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ORIGINAL ARTICLE

Circulating Long-Non Coding RNA RP11-445H22.4 as a Diagnostic and Prognostic Biomarker of Breast Cancer

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ABSTRACT

Background: The most frequent malignancy in women is breast cancer (BC). Biomarkers, like carbohydrate antigen (CA15-3) as well as carcino-embryonic antigen (CEA), remain of limited clinical value. Novel biomarkers are needed for early diagnosis. This study aimed at evaluating the role of circulating long non-coding RNA RP11-445H22.4 as a diagnostic marker of breast cancer and to assess its relation with prognostic characteristics of the disease. **Methods:** It was a case-control study conducted in Clinical Pathology and General Surgery Departments, Faculty of Human Medicine, Zagazig University. It involved 108 adult females: 36 females as healthy control, 36 newly diagnosed females with BC, and 36 females with benign breast lesions. Patients were diagnosed by mammography and biopsy. LncRNA RP11-445H22.4 was assessed in plasma by quantitative real-time polymerase chain reaction. **Results:** lncRNA RP11-445H22.4 was elevated among BC cases compared to controls as well as cases of benign breast lesions. The lncRNA RP11-445H22.4 revealed 95.9% sensitivity and 91.8% specificity for discriminating breast cancer vs. controls and benign breast lesions. CA15-3 had 77.8% sensitivity and 80.7% specificity. lncRNA RP11-445H22.4 showed 81.8% specificity versus 75.9% specificity for CA15-3 differentiating TNM stages; I and II vs. III and IV. **Conclusions:** LncRNA RP11-445H22.4 was up-regulated in breast cancer cases compared to controls and cases with benign breast lesions, it exhibited greater sensitivity and specificity compared to traditional biomarkers. Level of LncRNA RP11-445H22.4 was related to ER, PR and Her2/neu in BC patients. Consequently, it could serve as a potential biomarker for breast cancer diagnosis and prognosis.

Keywords: Breast cancer; biomarker; Long non coding RNA RP11-445H22.4

INTRODUCTION

Breast cancer (BC) is the most frequent malignancy among women around the globe, with newly diagnosed cases beyond two million in 2020. Additionally, it is the most important reason of cancer mortality in women, with deaths over 680,000 [1]. Although there is a variation in the frequency of BC among developed and developing countries [1, 2], BC is still the most frequent form of woman cancer among Egyptian females [3]. About 46,000 cases are predicted in 2050 [4]. Breast cancer is always silent. The majority of patients know about their disease through routine screenings. BC is diagnosed through physical examination, imaging, particularly mammography, and tissue biopsy [5]. Patients with breast cancer in Egypt are often diagnosed earlier at a young age, present with late stages, and have more aggressive biological subtypes when compared to patients in developed countries [6]. This indicates the necessity for new biomarkers to enable earlier detection of the disease, ultimately leading to improved survival outcomes.

The screening for breast cancer does not have biomarkers that offer high sensitivity and specificity. [7]. Serum biomarkers, like the

carbohydrate antigen (CA15-3) and carcino-embryonic antigen (CEA), are of limited clinical value. [8]. Consequently , it is crucial to identify new biomarkers .The levels of circulating lncsRNAs in patients with cancer have been proposed as potential noninvasive biomarkers[7]. Long non coding (lncsRNAs) are non-coding RNA that exceed two hundred nucleotides in length, found within eukaryotic cells and conserved among various mammalian species [9]. LncRNAs play essential roles in numerous biological processes, like the inflammatory response [10] and the invasion of cancer cells [11].

