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Evaluation of Circulating Long Non-Coding RNA HOTAIR as a Diagnostic and Prognostic Marker of Breast Cancer

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ABSTRACT

Background: Among female cancers, breast cancer accounts for the majority of cases and deaths. Trustworthy biomarkers to early detect and follow prognosis are of the utmost importance. New research suggests that HOX transcript antisense intergenic RNA (HOTAIR) and other long non-coding RNAs (lncRNAs) play a particular role in the initiation and progression of cancer**.**

Objective: This research aimed to evaluate the diagnostic and prognostic implication of plasma lncRNA HOTAIR in breast cancer patients.

Patients and methods: A controlled case-cohort study was conducted on 60 adult females, categorized into three groups: 30 newly diagnosed breast cancer patients, 15 with benign breast lesions, and 15 healthy controls. Plasma HOTAIR levels were measured by real-time PCR, and CA15-3 was assessed as a conventional marker. Statistical analyses included associations with clinicopathological data and ROC curve analysis for diagnostic performance.

Results: Plasma HOTAIR was found to be significantly higher among breast cancer patients than in both benign and healthy groups ($p < 0.001$). HOTAIR showed high diagnostic accuracy (sensitivity 93.3%, specificity 96.4%, AUC 0.957 at >1.085). Combining HOTAIR with CA15-3 further improved sensitivity. Elevated HOTAIR correlated with lymph node involvement, larger tumor size, metastasis, and poorer prognosis ($p < 0.001$).

Conclusion: Circulating lncRNA HOTAIR might be a promising, sensitive, and specific biomarker for breast cancer diagnosis and prognosis. Incorporating HOTAIR with traditional markers may enhance early detection and risk stratification.

Keywords: Long Non-Coding RNA, HOTAIR, Diagnostic, Prognostic, Breast cancer.

INTRODUCTION

Breast cancer (BC) remains the most diagnosed malignancy among females which is considered the leading cause of cancer-related morbidity and mortality worldwide, including Egypt. Recent national statistics reveal that Egypt alone reported over 22,000 new breast cancer cases and more than 9,000 associated deaths in 2020 [(1-3)].

In recent years, research has increasingly emphasized the critical functions of long noncoding RNAs (lncRNAs) in cancer, beyond traditional protein-coding genes. LncRNAs are transcripts longer than 200 nucleotides that do not encode proteins, yet they have been found to regulate gene expression at various levels, playing key roles in cancer biology. Among these, HOX transcript antisense intergenic RNA (HOTAIR) has attracted considerable interest for its ability to alter chromatin structure and affect transcriptional activity. Structurally, HOTAIR is a polyadenylated lncRNA with six exons, spanning 2,158 nucleotides [(4-5)].

On a molecular level, HOTAIR aids oncogenesis in breast cancer by interacting with PRC2, an important multi-protein complex that regulates transcription by condensing chromatin [(6)]. Clinical and molecular investigations have demonstrated that HOTAIR expression is frequently elevated in BC tissues, and its upregulation has been linked to enhanced tumor invasion, metastasis, and unfavorable patient outcomes [(7)]. Further, HOTAIR may promote tumor progression by modulating pathways such as the upregulation of *S100A4* and interaction with estrogen receptor pathways through microRNAs, including miR-568 and miR-148a [(8)].

Emerging evidence also supports the detection of circulating nucleic acids (CNA), including lncRNAs, in various body fluids. The quantification of CNAs in plasma or serum has shown promise as a minimally invasive biomarker for cancer diagnosis and real-time monitoring, including breast cancer [(9)]. However, despite the availability of traditional serum markers such as cancer antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA), these markers are limited by suboptimal sensitivity and specificity in early detection and prognostic assessment [(10)].

While traditional breast cancer biomarkers like CA15-3 are widely used, they lack the sensitivity and specificity needed for early diagnosis and prognosis. The clinical value of circulating lncRNAs, particularly HOTAIR, is not well established, as most research has focused on tissue samples rather than blood-based assays. There is also limited evidence on HOTAIR's ability to differentiate malignant from benign breast lesions or predict patient outcomes. Therefore, this study aimed to fill these gaps by investigating the diagnostic and prognostic potential of plasma HOTAIR as a non-invasive biomarker in breast cancer.

PATIENTS AND METHODS

This controlled case-cohort study was carried out at the Faculty of Medicine, Zagazig University. The research

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was carried out across multiple centers, including the Clinical Pathology Department, the laboratories of Zagazig University Hospital, Zagazig University Scientific and Medical Research Centre, and Surgery Department.

Study population: A total of 60 adult female participants were enrolled for the study after obtaining informed written consent for sample and clinical data usage, who were categorized into three groups:

Group I (breast cancer patients): Thirty women who were newly diagnosed with breast cancer and admitted to Surgery Department, Zagazig University Hospitals aged from 43 to 63 years.

Group II (disease control – benign lesions): Fifteen women diagnosed with benign breast lesions, aged from 41 to 57 years.

Group III (healthy controls): Fifteen apparently healthy women, aged from 40 to 55 years.

Inclusion criteria: Who had willingness to participate and provide written consent and newly diagnosed breast cancer patients with no prior clinical treatment before sample collection?

