polymerase chain reaction (qRT-PCR). Additionally, the correlation between HOTAIR expression levels and clinicopathologic characteristics was statistically evaluated.

Results: The serum levels of HOTAIR were significantly elevated in patients with esophageal squamous cell carcinoma (ESCC) compared to healthy controls ($P<0.001$). The receiver operating characteristic (ROC) curve analysis demonstrated an area under the curve (AUC) value of 0.770 (95% CI: 0.652 to 0.888, $P<0.001$).

Conclusion: This study suggests that circulating HOTAIR could potentially serve as a valuable biomarker for diagnosing ESCC and may offer a non-invasive approach for the early detection of esophageal cancer.

Keywords: Circulating Long Noncoding RNA, HOTAIR, Esophageal Cancer.

Introduction

Esophageal cancer (EC) ranks as the ninth most common malignancy worldwide, with over 0.6 million new cases and 0.54 million deaths reported in 2020.[1,2] Various forms of esophageal cancer exist, including squamous cell carcinoma (SCC), adenocarcinoma (AC), sarcomas, small cell carcinomas, and rare types like lymphomas and melanomas, with SCC and AC being the most prevalent types.[3] The distribution of esophageal cancer types varies geographically, with esophageal squamous cell carcinoma (ESCC) comprising 73% of cases in the north and northeast regions of Iran, while adenocarcinoma is more common in the United States.[4,5]

Early diagnosis of ESCC is crucial as most patients present with advanced metastatic disease, resulting in a low 5-year survival rate of less than 10%. Understanding the underlying mechanisms of EC remains a challenge, but emerging evidence suggests that noncoding RNAs, which regulate gene expression at various levels, play a significant role in EC pathophysiology.[6]

Long non-coding RNAs (LncRNAs) constitute a significant class of RNAs involved in numerous biological processes.[7] Noncoding RNAs (ncRNAs), including microRNAs, small interfering RNAs, and LncRNA, play crucial regulatory roles in the development of various diseases, particularly in cancers.[8-10] LncRNAs are RNA molecules longer than 200 nucleotides and have been implicated in oncogenic pathways at epigenetic, transcriptional, and posttranscriptional levels. One such LncRNA, HOX transcript antisense RNA (HOTAIR), functions by suppressing gene expression through the recruitment of chromatin modifiers. Studies have

demonstrated that HOTAIR promotes the migration and invasion of ESCC cells and plays a significant role in the Wnt/β-catenin signaling pathway.[8,11] Additionally, HOTAIR is involved in metastasis, angiogenesis, DNA repair, and tumor cell metabolism, suggesting its potential as a diagnostic target in patients with ESCC.[12]

Objectives

This study aims to assess serum HOTAIR expression in ESCC and explore its diagnostic value for early detection. Quantitative Reverse Transcriptase PCR (qRT-PCR) was employed to measure HOTAIR levels, and associations between HOTAIR expression and specific clinical features of ESCC were analyzed.

Methods

Patients and Blood Samples

Blood samples were collected from participants in this study over a period of 17 months, who were referred to the oncology department of Imam Ali Hospital and the oncology clinic in Bojnourd, located in North Khorasan province in northeastern Iran. All participants were clinically suspected of having esophageal cancer and underwent endoscopy, during which a biopsy specimen was taken from their esophageal tissue.

The case group consisted of individuals who tested positive for histopathological tests confirming esophageal cancer, while the control group tested negative in histopathological examinations. The control group was matched with the case group based on age and sex. In total, 47 participants were included in the case group and 50 in the control group. Clinical details such as age, sex, clinical signs, diagnosis, history of smoking, substance and alcohol use, endoscopic lesion area, and histological description of the lesion were recorded. Blood samples (5 ml) were collected and centrifuged at 3000 g for 10 minutes in test tubes. The serum was then transferred to RNase/DNase-free microtubes and stored at -70 °C [Table 1].

