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# Differential Expression of MALAT1 and HOTAIR lncRNAs in Early and Advanced Stage Breast Cancer Patients: Potential Prognostic Biomarkers

**Haider Ali [1,*], Tilekeeva UM [1,*], Dharmsheel Shrivastav [2], Masroor Ali Beg Mirza [3], Varun Kumar Sharma [2] and Amit Verma [4]**

[1] Department of Basic and Clinical Pharmacology, Kyrgyz State Medical Academy, Bishkek 720020, Kyrgyzstan

[2] Department of Biotechnology and Microbiology, Noida International University, Greater Noida 201003, India

[3] Faculty of Medicine, Alatoo International University, Bishkek 720048, Kyrgyzstan

[4] Division of Diagnostic Innovation, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104-6129, USA

[*] Correspondence: alicim01@gmail.com (H.A.); ulangul@mail.ru (T.U.)

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**Abstract:** Long non-coding RNAs (lncRNAs) have emerged as key regulators of gene expression involved in tumor initiation, progression, and metastasis. Among them, HOTAIR (HOX Transcript Antisense Intergenic RNA) and MALAT1 (Metastasis-Associated Lung Adenocarcinoma Transcript 1) have been implicated in several cancers, including breast cancer. However, their stage-specific expression patterns and prognostic relevance in breast cancer remain underexplored. This study aimed to evaluate the differential expression of HOTAIR and MALAT1 in early-stage (I–II) and late-stage (III–IV) breast cancers and to assess their association with clinicopathological and demographic parameters to determine their potential as prognostic biomarkers. Eighty breast cancer samples were analyzed for HOTAIR and MALAT1 expression using qRT-PCR. Clinicopathological data, including age, menopausal status, lymph node involvement, and hormone receptor status (ER, PR, HER2/neu), were collected. Logistic regression, ROC curve, and univariate analyses assessed their diagnostic and predictive significance. HOTAIR expression was significantly upregulated in late-stage (III–IV) breast cancer compared with early-stage (I–II) cases ($p < 0.05$), showing strong association with lymph node metastasis, postmenopausal status, and receptor negativity. ROC analysis demonstrated HOTAIR's predictive potential with an AUC of 0.73 (sensitivity: 64%; specificity: 86%). MALAT1 expression was non-significantly elevated in late-stage tumors. Logistic regression analysis we observed that overexpression of HOTAIR, lymph node involvement, and hormone receptor negativity as increases disease risk. The findings indicate that HOTAIR over expression serves as a potential prognostic biomarker for breast cancer at late stage and associated with clinicopathological features and disease progression.

**Keywords:** Breast Cancer; Long Non-Coding RNA; MALAT1; HOTAIR; Prognostic Markers

## 1. Introduction

Breast cancer remains one of the most prevalent and life-threatening malignancies affecting women worldwide, posing a significant global health challenge. It accounts for a substantial proportion of cancer-related morbidity and mortality and places a considerable burden on healthcare systems due to its high incidence, complex treatment

modalities, and psychosocial implications [1]. Breast cancer arises from the uncontrolled proliferation of atypical epithelial cells within breast tissue, leading to the development of malignant tumors [2]. it is a heterogeneous disease comprising multiple subtypes such as hormone receptor-positive, HER2-positive, and triple-negative breast cancers each with distinct etiological factors, genetic profiles, and therapeutic vulnerabilities [3]. Delayed diagnosis and inappropriate intervention, these tumors can invade surrounding tissues and metastasize to distant organs such as the lungs, liver, bones, and brain [4]. The classification of breast cancer is often based on histopathological and molecular characteristics that determine tumor behavior and treatment response. Early-stage tumors (Stages I–II) typically exhibit cellular features that closely resemble normal breast tissue and are associated with slower proliferation rates and better clinical outcomes. In contrast, late-stage tumors (Stages III–IV) are characterized by poorly differentiated cells, high mitotic activity, and an enhanced capacity for invasion and metastasis, reflecting an aggressive disease phenotype [5].

Studies suggested that breast cancer is often considered as a genetic disorder which occurs by genetic and epigenetic changes in oncogenes and tumor suppressor [6]. Additionally, dysregulated transcriptional and post-transcriptional mechanisms governed by non-coding RNAs (ncRNAs) may be responsible for breast cancer pathogenesis [7]. The long non-coding RNAs (lncRNAs) RNA transcripts more than 200 nucleotides that do not encode proteins have emerged as crucial regulators of gene expression. LncRNAs are involved in diverse cellular processes, including chromatin remodeling, transcriptional and post-transcriptional gene regulation, mRNA stability, and intracellular signaling pathways [8–11]. Their aberrant expression has been implicated in tumor initiation, progression, metastasis, and resistance to therapy across various cancer types.

Two of the most extensively studied lncRNAs in breast cancer are HOX Transcript Antisense RNA (HOTAIR) and Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1), both of which have been identified as key modulators of tumor aggressiveness and poor clinical outcomes [12–17]. HOTAIR, located on chromosome 12q13.13, is known to interact with chromatin-modifying complexes such as PRC2 and LSD1, thereby altering histone methylation patterns and repressing tumor suppressor genes. Elevated HOTAIR expression has been strongly correlated with increased invasiveness, metastasis, and unfavorable prognosis in breast cancer patients. Mechanistically, HOTAIR modulates several oncogenic signaling cascades, including the HIF1A, AP1, and FGFR pathways, and promotes epithelial-to-mesenchymal transition (EMT), a key process enabling cancer cells to acquire migratory and invasive properties [18].