Exclusion Criteria: Refusal to participate in the study, history of receiving any treatment before sample collection, and diagnosis of any other malignancy.

Breast cancer patients underwent standard treatment including surgical excision of the tumor and affected lymph nodes, followed by radiotherapy, chemotherapy, and hormonal therapy as clinically indicated.

Study procedures and assessments (Figure 1)
All participants underwent the following:

1. Clinical Assessment

Medical history and physical examination: A detailed history was obtained, and each participant underwent a clinical breast examination, including inspection and palpation of the breast and axillary regions to detect any lumps, changes, or abnormalities.

2. Histopathological Examination

Tumor tissues were subjected to histopathological evaluation in Pathology Department, Zagazig University to confirm diagnosis and assess tumor characteristics.

3. Laboratory Investigations

A. Routine laboratory tests: Complete blood count (CBC): Measured with an automated cell counter (Sysmex XS, Japan). **Liver and kidney function tests:** Performed utilizing a Roche Cobas 8000-c702 automated analyzer (Roche Diagnostics, Germany). **CA15-3:** Quantified via electrochemiluminescence immunoassay (Roche Cobas 8000-e602, Roche Diagnostics, Germany).

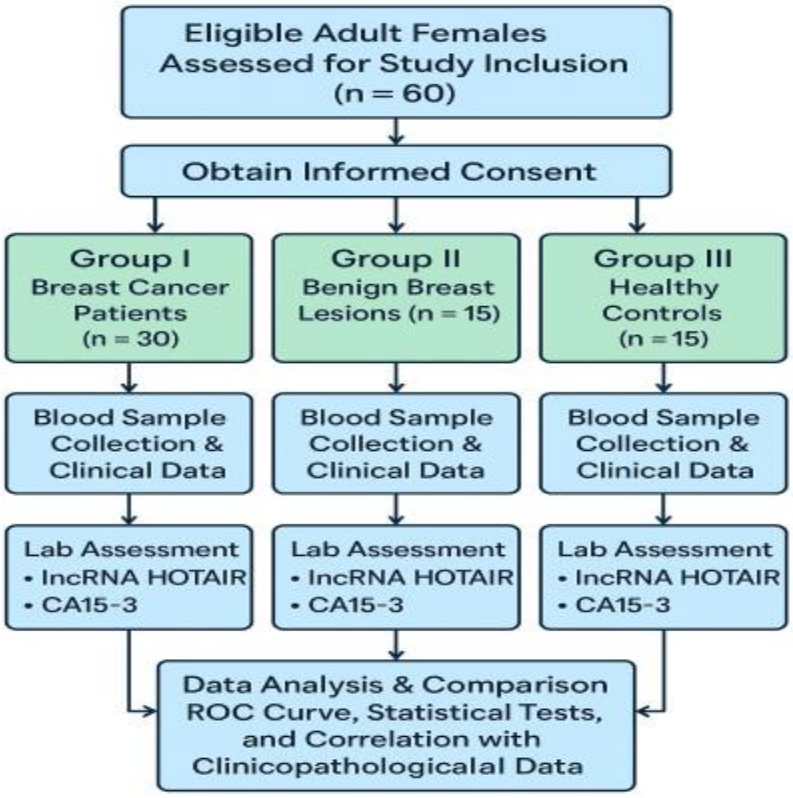


Figure (1): Flowchart of cases.

B. Specific laboratory test: Quantification of plasma lncRNA HOTAIR

Sample collection: Two milliliters of fresh blood were collected in EDTA tubes and gently mixed for lncRNA HOTAIR analysis.

Measurement method: Plasma lncRNA HOTAIR levels were assessed using Real-Time Polymerase Chain Reaction (RT-PCR) (Applied Biosystems, USA), following these steps:

a) RNA extraction: Total RNA isolation was performed utilizing the miRNeasy Mini Kit (Qiagen, Germany), following the manufacturer's instructions, which involved lysis, chloroform phase separation, ethanol precipitation, column purification, and final elution in RNase-free water. The purity of RNA was established spectrophotometrically (A260/A280 ratio between 1.8 and 2.1), and care was taken to avoid RNase contamination.

b) Reverse transcription: Total RNA was reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, USA), as per the provided protocol.

c) qPCR amplification and detection: Quantitative real-time PCR was performed utilizing SYBR® Green PCR Master Mix (Applied Biosystems, USA) with gene-specific primers for HOTAIR and GAPDH (internal control).
HOTAIR primers: Forward: 5′-GGCAAATGTCAGAGGGTT-3′ and Reverse: 5′-GTGTAACAGGCAGGTGGA-3′
GAPDH primers: Forward: 5′-CACCAGGGCTGCTTTTAACTC-3′, and Reverse: 5′-GACAAGCTTCCCGTTCTCAG-3′
Reactions were run with 40 cycles, denaturation at 95 ºC, and annealing/extension at 58ºC. Melting curve analysis was performed to ensure specificity. Data were normalized to GAPDH, and folds change in HOTAIR expression was evaluated utilizing the 2^-ΔΔCT method.

Prognostic assessment and follow-up: Patients were followed for one year to assess prognosis. Prognostic evaluation considered:

Age: Patients under 35 years often have more aggressive, high-grade tumors, leading to higher recurrence risk and poorer outcomes.