LncRNA RP11-445H22.4 gene is found on human chromosome 20 measuring 862 bp in length [12]. The lncRNA RP11-445H22.4 has a vital function in biological processes and its abnormal expression may be connected to different types of malignancies [13]. Moreover, osteoarthritis cartilage cells have been shown to express the LncRNA RP11-445H22.4 at high levels, which may be associated with osteoarthritis pathogenesis [14]. lncRNA RP11- 445H22.4 shows elevated expression within cells of BC, also associates with the inflammatory response triggered by tumor [12]. Consequently, this work aimed at evaluating the role of circulating long non-coding RNA RP11-445H22.4 (lncRNA RP11- 445H22.4) as a diagnostic marker of breast cancer and to assess its relation with the prognostic characteristics of the disease.

METHODS

This is a case-control study, conducted on 108 adult females classified into three age-matched groups: 36 females who served as a healthy control group, 36 newly diagnosed females with BC, and 36 females with benign breast lesions. Patients were recruited from the General Surgery Department, Zagazig University Hospitals, Faculty of Human Medicine, in the period from April 2022 to November 2023.

Informed written consents were collected from all participants after explaining the purpose of collecting their venous blood and gathering clinical data in this research. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research. The Institutional Review Board (IRB) approved this study (IRB No # ٥٥١١-29-7-2019).

Patients were diagnosed by radiological investigations and histological examination of biopsy samples. Newly diagnosed patients with BC were included in this study once their enrollment was approved. Individuals who underwent targeted therapy, chemotherapy, or radiation therapy prior to sample collection were not included in the study. Additionally, patients with other malignancies were excluded.

Every patient underwent a thorough history-taking, clinical examination, and radiological investigations (US and mammography). Staging of tumor was done according to the American Joint Committee on Cancer (AJCC) into; tumor, node, and metastasis (TNM) staging and immunophenotyping was done for estrogen receptor (ER), progesterone receptor (PR) as well as human epidermal growth factor receptor-2 (HER2/neu) [15].

Specimen collection:

Approximately four ml of venous blood were aseptically collected from all subjects by venipuncture and allocated as follows: two ml were delivered into a sterile tube containing gel separator for CA15-3 determination by electro-chemoluminescent automated analyzer "Roche Cobas 8000-e602" (Roche Diagnostics, Germany), and two ml were delivered into an ethylene diamine tetraacetic acid (EDTA) vaccutainer for extraction of lncRNA RP11-445H22.4. Samples were processed within an hour from collection.

Extraction of RNA:

Total RNA was extracted from fresh plasma samples by (GENEzol™ Reagent, Genaid, Taiwan), (catalogue number: GZR100). Reverse transcription reaction was done using TOPscript™ RT (Enzynomics, Korea: catalogue number: RT 220).

Quantitative Real Time PCR:

qPCR SYBR® Green PCR Master Mix with low ROX (Enzynomics Inc., Korea) was used to amplify cDNA. Real-time PCR was done on "Applied Biosystems, USA" using the subsequent cycling conditions; 15 minutes at 95°C for the initial activation step, then 40 cycles, with every single cycle consisted of; 10 seconds at 94 °C for denaturation, 15 seconds at 60 °C for annealing, and 30 seconds at 72°C for extension**.**

- Primers for LncRNA RP11-445H22.4 were: Forward primer 5′-GTAAAGCCATCACCAGGACAACC-3′ and reverse primer 5′-CTCCCTAACAGAAGCCCACCA-3′.
- Primers for GADPH were: Forward primer 5′-CACCAGGGCTGCTTTTAACTC-3′ and reverse primer 5′- GACAAGCTTCCCGTTCTCAG-3′ (Invetrogen by Thermo Fisher Scientific, USA).

Fold changes of lncRNA RP11-445H22.4 expressions were calculated using $2^{-\Delta\Delta CT}$ method [16].

STATISTICAL ANALYSIS

Microsoft Office Excel 2010 and the Statistical Package for Social Sciences, version 20, were used to tabulate and statistically analyze the data (SPPS: An IBM Company). Quantitative variables were described using the median and range, or means and standard deviations. The qualitative data, which was presented as frequencies and percentages, was compared using the chi-square test ($\chi 2$). To compare two means, the independent sample (t) test or the Mann-Whitney test (MW) were used if appropriate. When comparing more than two groups, the Kruskal Wallis test (KW) or analysis of variance ANOVA test (F) was utilized. The studied parameters were evaluated using the receiver operating characteristic (ROC) curve analysis. P-values less than 0.05 were deemed statistically significant.