Similarly, MALAT1 (also known as NEAT2) has garnered significant attention for its multifaceted role in breast cancer progression. Overexpression of MALAT1 enhances tumor cell proliferation, migration, and metastasis through the regulation of EMT and cytoskeletal dynamics [19]. Functionally, MALAT1 acts as a competing endogenous RNA (ceRNA) or "microRNA sponge," sequestering specific microRNAs that would otherwise suppress oncogenic transcripts. Under hypoxic conditions, MALAT1 expression is transcriptionally regulated by hypoxia-inducible factors (HIF-1α and HIF-2α), linking it to tumor adaptation in hypoxic microenvironments. Furthermore, MALAT1 influences angiogenesis, immune evasion, and resistance to chemotherapy, thereby representing a promising biomarker and potential therapeutic target in aggressive breast cancers [20,21]. Despite extensive research into their molecular mechanisms, the combined clinical relevance of HOTAIR and MALAT1 in distinguishing between early-stage (I–II) and late-stage (III–IV) breast tumors remains inadequately explored. The differential expression patterns of these lncRNAs across tumor grades may provide valuable insights into disease progression, metastatic potential, and patient prognosis. The present study aims to investigate the specific roles of HOTAIR and MALAT1 in differentiating early-stage from late-stage breast cancers and to evaluate their combined prognostic potential. By correlating their expression levels with clinicopathological parameters, this research seeks to bridge existing gaps in understanding their functional and clinical significance. Elucidating these molecular associations could contribute to the development of reliable prognostic biomarkers and novel therapeutic strategies tailored to breast cancer progression and metastasis.

## 2. Methodology

### 2.1. Study Design and Participants

This study was designed as a hospital-based analytical investigation to assess the expression of long non-coding RNAs (lncRNAs) MALAT1 and HOTAIR in breast cancer patients and to evaluate their potential as prognostic

biomarkers. A total of 80 newly diagnosed, histopathologically confirmed breast cancer patients were enrolled between January 2022 to July 2024. All participants were recruited from oncology departments affiliated with Alatoo International University, Bishkek, Kyrgyzstan, after obtaining informed written consent.

Patient classification was carried out based on the TNM staging system (Tumor–Node–Metastasis), in accordance with the American Joint Committee on Cancer (AJCC) guidelines [22]. The study included patients across all TNM stages (I–IV), representing a wide range of disease progression. Individuals with a prior history of malignancy, those undergoing chemotherapy or radiotherapy, and patients with metastases originating from non-breast primary cancers were excluded to avoid confounding effects. Demographic and clinicopathological information—including age, menopausal status, tumor grade, hormone receptor status (ER, PR, and HER2), and lymph node involvement was obtained from medical records and pathology reports.

## 2.2. Ethical Considerations

The research protocol was reviewed and approved by the Ethics Committee of Alatoo International University, Bishkek, Kyrgyzstan (Approval No. 69/k-22). All study procedures adhered to the principles of the Declaration of Helsinki (2013 revision). Each participant provided informed written consent prior to enrolment, after being informed about the study objectives, procedures, and confidentiality measures. Patient identities and clinical data were anonymized during analysis to maintain privacy.

## 2.3. Sample Collection and Processing

From each participant, 3 mL of peripheral venous blood was collected using sterile disposable syringes into plain vacutainer tubes. The samples were allowed to clot at room temperature for 20–30 min and then centrifuged at 1500 rpm for 5 min to separate the serum. The obtained serum was aliquoted into microcentrifuge tubes and stored at −80 °C until further molecular processing. All samples were handled using RNase-free conditions to prevent degradation of nucleic acids.

## 2.4. RNA Isolation and cDNA Synthesis

Total RNA was extracted from serum samples using the TRIzol reagent (Thermo Fisher Scientific, MA, USA; Catalog No. 15596018), following the manufacturer's protocol. The purity and concentration of isolated RNA were determined spectrophotometrically by measuring absorbance at 260 nm and 280 nm using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Samples with an A260/A280 ratio between 1.8 and 2.0 were considered of acceptable purity. To eliminate genomic DNA contamination, RNA samples were treated with DNase I prior to cDNA synthesis.

Complementary DNA (cDNA) was synthesized from 100 ng of total RNA using a commercial reverse transcription kit (Thermo Fisher Scientific, MA, USA; Catalog No. 18091200). The reverse transcription reaction was set up as follows: 1 µL of total RNA (100 ng), 2 µL of oligo (dT) primers, and 9 µL of nuclease-free water were incubated at 70 °C for 5 min, followed by cooling at 4 °C for 10 min to facilitate primer annealing. Subsequently, a reaction mixture containing 4 µL of 5X RT buffer, 2 µL of 5 mM dNTPs, 1 µL of RNase inhibitor, and 1 µL of reverse transcriptase enzyme was added to achieve a total volume of 20 µL. The reverse transcription was carried out at 42 °C for 60 min, and the reaction was terminated by heating at 95 °C for 5 min, followed by immediate cooling to 4 °C. The synthesized cDNA was stored at −20 °C for subsequent analysis.

## 2.5. Quantitative Real-Time PCR (qRT-PCR) Analysis

Quantitative real-time PCR (qRT-PCR) was performed using a Rotor-Gene Q system (Qiagen, Germany) with SYBR Green chemistry to quantify the expression levels of MALAT1 and HOTAIR lncRNAs. β-actin served as the internal control (housekeeping gene) to normalize gene expression levels. Each qPCR reaction was carried out in a 20 µL final volume containing 10 µL SYBR Green Master Mix, 1 µL cDNA template, 0.3 µL forward primer (25 pmol/µL), 0.3 µL reverse primer (25 pmol/µL), and 8.4 µL nuclease-free water.