TNM stage: Early-stage cancers are associated with better prognosis, larger tumors and lymph node involvement increase recurrence risk. **Tumor grade:** Low-grade tumors grow slower and are less likely to metastasize than high-grade tumors.

Hormone receptor status (ER/PR): Tumors positive for estrogen or progesterone receptors are typically less aggressive. **HER2 status:** HER2-positive cancers are more aggressive and likely to recur, but may respond better to targeted therapy.

Outcomes: Good prognosis: Absence of complications, metastasis, or recurrence during follow-up.

Poor prognosis: Development of metastasis or recurrence. (In this cohort, eight patients developed metastasis two after surgical excision and one patient had tumor recurrence during follow-up.)

Ethical approval: The Ethics Committee of the Zagazig Faculty of Medicine approved this investigation. Each participant completed a permission form when all information was received. Throughout its implementation, the study complied with the Helsinki Declaration.

Statistical analysis

We used SPSS version 20 and Microsoft Excel 2010 to analyze the data. For numerical data, descriptive statistics provided summary measures like medians and ranges, while for categorical data, they displayed frequency and percentage breakdowns. Group comparisons were conducted using the relevant tests: t-tests for parametric data across or within groups, Mann-Whitney and Kruskal-Wallis for non-parametric data, and chi-squared for categorical variables. Various assays' diagnostic and prognostic utility were evaluated using ROC curves. $P \leq 0.05$ was deemed significant.

RESULTS

All three groups were compared with respect to demographics and basic laboratory results. There were no notable variations in the majority of the baseline variables between the groups. These variables included age, marital status, BMI, age of menarche, hemoglobin, white blood cell count, and platelet count. There was a statistically significant difference between the groups with benign lesions and those with breast cancer in terms of blood albumin levels ($p = 0.016$). Compared to both benign and healthy controls, the breast cancer group had significantly higher levels of CA 15-3 ($p < 0.001$), although there were no significant differences between the groups in terms of other laboratory data (Table 1).

Table (1): Comparing the studied groups as regards the demographic and Laboratory data

	Group I	Group II	Group III	χ^2	P
	N=30(%)	N=15(%)	N=15(%)		
Marital status:					
Single	8 (26.7%)	5 (33.3%)	3 (20%)	0.68	0.71
Married	22 (73.3%)	10 (66.6%)	12 (80%)		
Post menopause	18(60%)	8(53.3%)	4(26.7%)	4.533	0.117
Breastfeeding	20(66.6%)	9(60%)	10(66.6%)	0.22	0.89
Parity:					
0	10 (33.3%)	5 (33.3%)	3 (20%)		
1	8 (26.7%)	4 (26.6%)	5 (33.3%)	2.38	0.96
2	8 (26.7%)	3(20%)	6 (40%)		
3	2 (6.7%)	2 (13.3%)	0(0%)		
4	2 (6.7%)	1 (6.6%)	1(6.6%)		
Age (year)	53.07±10.04	49.87 ± 8.42	47.87 ± 8.99	1.663	0.199
BMI (kg/m^2)	28.5 ± 1.94	27.27 ± 3.56	27.93 ± 3.2	1.028	0.364
Age at menarche (year)	12.07 ± 0.79	12.27 ± 0.96	12.4 ± 0.83	0.847	0.434
Hemoglobin(g/dl)	10.68 ± 1.0	10.35 ± 1.16	10.5 ± 1.02	0.512	0.602
WBCs (10^3/mm^3)	7.55 ± 1.04	7.03 ± 1.89	6.59 ± 1.91	1.236	0.298
Platelet(10^3/mm^3)	186.23 ± 38.4	177.2 ± 18.03	181.93 ± 26.39	0.417	0.661
Albumin (g/dl)	3.17 ± 0.35	3.54 ± 0.47	3.38 ± 0.45	4.483	**0.016***
LSD	P_1 0.005*	P_2 0.286	P_3 0.103		
BUN (mg/dl)	10.73 ± 2.87	12.33 ± 2.29	13.13 ± 2.66	1.856	0.166
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
ALT (U/L)	19(7.5 – 25)	19(9 – 28)	20(13 – 29)	0.465	0.793
AST (U/L)	18(11 – 23)	15(8 – 29)	19(15 – 25)	0.346	0.841
Bilirubin (mg/dl)	0.5(0.35– 0.7)	0.4(0.3 – 0.7)	0.5(0.2 – 0.6)	1.153	0.562
Creatinine(mg/dl)	0.5(0.4 – 0.8)	0.6(0.4 – 0.7)	0.6(0.4 – 0.8)	0.054	0.973
CA 15-3(ng\ml)	70(32 – 94.5)	19(15 – 25)	18(13 – 23)	30.045	<0.001**
Pairwise	P_1 <0.001**	P_2 0.687	P_3 <0.001**		

χ2: Chi square test, MC Monte Carlo test, §Chi square for trend test, KW Kruskal Wallis test, **F:** One way ANOVA test value, **LSD**: Fisher least significant difference test, **P1:** Comparing groups I and II, **P2:** Comparing II and III, **P3:** Comparing I and III.