RESULTS

-Participant's characteristics

The general features of studied groups are displayed in (Table 1). There were non-significant differences among studied groups as regard BMI, marital state, menarche age, or number of parity.

-Level of CA 15-3:

CA 15-3 was significantly increased in BC cases when compared to healthy controls and patients with benign breast diseases. Besides, it was significantly higher in benign group than control (Table 2, Figure 1).

-LncRNA RP11-445H22.4 expression in studied groups:

As regard lncRNA RP 11-445H22.4, it was significantly higher in BC cases compared to controls and cases of benign breast lesions; moreover, there were no significant difference between controls and benign cases (Table 2, Figure 1).

-Relation between pathological features and level of both lncRNA RP11- 445H22.4 and CA15-3 in BC cases

The expression of lncRNA RP11-445H22.4 was higher in BC cases who had stages III/IV or tumor grades II and III, as well as those with positive ER/PR and negative HER-2/neu expression. Furthermore, BC patients who had TNM stages III/IV or tumor grades II/III, as well as positive ER/PR expression exhibited a statistically significant increase of CA 15-3 level (Table 3).

-Diagnostic and prognostic potentials of circulating lncRNA RP11- 445H22.4 for BC patients

CA15-3 displayed 77.8% sensitivity and 80.7%, specificity, for the prediction of BC. However, lncRNA RP11-445H22.4 had 95.9% sensitivity, 91.8% specificity and 0.993 area under curve (AUC) (Table 4, Figure 2).

When lncRNA RP11-445H22.4 was combined with CA15-3, the sensitivity was 94.3% for prediction of BC with 86.4% specificity and this combination showed better performance than CA15-3 alone (Table 4).

LncRNA RP11-445H22.4 had 71.4% sensitivity and 81.8% specificity when differentiating between TNM stages (I/II) vs. (III/IV); however, CA15-3 revealed 71.4% sensitivity and 75.9% specificity (Table 5).

Table (1): General characteristics of studied groups.

	Studied groups			P
	Control (n=36)	**BC (n=36)**	**BBL (n=36)**	
Age (years) Mean ± SD	46.37±11	44.36±7.69	47.44 ± 10.84	0.415
Body mass index (BMI) Mean ± SD	28.44±2.98	27.36±3.41	28.03± 3.30	0.340
Menarche age (years) Mean ± SD	12.11±0.79	12.31±1.04	12.03± 0.81	0396
Marital state [number (%)]				
Single	8 (22.2%)	11 (30.6%)	13(36.1%)	0.430
Married	28 (77.8%)	25 (69.4%)	23(63.9%)	
n. of parity [number (%)]				
No	11(30.6%)	10 (27.8%)	13 (36.1%)	
1-2	11 (30.6%)	13 (36.1%)	14 (38.9%)	0.746
3-4	14 (38.9%)	13 (36.1%)	9 (9.25%)	

BC: breast cancer BBL: benign breast lesions P >0.05 is not significant.

Table (2): Levels of CA15-3 and expression of lncRNA RP11-445H22.4 in studied groups.

	Studied groups			P	P*
	Control (n=36)	BC (n=36)	BBL (n=36)		
CA 15-3 (IU/ml) Median (Range)	11.5(7-18)	50 (10-95)	20.5 (10-35)	0.001	<0.001**[1] <0.05*[2] <0.05*[3]
LncRNA RP11-445H22.4 Median (Range)	0.97 (0.94-1)	5 (3-10)	1 (.99-3)	<0.001	<0.001**[1] >0.05 [2] <0.001**[3]

*p<0.05 is statistically significant **p≤0.001 is statistically highly significant.
P1=(control) vs. (BC) P2=(control) vs. (benign lesions) P3=(BC) vs. (benign lesions).