The amplification conditions were as follows: an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s. A final extension step at 72 °C for 5 min was included. Non-template controls (NTCs) were included in each run to verify reaction

specificity and to detect possible contamination.

To ensure the specificity of amplification, a melting curve analysis was performed from 35 °C to 95 °C, and the presence of a single sharp peak confirmed the specificity of the PCR products. All reactions were performed in triplicate to ensure reproducibility. Primer sequences used for amplification are listed in **Supplementary Materials Table S1**. The amplification efficiency (E) for each target gene was determined using standard curves, with correlation coefficients ($R^2$) exceeding 0.98 indicating optimal performance.

The relative gene expression levels of MALAT1 and HOTAIR were calculated using the 2^−ΔΔCt method, where Ct represents the cycle threshold values obtained for both the target and reference genes. The mean Ct values from triplicate reactions were used for final analysis to minimize technical variability.

### 2.6. Statistical Analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Chicago, USA). Data were expressed as mean ± standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. The Shapiro–Wilk test was applied to assess the normality of data distribution. For normally distributed quantitative variables, comparisons between groups were made using the independent samples t-test, whereas the Mann–Whitney U test was employed for non-parametric data.

Associations between clinicopathological characteristics (such as age, tumor grade, hormone receptor status, and nodal involvement) and gene expression levels were examined using univariate logistic regression analysis to estimate odds ratios (ORs) with 95% confidence intervals (CIs). To evaluate the diagnostic and prognostic performance of MALAT1 and HOTAIR, Receiver Operating Characteristic (ROC) curve analysis was performed. The area under the curve (AUC) was calculated to determine sensitivity and specificity at optimal cutoff values. All experiments were performed in triplicate to ensure reproducibility, and $p$-values < 0.05 were considered statistically significant.

## 3. Results

### 3.1. Demographic and Clinical Characteristics

A total of 80 female patients diagnosed with breast cancer were included in the present study. Based on the TNM classification, patients were categorized into early-stage (I–II) and late-stage (III–IV) groups. Of the total, 15 patients (18.75%) were in the early stage, while 65 patients (81.25%) presented with late-stage disease, reflecting a predominance of advanced cases in the studied population.

The mean ± SD age of early-stage breast cancer patients was 46.33 ± 8.18 years (range: 37–58 years). Among them, 10 patients (66.7%) were below 50 years of age, while 5 (33.3%) were above 50 years. In contrast, the late-stage (III–IV) group had a mean age of 51.78 ± 6.97 years (range: 37–63 years), comprising 26 patients (40%) under 50 years and 39 (60%) aged 50 years or above. These findings indicate that breast cancer occurrence was more frequent in older age groups, especially in those presenting with advanced disease.

Menopausal status also showed a distinct pattern across stages. In the early-stage group, 9 of 15 patients (60%) were postmenopausal, whereas among late-stage patients, 51 of 65 (78.5%) were postmenopausal. Although this difference did not reach statistical significance ($p$ = 0.13), the trend suggests that menopause may be associated with disease progression or delayed diagnosis in some cases.

Lymph node involvement was observed in 38 of 65 (58.5%) late-stage patients compared to only 4 of 15 (26.7%) early-stage patients, representing a statistically significant association between nodal positivity and tumor stage ($p$ < 0.02). This strong correlation underscores lymph node metastasis as a key pathological hallmark of advanced breast cancer.

At the time of diagnosis, receptor status, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) was determined by a clinical pathologist using immunohistochemical (IHC) analysis. Among the 80 participants, 12 early-stage and 39 late-stage patients were ER-negative, whereas the remaining were ER-positive ($p$ = 0.14), indicating a slightly higher ER negativity in late-stage tumors.

A more pronounced pattern was seen with PR status. All 15 early-stage (I–II) tumors were PR-negative, and among late-stage patients, 41 of 65 (63%) were also PR-negative, suggesting a strong negative association between PR expression and tumor progression ($p$ < 0.005). Conversely, HER2 negativity was observed in 9 of 15 (60%) early-

stage and 37 of 65 (57%) late-stage patients ($p = 0.82$), indicating no significant relationship between HER2 status and stage in this cohort.

When stratified by metastatic status, all early-stage patients were non-metastatic, while among late-stage (III–IV) patients, 23 (35%) exhibited distant metastasis and 42 (65%) were non-metastatic. This difference was highly significant ($p < 0.001$), reflecting the expected clinical correlation between advanced tumor stage and metastasis.

A comprehensive summary of these demographic and clinicopathological characteristics is presented in **Table 1**, which highlights significant variations in tumor grade, receptor profile, and lymph node status between early and late stages of disease progression.

**Table 1.** Comparison of demographic characteristics between low- and high-grade breast cancer patients.

| Parameter | | Low Grade (N = 15) | High Grade (N = 65) | *p* Value |
|---|---|---|---|---|
| Age (in years) | | 46.33 ± 8.18 | 51.78 ± 6.97 | 0.01 |
| Age | <50 years<br>>50 years | 10<br>5 | 26<br>39 | 0.61 |
| Menopause (Yes/NO) | | 9/6 | 51/14 | 0.137 |
| Lymphnode (Yes/NO) | | 4/11 | 38/27 | 0.26 |
| Estrogen Receptor (Yes/NO) | | 3/12 | 26/39 | 0.14 |
| Progesterone Receptor (Yes/NO) | | 0/15 | 24/41 | 0.005 |
| Her2neu (Yes/NO) | | 6/9 | 28/37 | 0.82 |
| Metastasis status (Metastasis/non-metastasis) | | 15/0 | 23/42 | 0.006 |

## 3.2. Expression Analysis of lncRNAs HOTAIR and MALAT1

The relative expression levels of the long non-coding RNAs (lncRNAs) HOTAIR and MALAT1 were evaluated in all patient samples using quantitative real-time PCR (qRT-PCR), normalized against the housekeeping gene, with relative quantification determined using the 2^(−ΔCt) method.