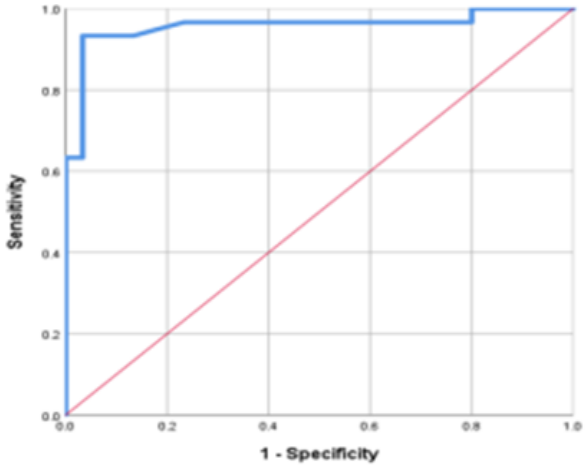
There was a highly significant difference in the amounts of circulating long non-coding RNA HOTAIR among the groups ($p < 0.001$), with group I exhibited much greater levels than groups II and III. Groups II and III did not differ significantly (Table 2).

Table (2): Comparing the studied groups regarding Circulating Long Non Coding RNA HOTAIR level

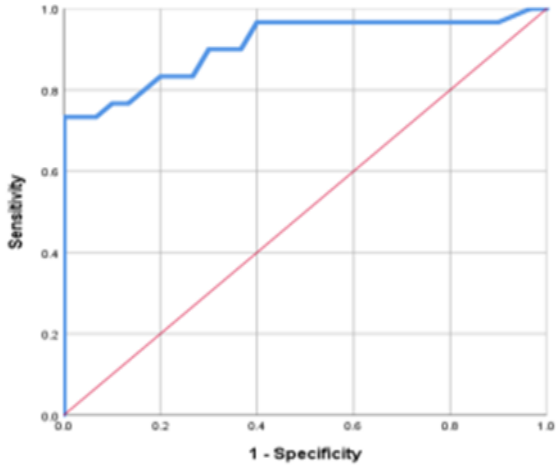
	Group I	Group II	Group III	KW	P
	Median (IQR)	Median (IQR)	Median (IQR)		
HOTAIR	2.35 (1.76 – 3.42)	0.44 (0.35 –0.73)	1 (0.98 – 1.02)	40.639	<0.001**
Pairwise	P_1 <0.001**	P_2 0.058	P_3 <0.001**		

Table (3): Diagnostic performance of circulating lncRNA HOTAIR, CA 15-3, and their combination in breast cancer detection among studied participants

Diagnostic Marker/Cutoff	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	P-value
HOTAIR (≥1.085)	0.957	93.3	96.7	96.6	93.5	95	<0.001**
CA 15-3 (≥24.5)	0.911	83.3	80.0	80.6	82.8	81.7	<0.001**
Combined (CA 15-3 ≥24.5 and/or HOTAIR ≥1.085)	1.000	100.0	81.8	76.7	100.0	88.3	<0.001**



(A)



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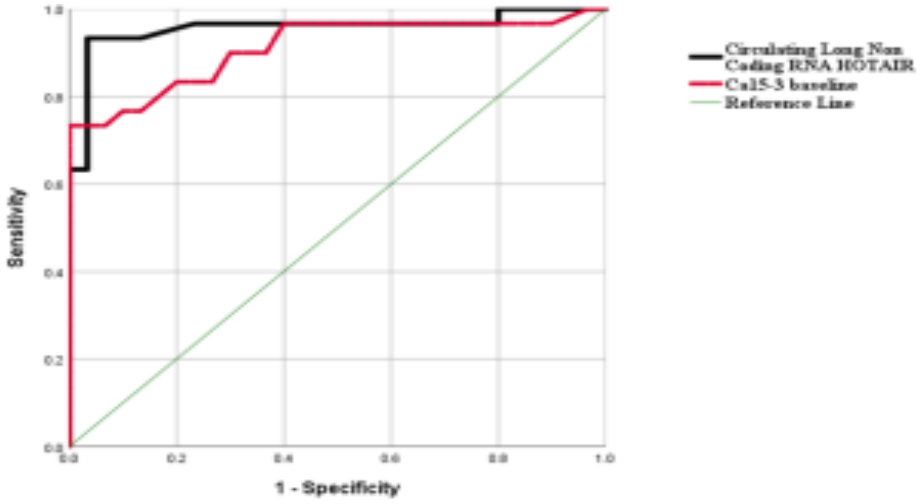

(C)

Figure (2): ROC curves showing (A): HOTAIR in diagnosing breast cancer among studied participants (B): CA 15-3 in diagnosing breast cancer among studied participants. (C): Combined CA 15-3 and HOTAIR in diagnosing breast cancer among studied participants.

Among breast cancer patients, circulating HOTAIR levels were significantly higher in those with negative estrogen receptor status ($p = 0.043$), with metastasis on follow-up ($p = 0.002$), and showed significant positive correlations with tumor (T) staging ($p < 0.001$), lymph node (LN) staging ($p = 0.019$), presence of metastasis ($p = 0.041$), and overall cancer staging ($p = 0.01$). Additionally, HOTAIR was negatively correlated with estrogen receptor status ($p = 0.041$) (Table 4).