Table (3): Levels of RP11-445H22.4 and CA 15-3 in BC patients in relation to pathological features.

	RP11-445H22.4		CA 15-3 (IU/ml)	
	Median (Range)	P*	Median (Range)	P*
TNM stage				
I-II	4 (3-10)	0.03*	25 (10-80)	0.003*
III-IV	8 (4-10)		90 (23-120)	
Grade				
I	4 (3-7)	0.03*	25 (10-80)	0.004*
II- III	8 (4-10)		90 (23-120)	
ER\ PR				
Negative	4 (3-5)	0.03*	24 (20-50)	0.03*
Positive	7 (3-10)		70 (10-120)	
HER 2				
Negative	5 (2-10)	0.006*	70 (11-120)	0.126
Positive	2 (1-4)		25 (10-85)	

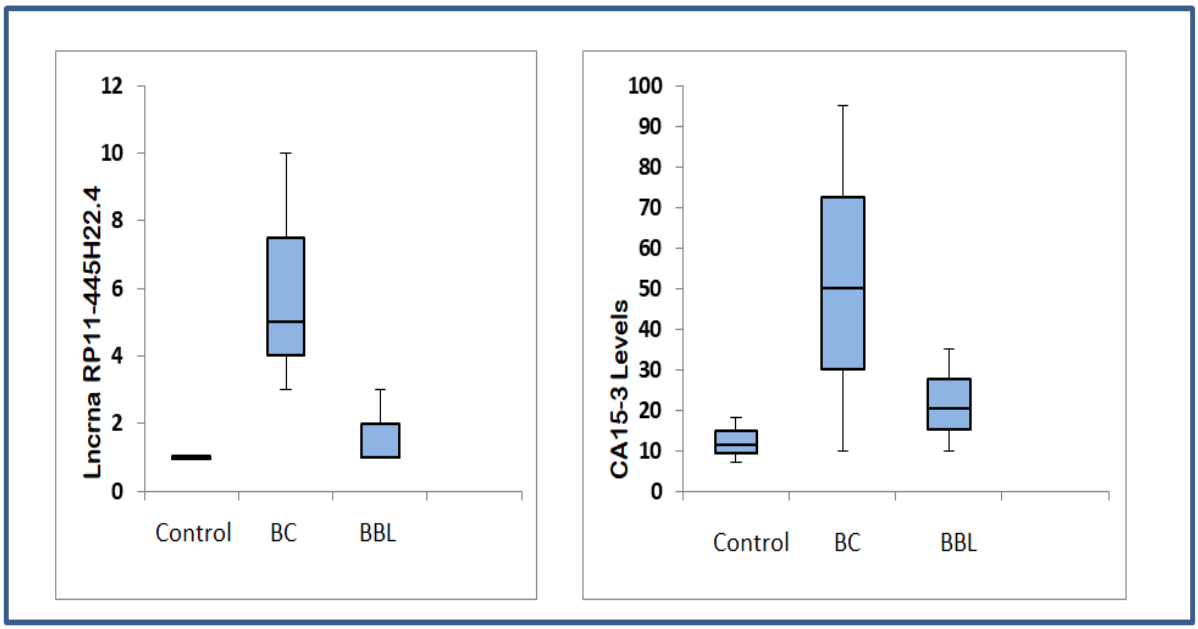
P-value>0.05 is not significant *P<0.05 is statistically significant

Table (4): Validity of CA 15-3 and RP11-445H22.4 within BC vs control and benign groups.

	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
CA 15-3	0.862	77.8%	80.7%	66.7%	87.9%	79.6%	<0.001
LncRNA RP11-445H22.4	0.993	95.9%	91.8%	85%	97.1%	92.5%	<0.001
LncRNA RP11-445H22.4 + CA15-3	0.906	94.3%	86.4%	77.2%	96.8%	88.9%	<0.001

Table (5): Reliability data of CA15-3 and RP11-445H22.4 to differentiate between TNM stages (I/II) and (III/IV) among BC patients.