RNA Extraction, cDNA Synthesis, and Real-Time PCR (qRT-PCR)

Total RNA was extracted from each serum sample using the QIAamp Circulating Nucleic Acid Total RNA Extraction kit following the manufacturer's instructions (Qiagen, Germany). The concentration and purity of RNA samples were determined using NanoDrop 8000 (Thermo Scientific, Waltham, MA, USA) at 260/280 nm and 260/230 nm ratios. Complementary DNA (cDNA) was synthesized using the bio cDNA synthesis protocol from South Korea. qRT-PCR was conducted using the Step One Real Time PCR System (Applied Biosystems, CA, USA). The fold-change gene expression in patient samples compared to the control group was calculated using the $2^{-\Delta\Delta CT}$ method, with U6 used as a normalization control for mRNA expression changes. Exon-exon binding primers were designed to minimize DNA presence in experiments where RNA extraction samples contained potential DNA. The primer sets used for qRT-PCR are listed in Table 2.

Statistical Analysis

Statistical analysis was performed using SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). Normal distribution tests were conducted for all data. Mean±standard deviation (SD) was used for normally distributed measurement data. Student's t-test was used to compare normally distributed measurement data. Pearson correlation analysis was employed to assess associations between continuous variables. Receiver operating characteristics (ROC) curves were plotted to evaluate the discriminatory ability of serum HOTAIR expression levels between tumor samples and healthy control samples. An AUC-ROC value greater than 0.7 indicated reasonable biomarker performance, with optimal cut-off values defined as the point maximizing the Youden index (sensitivity + specificity). A “P-value” less than 0.05 was considered significant.

Bioinformatics Analysis

Interaction between HOTAIR and miRNAs was analyzed using DIANA-LncBase v3.[13] Pathways involving these miRNAs were identified using DIANA-mirPath v.3.[14]

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval was obtained.

Results

Fold Change Analysis

After analyzing the results, the ΔCT of LncRNA HOTAIR was found to be 1.1 for the patient group and 5.14 for the control group (Lower ΔCT indicates higher expression). There was a significant difference in LncRNA HOTAIR expression between the patient and control groups ($P< 0.05$). The ΔCT analysis revealed that LncRNA HOTAIR expression in the patient group (individuals with esophageal cancer) was approximately 5.14 times higher compared to the control group. Statistical analysis using t-tests and Pearson correlation tests indicated a significant association between LncRNA HOTAIR expression and

esophageal cancer as well as aging. No significant correlations were observed with gender, family history of the disease, cancer type, or tumor area.

ROC Curve

The ROC curve analysis of serum HOTAIR levels was conducted to evaluate its diagnostic utility [Figure 1]. Serum HOTAIR levels were able to distinguish between ESCC patients and healthy controls, with an AUC of 0.770 (95% CI: 0.652 to 0.888, P < 0.001). The optimal cut-off values (ΔCT) were determined to be -1.3, with a sensitivity of 76.19% and specificity of 68.0% [Figure 1].

miRNAs Targeted by HOTAIR

Figure 2 displays miRNAs that can potentially be targeted by HOTAIR, influencing various pathways crucial in cancer initiation and progression, such as Pathways in cancer (hsa05200), Cell cycle (hsa04110), MAPK signaling pathway (hsa04010), PI3K-Akt signaling pathway (hsa04151), VEGF signaling pathway (hsa04370), and mTOR signaling pathway (hsa04150) [Figure 2].

Table 1. Summary of descriptive statistics information of variables

Variables		No. of patients and Frequency (%)	Serum HOTAIR expression (mean±SD)	P value
Age	≤ 60 years	15 (31.91%)	63.67±10	0.464
	> 60 years	32 (68.08%)		
Gender	Men	26 (55.3%)	2.269±0.511	0.027
	Women	21 (44.7%)	1.751±0.772	
Pathology	ESCC	34(72.34%)	1.97±0.852	0.605
	AC	13(27.66%)	2.155±0.512	
Histological grad	Grade l	32 (68.08%)	2.45±0.566	0.586
	Grade ll, lll	15 (31.92%)	1.24±0.694	
Expression level	Cancer	47	1.94±0.4	0.03
	Normal	30	5.98±1.04	

Table 2. sequence of primers

Gene	Forward/Revers	Sequence (5' to 3')
HOTAIR	F	AACAGAGTCCGTTCAGTGTC
HOTAIR	R	ACACAAGTAGCAGGGAAAGG
U6	F	CCAAAACTGGCTGGATTCCTAG
U6	R	CCAAGAAATGCAAAACAAACCC