Table (4): Associations and correlations of circulating long non-coding RNA HOTAIR levels with baseline, demographic, laboratory, and disease-specific data among breast cancer patients

Parameter	Subgroup/ Unit	HOTAIR Median (IQR)	Correlation Coefficient (r/Z)	P value	Statistical Test
Menopause	Pre	2.33 (1.67–3.38)	-0.106	0.917	Mann-Whitney Z
	Post	3.49 (2.66–6.64)			
ER receptor	Negative	3.04 (2.47–3.57)	-2.016	0.043*	Mann-Whitney Z
	Positive	2.07 (1.66–3.19)			
PR receptor	Negative	2.88 (1.8–4.23)	-0.88	0.397	Mann-Whitney Z
	Positive	2.33 (1.72–3.38)			
HER2 receptor	Negative	2.27 (1.68–3.4)	-1.162	0.25	Mann-Whitney Z
	Positive	3.05 (2.35–3.71)			
Metastasis on follow up	Absent	2.29 (1.67–3.12)	2.955	0.002*	Mann-Whitney Z
	Present	4.46 (2.92–6.05)			
Age (year)			0.032	0.865	Spearman r
BMI (kg/m²)			-0.201	0.286	
Age at Menarche (year)			0.186	0.325	
Parity (n)			0.147	0.439	
Hemoglobin (g/dl)			-0.045	0.817	
WBCs (10^3/mm³)			0.119	0.53	
Platelet (10^3/mm³)			0.088	0.644	
Albumin (g/dl)			-0.101	0.596	
ALT (U/L)			-0.182	0.337	
AST (U/L)			-0.15	0.429	
Bilirubin (mg/dl)			0.035	0.856	
BUN (mg/dl)			-0.264	0.159	
Baseline CA 15-3			0.276	0.14	
CA 15-3 on follow up			0.324	0.081	
T Staging			0.506	<0.001**	
LN Staging			0.426	0.019*	
Metastasis			0.376	0.041*	
Staging			0.463	0.01*	
Grading			0.263	0.16	
HER2			0.216	0.252	
ER			-0.374	0.041*	
PR			-0.163	0.388	

PR: Progesterone receptor, **ER:** Estrogen receptor, **HER2:** Human epidermal growth factor receptor 2, **WBCs:** White blood cells, **ALT:** Alanine aminotransferase, **AST:** Aspartate aminotransferase, **BUN:** Blood urea nitrogen, **CA 15-3:** Cancer antigen 15-3, **LN:** Lymph node, **IQR:** Interquartile range, **Mann-Whitney Z:** Mann-Whitney U test statistic (Z), **r:** Spearman rank correlation

coefficient, **n:** Number, **g/dl:** grams per deciliter, **U/L:** units per liter, **mg/dl:** milligrams per deciliter, **kg/m²:** kilograms per square meter.

Among factors significantly correlated with circulating long non-coding RNA HOTAIR and disease-specific data among breast cancer patients, T staging (unstandardized β=1.014, p=0.004) and staging (unstandardized β=0.407, p=0.031) significantly independently associated with it (Table 5).

Table (5): Linear stepwise regression analysis of factors associated with circulating long non coding RNA HOTAIR level and disease-specific data among breast cancer patients

	Unstandardized Coefficients		Standardized Coefficients	t	P	95.0% Confidence Interval	
	B	Std. Error	Beta			Lower	Upper
(Constant)	-1.285	0.895		-1.435	0.163	-3.122	.552
T staging	1.014	0.317	0.481	3.196	0.004*	0.363	1.664
Staging	0.407	0.178	0.344	2.282	0.031*	0.041	0.772

Patients with breast cancer who had a bad prognosis had considerably greater levels of circulating HOTAIR compared to those with a good prognosis ($p < 0.001$), suggesting a strong correlation between raised HOTAIR and a negative result (Table 6).

Table (6): Relation between prognosis of breast cancer and circulating Long Non-Coding RNA HOTAIR

	Good prognosis (N=21)	Poor prognosis (N=9)	Z	P
	Median (IQR)	Median (IQR)		
HOTAIR	2.27(1.67 – 2.9)	3.95(2.95 – 5.73)	-3.281	<0.001**

Z: Mann Whitney test.

Breast cancer patients with a poor prognosis had significantly greater CA 15-3 levels at baseline and during follow-up than who had a good prognosis ($p = 0.019$ and $p < 0.001$ respectively). Moreover, CA 15-3 levels declined noticeably with time in patients who had a favorable prognosis, whereas they surged in patients who had a negative prognosis (both $p < 0.001$) (Table 7).

Table (7): Relation between prognosis of breast cancer and Ca 15-3 baseline and on follow up

	Good prognosis	Poor prognosis	Z	P
	Median (IQR)	Median (IQR)		
CA 15-3 baseline	50(25 – 81)	90(74.5 – 108.5)	-2.355	0.019*
CA 15-3 on follow up	31(21.5 – 34.5)	152(134.5 – 187.5)	-3.693	<0.001**
P (Wx)	<0.001**	<0.001**		

Z: Mann Whitney test, Wx: Wilcoxon signed rank test.