	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
CA15-3	0.799	71.4%	75.9%	62.5%	80.0%	72.2%	0.04
LncRNA RP11-445H22.4	0.812	71.4%	81.8%	71.4%	81.8%	77.8%	0.031



BC; breast cancer, BBL; benign breast lesion

Figure (1): Expression of RP11-445H22.4 and CA15-3 among studied groups.

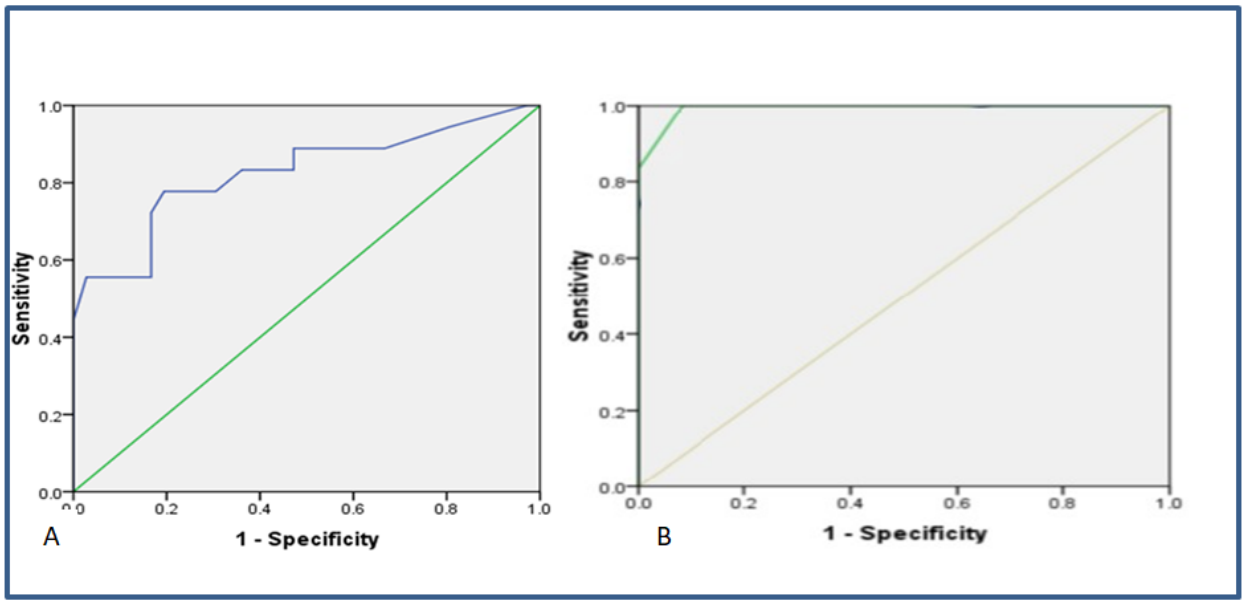


Figure (2): Receiver operating characteristics (ROC) curve.
(A) Validity of CA15-3 in BC versus control and benign groups.
(B) Validity of RP11-445H22.4 in BC versus control and benign groups

DISCUSSION

Women get breast cancer at any age after puberty, while the incidence rises with age [1]. Early diagnosis is essential for the best chance of recovery. Screening tests can save lives by detecting cancers at an early stage [17]. Long non-coding RNAs (LncRNAs) are RNAs longer than two hundred nucleotides which are not converted into proteins and are commonly copied via RNA polymerase II from various genomic regions [18]. Under both normal and pathologic conditions, as well as cancer, they are acknowledged as crucial controllers of cellular function over different mechanisms like epigenetic or nonepigenetic mechanisms [19, 20]. On human chromosome 20, there is a gene called LncRNA RP11-445H22.4. It is 862 bp long. It has been linked to tumor-induced inflammatory response and is notably expressed in cells of breast cancer [12]. Its inhibition considerably salvaged lipopolysaccharide (LPS) induced cell injuries via encouraging viability of cells, suppression of apoptosis and secretions of

inflammatory cytokines. Additionally, lncRNA RP11-445H22.4 functions like molecular sponge of miR-301a, besides, the suppression of miR-301a considerably removed the protective roles of RP11- 445H22.4 silencing against LPS induced damage [14].