The results demonstrated that HOTAIR was significantly upregulated in late-stage breast cancer compared to early-stage disease. The mean ± SD relative expression level of HOTAIR was 13.81 ± 7.22 in late-stage (III–IV) patients versus 8.71 ± 4.19 in early-stage (I–II) patients ($p < 0.01$). This marked increase in HOTAIR expression in advanced tumors supports its putative role as an oncogenic lncRNA associated with tumor aggressiveness, invasion, and metastatic potential. Similarly, the mean expression level of MALAT1 was found to be higher in late-stage patients (7.61 ± 4.12) than in early-stage patients (6.30 ± 2.02), although the difference did not reach statistical significance ($p = 0.624$). These findings suggest that, while MALAT1 may contribute to tumor biology, its expression pattern is less distinctly correlated with disease stage compared to HOTAIR. When expression levels were further analyzed within the late-stage cohort, a striking difference emerged between metastatic and non-metastatic cases. HOTAIR expression was significantly elevated in metastatic late-stage tumors (16.24 ± 6.36) compared to non-metastatic late-stage tumors (11.49 ± 6.87, $p < 0.01$), indicating that HOTAIR expression correlates not only with tumor stage but also with metastatic progression. In contrast, MALAT1 showed a non-significant trend toward higher expression in non-metastatic tumors (7.57 ± 3.97) compared to metastatic cases (6.8 ± 3.53, $p = 0.37$), suggesting that MALAT1 may have a context-dependent regulatory role. **Figure 1** illustrates the comparative expression of HOTAIR and MALAT1 across tumor stages, while **Figure 2** presents the relative expression levels of both lncRNAs in metastatic versus non-metastatic breast cancer. Collectively, these results underscore the potential diagnostic and prognostic utility of HOTAIR as a molecular marker of breast cancer aggressiveness.

## 3.3. Odds Ratio and Risk Estimation

To further assess the prognostic relevance of clinicopathological variables and lncRNA expression levels, univariate logistic regression analyses were performed. The analysis calculated odds ratios (ORs), 95% confidence intervals (CIs), and *p*-values for each variable to determine their association with breast cancer outcomes. Menopausal status yielded an OR of 2.33 ($p = 0.82$), indicating no significant association with disease outcome in this cohort.

However, lymph node positivity emerged as a strong predictor of poor prognosis, with an OR of 2.45 (95% CI: 1.21–4.98, $p = 0.012$). Similarly, hormone receptor status showed significant predictive power: ER negativity was associated with an OR of 8.66 (95% CI: 2.66–28.63, $p = 0.001$), and PR negativity exhibited an OR of 2.73 (95% CI: 1.51–4.93, $p = 0.001$). These findings are consistent with previous studies identifying hormone receptor-negative breast cancers as biologically more aggressive and clinically challenging to treat. For HER2/neu negativity, the OR was 4.11 (95% CI: 1.93–11.27, $p = 0.001$), suggesting that HER2-negative status is also linked to adverse outcomes in this population. Regarding molecular markers, HOTAIR expression showed a significant positive association with disease progression, with an OR of 1.146 (95% CI: 1.085–1.210, $p = 0.001$), implying that for each unit increase in HOTAIR expression, the risk of developing late-stage or metastatic disease increases by approximately 14.6%. In contrast, MALAT1 expression had an OR of 1.126 (95% CI: 0.924–1.373, $p = 0.240$), which was not statistically significant, further reinforcing the notion that HOTAIR is the more potent prognostic indicator among the two lncRNAs analyzed.

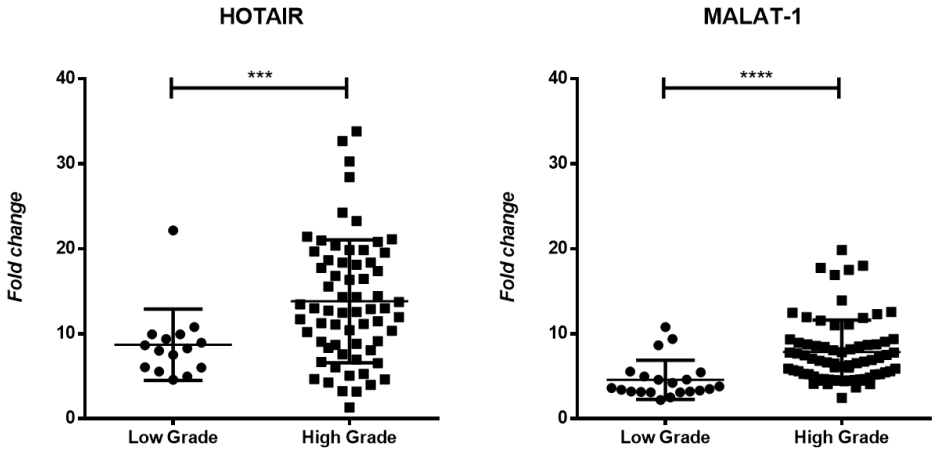


**Figure 1.** Comparison of HOTAIR and MALAT1 gene expression between low- and high-grade breast cancer patients. As shown, the expression levels of MALAT1 and HOTAIR were elevated in high-grade tumors compared to low-grade tumors.