HOTAIR demonstrated strong diagnostic performance for predicting poor prognosis in breast cancer patients, with an AUC of 0.884, specificity of 76.2% and sensitivity of 88.9% at a cutoff of ≥ 2.81 ($p < 0.001$). CA 15-3 at a baseline cutoff of ≥ 68.5 also showed significant, but lower, accuracy (AUC 0.775, sensitivity 88.9% and specificity 66.7%, $p = 0.019$). The combination of HOTAIR and/or CA 15-3 yielded perfect sensitivity (100%) but lower specificity (47.6%), with an AUC of 1.000 ($p < 0.001$) (Table 8 and figure 3).

Table (8): Diagnostic Performance of HOTAIR, CA 15-3, and Their Combination for Poor Prognosis in Breast Cancer Patients

Marker/Combination	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
HOTAIR	≥2.81	0.884	88.9%	76.2%	61.5%	94.1%	80%	<0.001**
CA 15-3 (baseline)	≥68.5	0.775	88.9%	66.7%	53.3%	93.3%	73.3%	0.019*
Combined (HOTAIR ≥2.81 and/or CA 15-3 ≥68.5)		1.000	100%	47.6%	45%	100%	63.3%	<0.001**

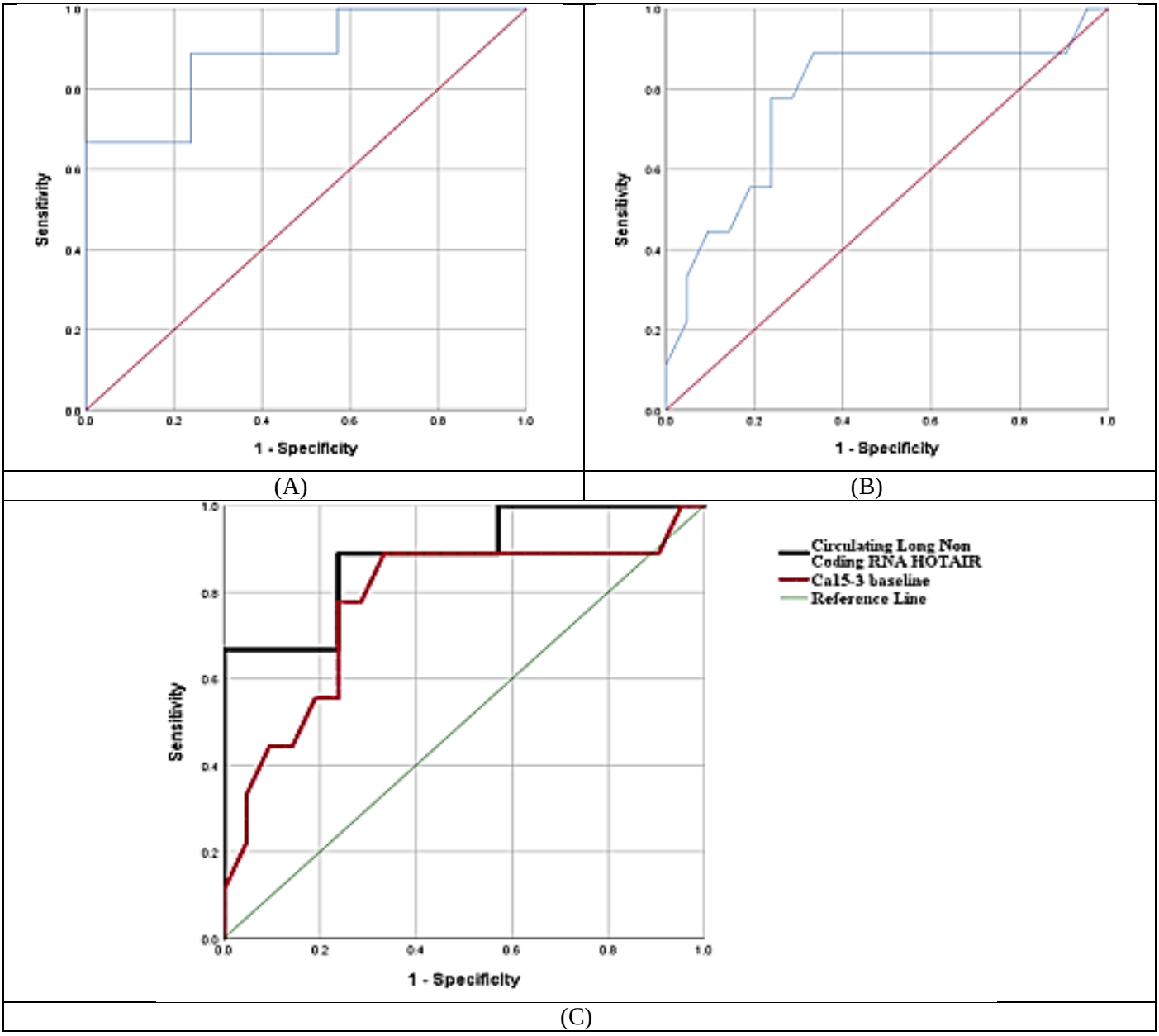

Figure (3): ROC curves showing: (A) Performance of HOTAIR in diagnosis of poor prognosis breast cancer (B) Performance of CA 15.-3 in diagnosing poor prognosis breast cancer (C) Performance of combined CA 15.-3 and HOTAIR in diagnosis of poor prognosis breast cancer.