This work aimed at evaluating the role of circulating long non-coding RNA RP11- 445H22.4 (lncRNA RP11-445H22.4) as a diagnostic marker of breast cancer and to investigate its association with the prognostic characteristics of the disease. CA15-3 is a form of the epithelial surface glycoproteins in cells of the breast; it was initially presented on the pellicle of the breast cancer cells, and highly expressed in breast cancer tissue, making it one of the most frequently used serum markers to assess the status of breast cancer [21].

In the present study, CA15-3 showed statistically significant increase among BC cases in comparison with healthy control group and cases with benign breast lesions. Also, it was significantly higher in cases with benign lesions than controls. So, these results agreed with Xue et al. [22] and also with Braihan et al. [23]. In contrast, Atoum *et al*. [24] identified that the control group had a greater level of CA15-3 than cases with benign breast lesions; this may be due to variation in age among their people and different method of determination by microparticle enzyme immunoassay (MEIA).

According to the current study, patients with BC had higher levels of lncRNA RP11-445H22.4 when compared to controls or cases who had benign breast lesions ($p<0.001$). Our results agreed with studies reported by Jiao *et al*. [25] and Moradi and Rahimi [26], who revealed there was overexpression of lncRNA RP11-445H22.4 in patients with BC. Regarding the relation between CA15-3 level and pathological features in BC patients, our study displayed that there was a statistically significant elevation in CA15-3 in patients presented with TNM stages III/IV or tumor grades II/III, as well as with positive ER/PR, while there was no significant difference among patients with positive or negative Her2/neu. These results went with that of Atoum *et al*. [24], who found substantial elevation in CA15-3 among grade II/III but disagreed in TNM stages as they revealed an increase in CA15-3 among patients with stage II/III.

Concerning the relation between lncRNA RP11-445H22.4 expression and pathological features in BC patients; our study revealed a significant increase in expression of RP11-445H22.4 with stage III/IV, tumor grade II/III, positive ER/PR, and negative Her2/neu. Our results agreed with Xu *et al*. [12], who revealed a significant increase in the expression of RP11-445H22.4 in BC cases with positive ER/PR and negative Her2/neu; however, these results disagreed with those of Moradi and Rahimi [26], who found no significant relation between expression of RP11-445H22.4 and the status of ER/PR, Her2/neu, and TNM stages. The receiver operating characteristic (ROC) curve analyzed the diagnostic performance for CA15-3 in differentiating cases that had BC from cases of benign breast lesions and controls. The present study revealed that the area under curve (AUC) for CA15-3 was (0.862) at cutoff level > (22.5 IU/ml) with 77.8% sensitivity and 80.7% specificity. Our results went with that of Zhong et al. [27], who reported that AUC for CA15-3 was (0.822), while results of Braihan et al. [23] showed lower sensitivity of CA15-3 (60%) and AUC was (<0.7).

In the present study, lncRNA RP11-445H22.4 ROC curves showed a 95.9% sensitivity, 91.8% specificity and an AUC of 0.991 at cutoff > 1.5. A study reported by Xu *et al*. [12] was in accordance with our study; the sensitivity was 92% and specificity was 74% for lncRNA RP11-445H22.4 as a diagnostic marker of BC. Moreover, Moradi and Rahimi [26] revealed that the sensitivity was 95% and specificity was 80% for RP11-445H22.4, while CA15-3 displayed 87% sensitivity and 77% specificity.