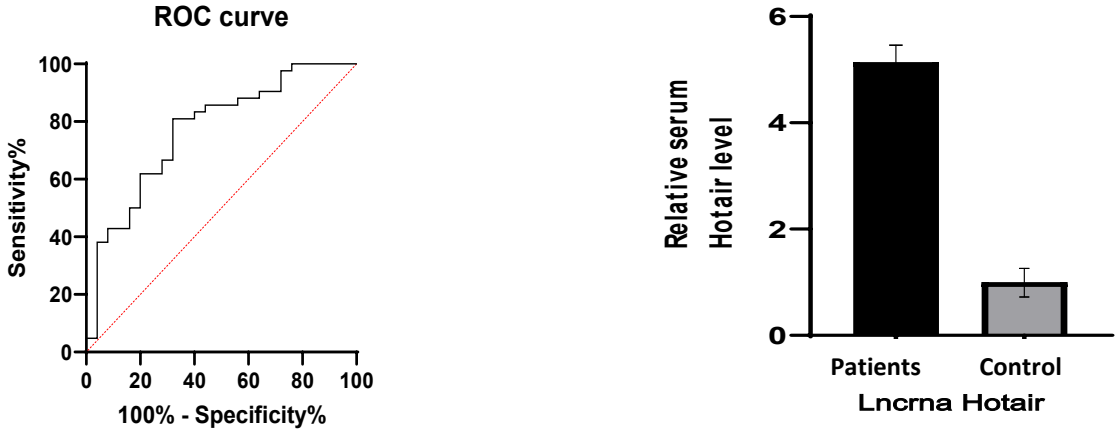

Figure 1. HOTAIR expression levels from different sources and patients. A Pearson correlation analysis of HOTAIR expression levels in ESCC serum; b Comparison of serum HOTAIR expression level between ESCC patients and healthy controls (***p<0.001).

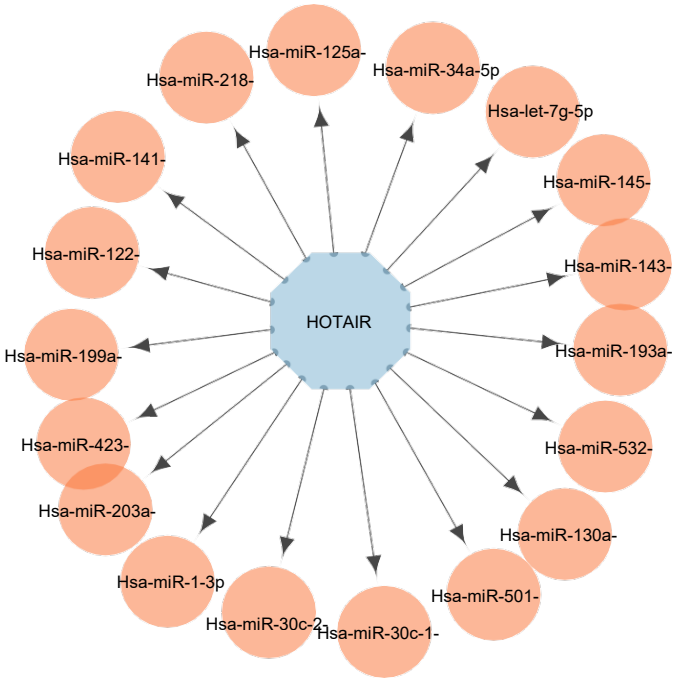

Figure 2. MicroRNAs- LncRNA interaction

Discussion

Esophageal cancer ranks as the third most prevalent cancer globally and stands out as a common malignant disease affecting the gastrointestinal tract, yet effective treatment options remain elusive. The worldwide incidence of esophageal cancer is notably high, underscoring the critical need for identifying a reliable biomarker for ESCC to facilitate early intervention. Recent studies have highlighted the stability of circulating LncRNAs in serum, despite the presence of high levels of RNase in the blood of cancer patients.[15] Previous research has identified HOTAIR, an LncRNA, as a prognostic marker for ESCC.[5] This study represents the first investigation in Iran to assess serum levels of HOTAIR LncRNA. The detection of these long non-coding RNAs in tissues and organs typically involves the qPCR technique. Our findings demonstrate that serum HOTAIR shows promise as a novel diagnostic biomarker for ESCC. These results align with a study conducted by Wenjian Wang in China, where elevated levels of HOTAIR LncRNA were observed in the serum samples of esophageal cancer patients compared to healthy individuals. While their study included approximately 50 patient samples, our study encompassed twice that number. The sensitivity and specificity of their test were reported as 56% and 90%, respectively, whereas our study yielded values of 76.19% and 68%, respectively, with an optimal delta CT value of -1.335.[16]