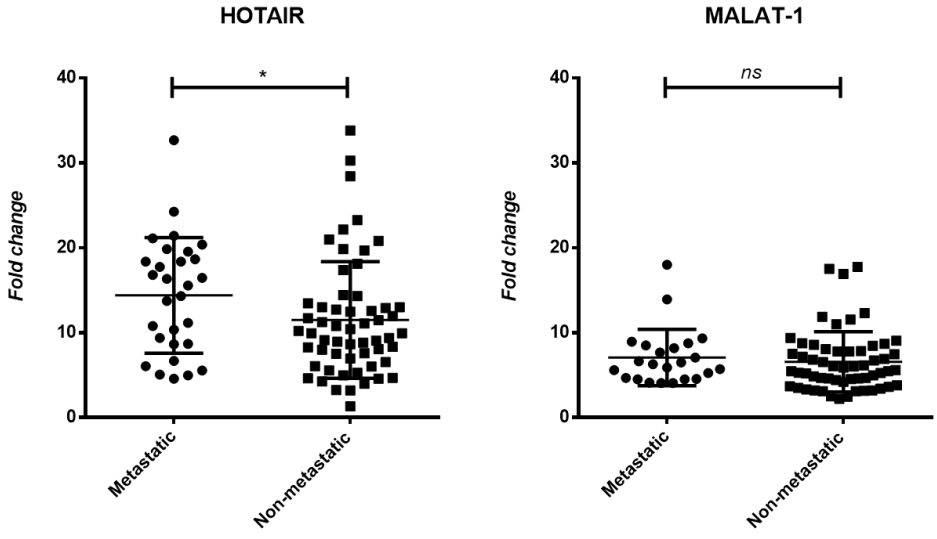


**Figure 2.** Comparison of MALAT1 and HOTAIR Gene Expression Between Metastatic and Non-Metastatic High-Grade Breast Cancer Patients. As shown, HOTAIR expression was elevated in high-grade metastatic breast cancer compared to high-grade non-metastatic breast cancer; however, MALAT1 expression was higher in high-grade non-metastatic cases compared to high-grade metastatic cases.

Logistic regression analysis (**Table 2**) identified several key variables menopausal status, lymph node involvement, absence of ER, PR, and HER2/neu expression, and elevated HOTAIR and MALAT1 levels as significant predictors of late-stage breast cancer.

**Table 2.** Outcome of univariate analysis evaluating the association between clinicopathological and demographic variables and the presence of high-grade breast cancer.

| S. No. | Parameter | Odd Ratio (95% CI) | *p* Value |
|---|---|---|---|
| 1. | Menopause (YES) | 2.33 (0.89–6.07) | 0.82 |
| 2. | Lymph node (YES) | 2.45 (1.21–4.98) | 0.012 |
| 3. | Estrogen Receptor expression (NO) | 8.66 (2.66–28.63) | 0.001 |
| 4. | Progesterone Receptor expression (NO) | 2.733 (1.51–4.93) | 0.001 |
| 5. | Her2/neu expression (NO) | 4.111 (1.93–11.27) | 0.001 |
| 6. | HOTAIR | 1.146 (1.085–1.210) | 0.001 |
| 7. | MALAT1 | 1.126 (0.924–1.373) | 0.240 |

### 3.4. Prognostic Significance of lncRNAs HOTAIR and MALAT1

To determine the prognostic potential of HOTAIR and MALAT1 expression levels in predicting breast cancer stage and progression, Receiver Operating Characteristic (ROC) curve analyses were performed. As shown in **Figure 3a**, the ROC curve for HOTAIR revealed an Area Under the Curve (AUC) value of 0.73 (95% CI: 0.69–0.86, $p < 0.001$), indicating good discriminatory ability between early- and late-stage tumors. The optimal cutoff value for HOTAIR expression was 10.59, which provided a sensitivity of 64% and a specificity of 86% for predicting advanced-stage disease. These results strongly support HOTAIR's potential as a reliable prognostic biomarker for breast cancer progression. In contrast, as illustrated in **Figure 3b**, the ROC curve for MALAT1 yielded an AUC of 0.55 (95% CI: 0.41–0.70), with a sensitivity and specificity of approximately 60% each. The lack of statistical significance suggests that MALAT1 alone may have limited predictive utility in distinguishing between early and late disease stages. Overall, these findings suggest that elevated HOTAIR expression is significantly associated with tumor advancement, lymph node metastasis, and hormone receptor negativity, making it a promising molecular marker for aggressive breast cancer phenotypes. While MALAT1 may play a role in tumor biology, its prognostic value appears less robust in this clinical context. In summary, the present study demonstrates that clinicopathological parameters, including lymph node involvement, hormone receptor status, and metastatic spread are closely linked with breast cancer stage. Among the molecular markers investigated, lncRNA HOTAIR showed a strong and statistically significant correlation with disease progression, while MALAT1 exhibited a weaker and non-significant trend. The combination of HOTAIR expression profiling with conventional pathological features may therefore improve the accuracy of prognostic assessment and facilitate early identification of high-risk breast cancer patients.

## 4. Discussion

Non-coding RNAs (ncRNAs) have emerged as pivotal regulators in the development and progression of breast cancer, influencing gene expression at transcriptional, post-transcriptional, and epigenetic levels. Among these, long non-coding RNAs (lncRNAs) such as HOTAIR and MALAT1 have gained significant attention due to their multifaceted roles in oncogenesis, metastasis, and treatment resistance [23]. The present study aimed to elucidate the expression patterns of HOTAIR and MALAT1 in low-stage (I–II) and late-stage (III–IV) breast cancers, and to explore their association with clinicopathological features, disease progression, and potential as diagnostic or prognostic biomarkers.