Combined ROC curve analysis done for both lncRNA RP11-445H22.4 and CA15-3 demonstrated 94.3% sensitivity and 86.4% specificity, so it can be used as a promising marker in diagnosis of BC.

In the current study, ROC curve was used to distinguish between TNM stages (I/II) vs. (III/IV) among cases of BC; the sensitivity for lncRNA RP11-445H22.4 and CA15-3 were 71.4% for both, while the specificity for them were 81.8% and 75.9% respectively. LncRNA RP11-445H22.4 had better sensitivity as well as specificity, it means that lncRNA RP11- 445H22.4 may act as a crucial biomarker that can be used in diagnosis of BC and show better performance than conventional circulating markers.

The study's strengths include being one of the recent studies to evaluate the role of lncRNA RP11- 445H22.4 as a biomarker that helps in diagnosis of BC better than current circulating markers.

Limitations:

The study included relatively small number of subjects (total of 108 subjects), and it was done in a single center, so multicenter studies with larger numbers are needed. Also, further studies are requisitioned to reveal the relation of lncRNA

RP11-445H22.4 with tumor burden and its association with response to treatment. Follow-up of patients to assess overall survival and disease-free interval is recommended to clarify the prognostic value of this marker.

Declaration of interest:

The authors report no conflicts of interest.

Funding information:

This study was not supported by any source of finding.

CONCLUSION

LncRNA RP11-445H22.4 was up-regulated in plasma from patients with BC when compared to normal controls and cases with benign breast lesions, in comparison to traditional biomarkers; it exhibited greater sensitivity and specificity. Moreover, LncRNA RP11-445H22.4 expression levels were related to ER, PR and Her2/neu status in BC patients. According to the results of the current study, women with BC who had advanced stages and higher tumor grades also had raised lncRNA RP11-445H22.4 levels. Consequently, it could serve as a potential biomarker for breast cancer diagnosis and prognosis.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71(3):209-49.
2. Azim HA, Ibrahim AS. Breast cancer in Egypt, China and Chinese: Statistics and beyond. J Thorac Dis 2014; 6(7):864-6.
3. International cancer control partnership: WHO cancer country profiles 2020. March 9, 2020. https://www.iccp-portal.org/news/who-cancer-country-profiles-2020
4. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: results of the national population-based cancer registry program. J Cancer Epidemiol 2014;2014:437971. doi:10.1155/2014/437971.
5. Mahvi DA, Liu R, Grinstaff MW, Colson YL, Raut CP. Local cancer recurrence: the realities, challenges, and opportunities for new therapies. CA Cancer J Clin 2018; 68(6):488–505. doi:10.3322/caac.21498.
6. Azim HA, Elghazawy H, Ghazy RM, Abdelaziz AH, Abdelsalam M, Elzorkany A et al. Clinicopathologic features of breast cancer in Egypt-contemporary profile and future needs: A systematic review and meta-analysis. JCO global oncology 2023; 9:e2200387. doi:10.1200/GO.22.00387.
7. Oloomi M, Bouzari S, Mohagheghi MA, Khodayaran-Tehrani H. Molecular markers in peripheral blood of Iranian women with breast cancer. Cancer Microenviron 2013;6(1):109-16. doi:10.1007/s12307-012-0118-7.
8. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PLoS One 2015;10(7):e0133830. doi:10.1371/journal.pone.0133830.
9. Angrand PO, Vennin C, Le BX, Adriaenssens E. The role of long non-coding RNAs in genome formatting and expression. Front Genet 2015;6:165. doi:10.3389/fgene.2015.00165.
10. Pearson MJ, Jones SW. Review: Long non-coding RNAs in the regulation of inflammatory pathways in rheumatoid arthritis and osteoarthritis. Arthritis Rheumatol 2016;68(11):2575-83. doi:10.1002/art.39759.
11. Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: A new paradigm. Cancer Res 2017;77(15):3965-81. doi:10.1158/0008-5472.CAN-16-2634
12. Xu N, Chen F, Wang F, Lu X, Wang X, Lv M et al. Clinical significance of high expression of circulating serum lncRNA RP11-445H22.4 in breast cancer patients: a Chinese population-based study. Tumor Biol 2015;36:7659-65. doi:10.1007/s13277-015-3469-0.
13. Xu N, Wang F, Lv M, Cheng L. Microarray expression profile analysis of long non-coding RNAs in human breast cancer: A study of Chinese women. Biomed Pharmacother 2010; 69: 221-227. doi: 10.1016/j.biopha.2014.12.002
14. Sun T, Yu J, Han L, Tian S, Xu B, Gong X et al. Knockdown of long non-coding RNA RP11-445H22.4 alleviates lPS-induced injuries by egulation of MiR-301a in osteoarthritis. Cell Physiol Biochem 2018;45(2):832-43.doi: 10.1159/000487175.
15. American Joint Committee on Cancer. Breast. In: AJCC Cancer Staging Manual. 8th ed. New York, NY: Springer; 2017:589.
16. Livak K and Schmittgen T. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4):402-8. doi: 10.1006/meth.2001.1262.
17. Hazelton WD, Luebeck EG. Biomarker-based early cancer detection: is it achievable?. Sci Transl Med 2011;3(109):109fs9. doi:10.1126/scitranslmed.3003272.
18. Mattick JS, Amaral PP, Carninci P, Carpenter S, Chang HY, Chen LL et al. Long non-coding

RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol 2023;24(6):430-47. doi:10.1038/s41580-022-00566-8
19. Kurokawa R. Long noncoding RNA as a regulator for transcription. Prog Mol Subcell Biol 2011;51:29-41. doi:10.1007/978-3-642-16502-3_2
20. Nie L, Wu HJ, Hsu JM, Chang SS, Labaff AM, Li CW et al. Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer. Am J Transl Res 2012;4(2):127-50.
21. Araz M, Beypinar I, Kazan S, Inci F, Celiker M, Uysal M. Are preoperative serum CA15-3 levels different in breast cancer subgroups?. Curr Probl Cancer 2019;43(2):115-22. doi:10.1016/j.currproblcancer.2018.06.011.
22. Xue F, Meng Y, Jiang J. Diagnostic value of dynamic enhanced magnetic resonance imaging combined with serum CA15-3, CYFRA21-1, and TFF1 for breast cancer. J Healthc Eng 2022;2022:7984591.doi:10.1155/2022/7984591.
23. Braihan H, Al-Rayahi I and Muhsin S. Evaluation of preoperative CA15-3 level and its relationship with clinicopathological characteristics in primary breast cancer patients. Journal of Techniques 2022;4(2):21–6. https://doi.org/10.51173/jt.v4i2.480
24. Atoum M, Nimer N, Abdeldayem S, Nasr H. Relationships among serum CA15-3 tumor marker, TNM staging, and estrogen and progesterone receptor expression in benign and malignant breast lesions. Asian Pac J Cancer Prev 2012;13(3):857-60. doi:10.7314/apjcp.2012.13.3.857.
25. Jiao ZY, Tian Q, Li N, Wang HB, Li KZ. Plasma long non-coding RNAs (lncRNAs) serve as potential biomarkers for predicting breast cancer. Eur Rev Med Pharmacol Sci 2018;22(7):1994-9. doi:10.26355/eurrev_201804_14727.
26. Moradi M, Rahimi Z. Circulating RP11-445H22.4 and TINCR are potential biomarkers for invasive ductal carcinoma of the breast. Preprints.org; 2020. DOI: 10.20944/preprints202008.0605.v1.
27. Zhong G, Wang K, Li J, Xiao S, Wei W, Liu J. Determination of serum exosomal H19 as a noninvasive biomarker for breast cancer diagnosis. Onco Targets Ther 2020;13:2563-71. doi:10.2147/OTT.S243601.

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