We analyzed the expression of HOTAIR in the serum of 47 ESCC patients, revealing a significant increase in HOTAIR expression in the serum of ESCC patients compared to the healthy control group. Our results suggest that serum HOTAIR holds promise as a potential biomarker for ESCC. Using qRT-PCR, we assessed serum HOTAIR expression levels in 47 ESCC patients and 50 healthy controls to ascertain whether patients with ESCC exhibit higher serum HOTAIR expression levels. The results demonstrated significantly elevated HOTAIR expression in the serum of ESCC patients compared to healthy controls. Furthermore, we observed a significant correlation between serum HOTAIR levels and the M stage, indicating a crucial role for HOTAIR in ESCC progression.

ROC curve analysis indicated that serum HOTAIR could effectively differentiate between ESCC patients and healthy controls, with an AUC of 0.770, underscoring its potential as a diagnostic indicator for ESCC patients. These findings align with studies by Kewei Ren and Hiroshi Ono, which highlighted increased HOTAIR expression in cancer cells and its association with ESCC progression. HOTAIR's oncogenic properties suggest its utility as both a prognostic biomarker and a potential therapeutic target.[17-19]

Semnani et al. stated in their study that esophageal cancer is more prevalent in the north and northeast regions of Iran, with ESCC being the predominant type (ESCC= 73% _ EAC = 27%). In Western societies like the United States, adenocarcinoma is the predominant form of esophageal cancer.[4,5,15]

The release of nucleic acids into the blood is believed to be associated with necrotic tumor cells in the tumor microenvironment. Our results indicated a significant increase in serum HOTAIR expression levels in patient samples compared to control specimens. Therefore, serum HOTAIR expression levels could potentially serve as an indicator for cancer recurrence in ESCC patients post-tumor resection.[20,21] However, certain limitations in our study need addressing. The relatively small population of enrolled patients and controls could introduce bias into the final results.

Studies conducted in Iran have shown that the northern and northeastern regions have a high incidence of esophageal cancer.[15,22] The rate of esophageal cancer in northeastern Iran is one per 23,459 people, necessitating further investigation into epidemiological and other health aspects.[23,24]

This study has some limitations. The limited number of patients was a major challenge, and the extended duration of sample collection was unavoidable.

Conclusions

In conclusion, given the global prevalence of esophageal cancer and the lack of early disease signs, our statistical analysis revealed a significant increase in HOTAIR LncRNA levels in the blood serum of individuals with esophageal cancer compared to controls, with HOTAIR levels being 5.14 times higher in the cancer group. The upregulation of these genes correlates with different stages of cancer progression, suggesting HOTAIR's potential as a prognostic indicator for diagnosing esophageal cancer. Additionally, this study established a link between heredity, age, and sex with esophageal cancer. Considering the diagnostic significance and cost-effectiveness of detecting LncRNA compared to tissue sampling, first-degree relatives of affected individuals could benefit from monitoring based on the findings of this study.

Acknowledgment

The authors take this opportunity to thank the Department of Clinical Biochemistry, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran for their financial and technical support.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Esophageal cancer: EC;
Esophageal squamous cell carcinoma: ESCC;
Squamous cell carcinoma: SCC;
Adenocarcinoma: AC;
Long non-coding RNAs: LncRNAs;
Noncoding RNAs: ncRNAs;
HOX transcript antisense RNA: HOTAIR;
Quantitative Reverse Transcriptase PCR: qRT-PCR;
Complementary DNA: cDNA;
Receiver operating characteristics: ROC.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

None.

Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Institutional Review Board approval was obtained. The present study did not interfere with the process of diagnosis and treatment of patients and all participants signed an informed consent form.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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How to Cite this Article:
Kazemi H, Assaran R, Salarinia R, Abbaspour A. Circulating LncRNA HOTAIR as tumor markers in the serum of patients with esophageal cancer. Basic Clin Biochem Nutr. 2024; 1 (1): 10-15. doi: 10.48307/bcbn.2024.190503