Our findings demonstrated that both HOTAIR and MALAT1 were differentially expressed between early and late stages of breast cancer, with significantly higher expression observed in late-stage (III–IV) tumors. These results underscore their potential contribution to tumor aggressiveness and disease advancement, aligning with the growing body of literature that implicates lncRNAs in breast cancer pathobiology and clinical outcomes. Analysis of clinicopathological variables (**Table 1**) revealed that late-stage breast cancer was more prevalent among older patients, particularly those aged above 50 years. This age association is consistent with global cancer epidemiology. Abba et al. in 2021 projected that cancer incidence among individuals aged over 65 years will reach nearly 60% by

2035, reflecting the strong correlation between aging and cancer development [24]. Similarly, Zhang et al. in 2020 emphasized that breast cancer is predominantly an age-related disease, with incidence rates increasing sharply from midlife due to cumulative genetic damage, hormonal changes, and prolonged exposure to environmental risk factors [25].

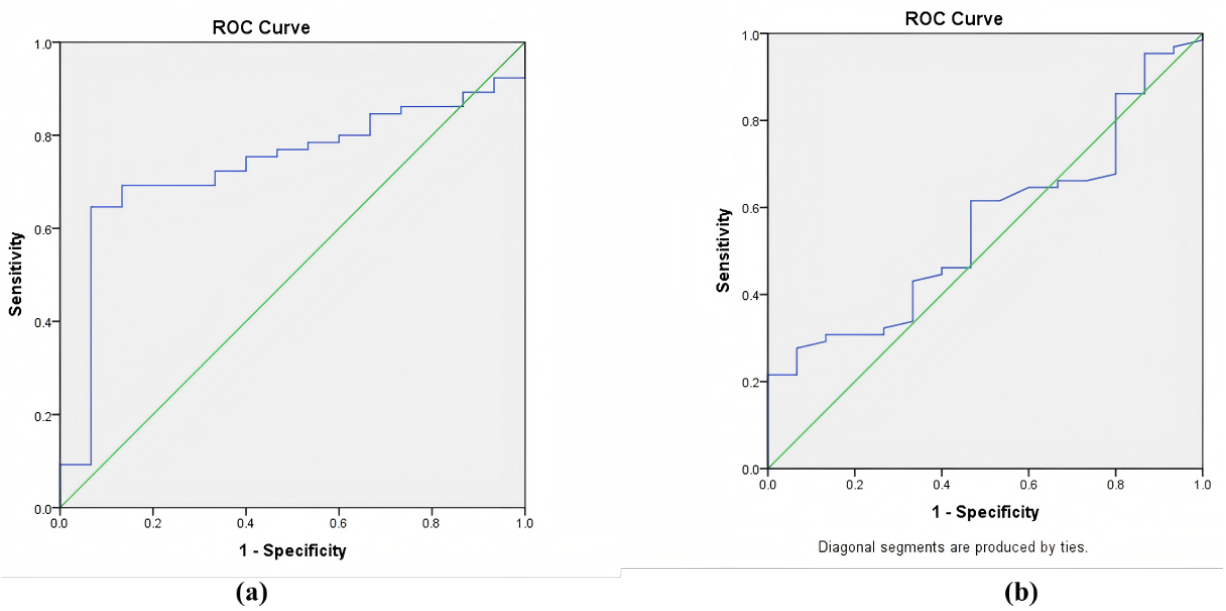


**Figure 3.** Receiver Operating Characteristic (ROC) curves for prognostic markers in high-grade breast cancer: (**a**) HOTAIR and (**b**) MALAT1.

We also observed that postmenopausal status was more common among patients with late-stage disease, indicating a potential hormonal influence on tumor aggressiveness and progression. Menopausal hormonal alterations, particularly reduced estrogen and progesterone levels—are known to reshape the tumor microenvironment and may promote the emergence of more aggressive subtypes, such as triple-negative breast cancer (TNBC). Previous studies have demonstrated that menopausal status influences the expression of hormone receptors and HER2/neu, thereby impacting disease progression and therapeutic response. These hormonal shifts may contribute to increased tumor heterogeneity and resistance to endocrine therapies in postmenopausal women [26].

Furthermore, our study identified a significant increase in lymph node involvement among late-stage breast cancer patients compared to those with early-stage disease. This observation suggests a strong link between tumor grade and lymphatic metastasis. Recent advances in molecular imaging, such as near-infrared (NIR-IIb) lymphatic imaging and deep learning-assisted diagnostic models, have further confirmed the prognostic value of lymph node status in predicting disease aggressiveness and guiding personalized treatment decisions [27]. Thus, our findings corroborate existing evidence that lymph node metastasis remains a key determinant of poor prognosis and advanced disease staging.

Our gene expression analysis (**Figure 1**) revealed a significant upregulation of HOTAIR in late-stage (III–IV) breast cancer tissues compared to early-stage tumors. This observation aligns with previous studies demonstrating that HOTAIR expression correlates strongly with tumor size, lymph node metastasis, poor histological differentiation, and decreased overall survival. Gupta 2010 first reported that HOTAIR reprograms chromatin organization by interacting with the Polycomb Repressive Complex 2 (PRC2), leading to epigenetic silencing of tumor suppressor genes such as PTEN and BRCA1. This interaction enhances cancer cell invasiveness and metastasis, establishing HOTAIR as a key driver of breast cancer progression [28].