Logistic regression analysis revealed that circulating lncRNA HOTAIR was a significant independent predictor of breast cancer, with an adjusted odds ratio of 12.8 (95% CI: 1.11–147, p = 0.041), while age, parity, and CA 15-3 were not significantly associated with breast cancer risk in both univariate and multivariate models (Table 9).

Table (9): Regression analysis of Breast cancer prediction

Parameters	Univariate		Multivariate	
	OR (95%CI)	p-value	AOR (95%CI)	p-value
Age	1.04 (0.94-1.14)	0.447	1.02 (0.91-1.15)	0.741
Parity	1.62 (0.77-3.42)	0.205	0.71 (0.04-11.3)	0.810
CA15-3	1.66 (0.16-17.3)	0.670	1.62 (0.6-4.4)	0.340
lncRNA HOTAIR	12.1 (1.19-123.6)	0.035*	12.8 (1.11-147)	0.041*

OR: Odds ratio, **CI:** Confidence interval, **AOR:** Adjusted OR.

DISCUSSION

Worldwide, breast cancer (BC) is still the most common cancer diagnosis and the top killer of female cancer patients [(1)], including Egypt [(2-3)]. The subtle and often nonspecific symptoms of early-stage BC frequently result in delayed diagnosis, contributing to poorer outcomes and limited treatment efficacy [(1-2)]. Standard imaging modalities such as mammography, ultrasonography, and MRI are routinely employed to detect breast lesions. However, mammography has only moderate sensitivity, particularly in younger women or those with dense breast tissue, which may hinder early detection. Similarly, breast ultrasonography is associated with a high false-negative rate, especially among women with dense breast tissue, while the high cost of MRI restricts its use for population-level screening [(4)].

Given these limitations, there is a clear need to identify novel biomarkers and achieve a deeper understanding of breast cancer biology. Recent advances highlight the critical role of molecular mechanisms in disease pathogenesis. Long non-coding RNAs (lncRNAs), which have emerged as key regulatory molecules, show dysregulation across a spectrum of cancers, including BC, and often exhibit a higher degree of tissue specificity than protein-coding transcripts [(4-5)]. Notably, HOX transcript antisense intergenic RNA (HOTAIR)—a polyadenylated lncRNA—has gained attention for its function in chromatin architecture modulation and transcriptional control [(5-6)]. Elevated HOTAIR levels have consistently been detected in both BC tissue specimens and cell lines [(7-8)].

This research was performed to determine whether plasma lncRNA HOTAIR can serve as a reliable biomarker in diagnosis and prognosis of breast cancer. We quantified HOTAIR gene expression and explored its relationship with clinical features and laboratory results, employing ROC analysis to assess its diagnostic as well as prognostic accuracy.

The study cohort comprised 60 individuals, categorized into three groups: 30 patients newly diagnosed with BC, 15 individuals with benign breast conditions, and 15 healthy volunteers. Our data revealed a highly significant disparity in serum CA15-3 concentrations across these groups (*p* < 0.001), aligning with earlier reports that link elevated CA15-3 with heightened BC risk [(9-12)]. Additionally, plasma HOTAIR concentrations were markedly increased in the BC cohort compared to both benign and healthy controls (*p* < 0.001), while no significant distinction was observed between the benign and control groups (*p* = 0.058). These findings parallel those of **El-Fattah *et al.*** [(13)] who demonstrated that serum HOTAIR levels are significantly raised in breast cancer patients relative to those with fibroadenoma or healthy status (*p* < 0.0001). Likewise, **Zhang *et al.*** [(8)] documented a pronounced HOTAIR upregulation in breast cancer tissue and plasma ($p < 0.05$). Also, **Arshi *et al.*** [(14)] confirmed increased HOTAIR expression in tumor tissue using qRT-PCR (*p* < 0.01). Moreover, exosomal HOTAIR originating from primary tumor tissue and released into circulation—has also been reported as significantly elevated in BC patients ($p < 0.001$) [(15)].

To evaluate the diagnostic potential of plasma HOTAIR, ROC curve analysis indicated strong discrimination between BC and non-cancer cases at a threshold exceeding 1.085, getting a sensitivity of 93.3%, specificity of 96.4%, and an AUC of 0.957. Consistently, **El-Fattah *et al.*** [(13)] found that serum HOTAIR could differentiate BC from controls (sensitivity 62%, specificity 64%, AUC 0.65), and **Zhang *et al.*** [(8)] also reported the ability of plasma HOTAIR to distinguish BC cases from healthy individuals (AUC 0.80; sensitivity 69.2%; specificity 93.3%), thus reinforcing its utility as a novel biomarker.

For CA15-3, ROC analysis revealed a cutoff of >24.5 that yielded specificity of 80%, a sensitivity of 83.3%, and an AUC of 0.911. Notably, the combined assessment of plasma HOTAIR with CA15-3 enhanced diagnostic sensitivity to 100% with specificity of 81.1%.