Mechanistically, HOTAIR acts as a competing endogenous RNA (ceRNA), sequestering tumor-suppressive microRNAs such as miR-129-5p, miR-34a, and miR-7, thereby relieving repression of oncogenic targets including ZIC2, CD24, and IYD [29]. This results in enhanced proliferation, epithelial–mesenchymal transition (EMT), and invasion. Furthermore, HOTAIR is implicated in therapy resistance, including decreased radiosensitivity and chemoresis-

tance, through modulation of DNA repair pathways and drug efflux mechanisms.

Consistent with our findings, several clinical studies have demonstrated that high HOTAIR expression predicts poor disease-free and overall survival, suggesting its potential as a diagnostic and prognostic biomarker. For example, Solak et al. in 2015 showed that serum HOTAIR levels could effectively distinguish between early and late-stage breast cancers, underscoring its value in non-invasive disease monitoring [30]. Collectively, these findings suggest that HOTAIR not only promotes tumor aggressiveness but could also serve as a therapeutic target for novel anti-metastatic strategies. Our results also indicated elevated expression of MALAT1 in late-stage breast cancer, suggesting its involvement in tumor progression and metastasis. However, literature reports on MALAT1's role remain somewhat context-dependent and heterogeneous. Some studies, such as those by Saltzman et al. in 2012, have associated MALAT1 overexpression with advanced stage, larger tumor size, and poor prognosis, while others propose a possible tumor-suppressive role under specific genetic or microenvironmental conditions [31]. Functionally, MALAT1 is known to regulate alternative splicing, mRNA stability, and gene transcription by interacting with serine/arginine-rich splicing factors (SR proteins) and transcriptional regulators. It modulates pathways central to cell cycle progression, EMT, angiogenesis, and immune evasion, particularly through PI3K/AKT and Wnt/β-catenin signaling cascades [32]. Elevated MALAT1 has also been linked to chemoresistance and immune checkpoint modulation, influencing the efficacy of targeted therapies [33]. Interestingly, while MALAT1 was upregulated in our late-stage cohort, its predictive value for disease stage was not statistically significant in logistic regression analysis. This may reflect MALAT1's dual functionality acting as either an oncogene or tumor suppressor depending on tumor subtype, cellular context, and post-transcriptional modifications. Nonetheless, meta-analyses have consistently shown that high MALAT1 expression correlates with poor overall and disease-free survival. Thus, further research is warranted to delineate MALAT1's grade-specific roles and its potential utility as a co-biomarker alongside HOTAIR in breast cancer prognosis [34].

The association between menopause and advanced disease reflects the complex interplay between hormonal milieu and tumor biology. Although menopause itself does not directly cause breast cancer, factors such as age at natural menopause, use of hormone replacement therapy (HRT), body mass index, and circulating sex hormone levels in postmenopausal women influence disease risk and progression. These variables may contribute to the hormonal dependency of certain tumor subtypes and influence treatment outcomes [35,36].

Consistent with prior studies, lymph node involvement emerged as a strong independent risk factor. Shi et al. in 2021 reported that the lymph node ratio (LNR) the proportion of positive nodes to total nodes examined provides superior prognostic information compared to conventional nodal staging. Particularly in triple-negative breast cancer (TNBC), LNR was identified as a robust predictor of survival, underscoring the importance of nodal assessment in disease management [37]. Our analysis also indicated that absence of ER, PR, and HER2/neu expression markedly increased the likelihood of late-stage disease. Tumors lacking these receptors, categorized as TNBC, comprise 15–20% of breast cancers and are characterized by rapid progression, early metastasis, and limited treatment options. Although TNBCs respond relatively well to chemotherapy, their molecular heterogeneity has spurred extensive research into novel targeted approaches, including PARP inhibitors, VEGFR/EGFR antagonists, and immune checkpoint inhibitors [38]. At the molecular level, both HOTAIR and MALAT1 exert profound influence on epigenetic regulation, chromatin remodeling, and signaling transduction. HOTAIR interacts with PRC2 components (EZH2, SUZ12, and EED), leading to trimethylation of histone H3 lysine 27 (H3K27me3) and subsequent silencing of tumor suppressor genes [39]. This epigenetic reprogramming facilitates cell motility, invasion, and metastasis. Additionally, HOTAIR has been implicated in angiogenesis, stemness, and immune evasion, contributing to treatment resistance and poor prognosis [33]. Conversely, MALAT1 modulates alternative splicing of pre-mRNAs by regulating SR protein phosphorylation, thereby influencing the expression of genes associated with EMT and cell proliferation [40]. Through its interaction with the PI3K/AKT and Wnt/β-catenin pathways, MALAT1 supports angiogenesis and apoptosis resistance. Both lncRNAs are involved in cross-talk with key transcription factors, such as STAT3 and NF-κB, thereby amplifying oncogenic signaling cascades [41]. Recent evidence also highlights the cooperative effects of HOTAIR and MALAT1 in cancer progression. Their simultaneous overexpression has been observed in several malignancies, suggesting potential synergistic regulation of metastatic and drug-resistance pathways. In breast cancer, this combined deregulation may potentiate EMT and enhance metastatic competence, as supported by studies in lung and gastric cancers [42].

Our study reinforces the potential of HOTAIR as a robust prognostic biomarker for late-stage breast cancer.