Multiple studies have pointed to the role of lncRNAs, and HOTAIR in particular, in breast tumorigenesis and their value as diagnostic candidates [(4, 16)]. Important tumor processes including proliferation, migration, invasion, apoptosis, and radiation response are regulated by the complex axis including HOTAIR, miRNAs, and mRNAs [(17)]. Enhanced HOTAIR expression has been linked to more aggressive tumor phenotypes and reduced sensitivity to radiotherapy in breast cancer cells [(18)], highlighting its contribution to disease progression.

On a mechanistic level, HOTAIR exerts its oncogenic influence through binding to polycomb repressive complex 2 (PRC2), facilitating H3K27 trimethylation at the promoter region of *WIF1*—a known Wnt pathway antagonist. This epigenetic silencing of *WIF1* leads to β-catenin pathway activation, which in turn drives tumor proliferation, invasion, and metastatic behavior [(6)]. Within our patient population, we identified significantly elevated circulating HOTAIR in estrogen receptor–negative BC cases (*p* = 0.043), whereas no notable association emerged with progesterone receptor (PR) or HER2 status. In contrast, **Shi *et al.*** [(17)] observed higher HOTAIR in HER2-positive BC (*p* = 0.006), which may reflect population-specific differences. **Zhang *et al.*** [(8)] also reported plasma HOTAIR correlations with

ER, HER2 expression, and lymph node metastases. No significant relationship was found between HOTAIR levels and demographic or laboratory markers in our cohort. We found that serum HOTAIR expression positively linked with tumor size ($p < 0.001$), lymph node involvement ($p= 0.019$), and metastasis ($p= 0.041$) when we examined clinicopathologic characteristics. This evidence supports the conclusions drawn by **Collina *et al.*** [7] and **Wang *et al.*** [20] who found that greater HOTAIR levels were associated with higher TNM stages and nodal metastases in breast cancer patients ($p< 0.05$). The strong diagnostic utility for HOTAIR in BC identified in our cohort echoes previous reports is consistent with **Sørensen *et al.*** [21] who demonstrated that high HOTAIR expression independently predicts metastatic risk in ER-positive breast cancer. While, other study have implicated HOTAIR overexpression in the development of resistance to tamoxifen therapy [22].

With regard to prognosis, we observed significantly greater HOTAIR expression among patients with poor prognostic features compared to those with more favorable outcomes ($*p* < 0.001$). Supporting this, **Tang *et al.*** [15] indicated that elevated exosomal HOTAIR serves as a potent indicator of adverse survival and therapeutic response in BC, independent of subtype. Mechanistic work by **He *et al.*** [23] elucidated that HOTAIR can drive proliferation and metastasis via the miR-130a-3p/Suv39H1 regulatory axis, reinforcing its emerging position as both a prognostic indicator and potential therapeutic target. Similarly, **Raju *et al.*** [24] reported an association between HOTAIR upregulation, aggressive tumor subtypes such as triple-negative breast cancer, and poorer clinical outcomes.

In our dataset, HOTAIR demonstrated prognostic accuracy with a sensitivity of 88.9%, specificity of 76.2%, and an AUC of 0.884. These findings are consistent with **Ma *et al.*** [25] who proposed that HOTAIR-targeted interventions may provide a promising avenue for impeding BC progression. **Xin *et al.*** [26] also emphasized HOTAIR's potential utility as a prognostic and therapeutic marker in multiple cancer types, including breast cancer.

ADVANTAGES

A key advantage of this study is the use of plasma HOTAIR measurement, offering a minimally invasive approach for assessing breast cancer risk and prognosis. Plasma-based assays are generally easier to perform and more acceptable to patients compared to tissue biopsies. Additionally, evaluating HOTAIR alongside conventional markers such as CA15-3 enhances diagnostic accuracy, potentially improving early detection and risk stratification. By establishing a strong link between plasma HOTAIR levels and tumor characteristics, the study provided valuable insight into the biomarker's clinical relevance and utility in patient management.

LIMITATIONS

But it is important to recognize that there were limitations of the current study which could affect generalizability due to the limited sample size. The case-cohort design is susceptible for selection bias, and the cross-sectional nature precludes evaluation of longitudinal changes or predictive value for long-term outcomes. Larger studies, ideally with a multicenter and longitudinal approach, are warranted for validation and confirmation of these findings and clarify the broader clinical applicability of plasma HOTAIR in breast cancer.

CONCLUSION

Plasma HOTAIR expression achieved a fine diagnostic accuracy in discriminating BC from benign breast conditions and healthy controls, also high plasma HOTAIR levels were associated with poor overall prognosis, suggesting that plasma HOTAIR may act as a novel diagnostic and prognostic biomarker for BC.

Author contribution: Azza Moustafa Ahmed conceptualized and designed the study, coordinated research activities, and drafted the manuscript. Nashwa Mohammad Alazizi and Hanan Samir Ahmed contributed to sample collection, laboratory analysis, and data acquisition. Hatem Mohammad Abd El-Monaem provided surgical expertise and assisted with clinical data interpretation. Basma Farag Mousa participated in data analysis, statistical evaluation, and manuscript revision.

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