Its high expression levels were significantly associated with advanced disease, supporting its utility in stratifying patients at higher risk for metastasis or recurrence. The integration of HOTAIR expression profiling into routine diagnostic workflows, potentially through liquid biopsy-based assays could enhance early detection of aggressive disease phenotypes and guide personalized therapy [43]. Studies suggested that HOTAIR levels are strongly associated to advanced disease stages and poor prognosis, particularly in estrogen receptor-positive breast cancers, where it interacts with estrogen receptor signaling to regulate gene expression and cancer cell proliferation. Conversely, microRNA-130a functions as a tumor suppressor in breast cancer by inhibiting cancer cell proliferation, invasion, and migration. It negatively regulates targets like RAB5A, which is upregulated in breast cancer and promotes metastasis. Lower miR-130a levels are associated with advanced stage and metastatic breast cancer, reflecting its role in disease suppression. Studies indicate a complex interplay between HOTAIR and miR-130a, where HOTAIR may downregulate miR-130a, contributing to tumor aggressiveness. This functional antagonism suggests that HOTAIR enhances breast cancer progression partly by suppressing tumor-suppressive microRNAs like miR-130a. Although MALAT1 did not exhibit independent predictive power in our cohort, its biological significance remains noteworthy. Its regulation of gene networks involved in cell migration, proliferation, and immune modulation positions it as a complementary biomarker, particularly in hormone receptor–positive cancers [44]. The apparent discrepancy in MALAT1's prognostic value across studies underscores the need for multi-omics approaches and larger population-based analyses to clarify its context-specific role [45]. Genetic variation in lncRNA loci also contributes to interindividual differences in cancer susceptibility. For instance, in a Southeast Iranian population, specific HOTAIR polymorphisms (rs920778 T>C) were significantly associated with increased breast cancer risk, while other variants such as rs1899663 G>T and rs12826786 T>C conferred a protective effect [46]. Together, these molecules hold promise as non-invasive biomarkers for breast cancer diagnosis, staging, and prognostication, and they represent potential therapeutic targets to inhibit tumor growth and metastasis by restoring microRNA function or silencing oncogenic lncRNAs like HOTAIR [47].

These findings suggest that both HOTAIR genetic variants and expression profiles could be harnessed to refine risk prediction models. These observations underscore the potential of lncRNAs as emerging biomarkers in precision oncology and highlight the need for larger, multi-center studies to validate their prognostic and therapeutic relevance in diverse breast cancer subtypes.

## 5. Limitation

Despite its valuable insights, this study has several limitations. The relatively small sample size, particularly in early-stage cases, may limit statistical power and generalizability. Additionally, the single-center observational design restricts the applicability of findings across diverse populations. The study also lacks functional validation experiments (e.g., knockdown or overexpression assays) that could confirm the mechanistic roles of HOTAIR and MALAT1 in breast cancer progression. Future multicentric studies with larger cohorts and experimental validation are warranted to substantiate these findings.

## 6. Conclusions

This study highlights the pivotal role of lncRNAs, particularly HOTAIR and MALAT1, in breast cancer progression and stage differentiation. Among the 80 histopathologically confirmed breast cancer patients analyzed, HOTAIR expression was significantly elevated in late-stage (III–IV) compared to early-stage (I–II) disease, demonstrating strong predictive value for tumor aggressiveness, metastasis, and poor prognosis. Receiver Operating Characteristic (ROC) analysis further supported HOTAIR's diagnostic accuracy (AUC = 0.73, sensitivity = 64%, specificity = 86%), indicating its potential as a non-invasive biomarker for disease staging and risk stratification. In contrast, although MALAT1 expression was higher in late-stage patients, its association with disease stage was not statistically significant, suggesting a more context-dependent role in breast tumor biology.

Clinicopathological correlations revealed that late-stage breast cancer was more prevalent among older and postmenopausal women, often accompanied by lymph node involvement and hormone receptor negativity (ER, PR, HER2/neu) factors strongly linked with aggressive tumor behavior. Logistic regression confirmed that elevated HOTAIR expression, lymph node metastasis, and receptor negativity were independent predictors of advanced disease. Mechanistically, HOTAIR is implicated in epigenetic reprogramming, EMT induction, and chromatin remodeling via

interaction with PRC2, contributing to enhanced invasion and metastasis. These findings underscore HOTAIR's dual potential as a prognostic biomarker and therapeutic target. In summary, this study identifies HOTAIR as a robust molecular marker distinguishing late-stage breast cancer, offering valuable insights into its biological and clinical significance. Further multicentric and functional studies are warranted to validate its prognostic utility and explore its integration into precision oncology frameworks.

## Supplementary Materials

The supporting information can be downloaded at https://ojs.ukscip.com/files/TI-1521-Supplementary-Materials.pdf.

## Author Contributions

Conceptualization, H.A., T.U. and M.A.B.M.; methodology, H.A., T.U. and M.A.B.M.; software, M.A.B.M., D.S., V.K.S. and A.V.; validation, T.U., M.A.B.M., D.S., V.K.S. and A.V.; formal analysis, H.A., T.U., M.A.B.M., D.S. and V.K.S.; investigation, H.A. and T.U.; resources, M.A.B.M., D.S. and V.K.S.; data curation, H.A., T.U., A.V. and M.A.B.M.; writing—original draft preparation, H.A., M.A.B.M., D.S. and A.V.; writing—review and editing, H.A., T.U., M.A.B.M., D.S., V.K.S. and A.V.; visualization, M.A.B.M.; supervision, T.U.; project administration, H.A. and T.U.; funding acquisition, H.A. and T.U. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

Present study was ethically approved by Institutional ethics board (Approval No. 69/k-22).

## Informed Consent Statement

Informed consent was obtained from all subjects.

## Data Availability Statement

Data could be provided on reasonable request from corresponding author if needed.

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## Conflicts of Interest

The authors declare no conflict of interest.

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