Egyptian Journal of Medical Research

Print ISSN: 2682-4396 / Online ISSN: 2682-440X

Original article

LncRNAs AK058003 Overexpression in the Blood of a Group of Breast Cancer Egyptian females

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Article Info

Article history:

Received 1 August 2023

Accepted 17 April 2024

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Keywords

LncRNAs AK058003,

Breast, Cancer,

qRT-PCR

Abstract:

Background: Breast cancer (BC) is one of commonest malignancies among females. Many factors play a role in breast cancer like age at menarche and menopause, breastfeeding, physical activity, and dietary habit. However, genetics still play a significant role as a key player in breast cancer occurrence. **Objectives:** This research aimed to assess the gene expression of Lnc RNAs AK058003 and to analyze their dysregulation with clinical features in Egyptian BC patients. **Methods:** A case-control research was done with 27 breast cancer patients ages 30 to 80 years old at Beni-Suef University Hospital, Clinical Pathology Department & Oncology Department .The average participation ages of 27 patients were matched with 27 healthy controls. Using the qRT-PCR kit, we detected LncRNAs AK058003 gene expression levels in the blood. **Results:** BC patients had a higher level of LncRNAs AK058003 gene expression than healthy controls (P =0.025) , there was a correlation between LncRNAs AK058003 and family history (P- value=0.010). The sensitivity and specificity of AK058003 is 74.1% and 77.8% respectively at cut off value 0.42 (P= 0.025) was found among breast cancer. The level of the gene expression was not linked to patient age, parity, or grade &type of BC (P>0.05). **Conclusion:** There was a significant association between LncRNAs AK058003 expression level in the blood of BC females, but further research is needed to completely understand LncRNA's molecular mechanisms for future incidence and prognosis.

1-Introduction

Breast Cancer is the most common female malignancy around the globe with increased incidence in less developed areas than in highly developed countries [1]. BC is accounted for up to two million in 2018 and it is expected to be around three million worldwide by 2040 [2].

Breast cancer is caused by environmental and genetic factors. Risk factors include a high-fat diet, smoking, and family history. Stress and mental illnesses contribute to the development of breast cancer. 5-10% of breast cancer cases are attributed to mutations in autosomal dominant genes. Breast cancer has been linked to a loss of function mutation in a tumor suppressor gene, which results in uncontrolled cell growth and division, disruption of the cell cycle checkpoints, and a breakdown in the DNA repair mechanism. A genetic mutation that causes function loss has a 70% risk of causing breast cancer in women under the age of 70 [3].

For breast cancer; immunotherapy, gene therapy, and targeted molecular treatment are being developed. Despite breakthroughs in therapy, (BC) continues to be the most prevalent cancer in women [4]. Noncoding RNAs are RNA transcripts that are not translated. Long ncRNAs AK058003 participate in several pathological and physiological processes, such as differentiation, proliferation, and cellular metabolism [5].

Despite their specificity and sensitivity, genetic testing and mammography screening are just two factors in assessing breast cancer risk. Researches demonstrate the feasibility of employing single nucleotide polymorphism genotyping to predict breast cancer risk and target therapy [6].

Unregulated LncRNA- AK058003 stimulates Syncline gene , hence enhancing BC proliferation, invasion, and migration [7].

LncRNAAK058003 is a precursor of the microRNA miR-15a, which affects Synuclein gene (SNCG) expression. It binds human antigen R, an essential regulator of RNA metabolism [8].

This research aimed to assess the gene expression of Lnc RNAs, AK058003 and to analyze their dysregulation with clinical features in Egyptian BC patients.

2-Subjects and methods:

2-1 Subjects: The current study is a case-control study that was conducted on breast cancer female patients from the Department of Oncology at Beni-suef University Hospital after the approval of the ethical committee (No:FMBSUREC/05122021/Hussein). The study included 54 females divided into 2 groups;

Group (I): Included 27 female patients with breast cancer, confirmed based on the evidence of histopathological examination.

Group (II): including 27 healthy subjects presented as controls who were age matched females, giving no personal or family history of breast cancer, or any other malignant tumors.

2-2. Exclusion criteria: The study excluded

patients who had received preoperative cancer treatments, including radiotherapy or chemotherapy, or females with a history of other types of cancers.

2-3-Data collection: included full history taking including hormonal history, Family history (list of all relatives who had breast cancer or other cancers), Social history (single or married, smoker or not), and history of diseases such as DM, HTN, asthma, HCV, and bilharziasis.

2-4 Grading and staging: Breast cancer staging was based on the TNM system and grading depended on breast cancer cell differentiation, and were determined according to European Society of Medical Oncology (ESMO)[9].

2-5 Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) analyses

The analysis of ER, PR and HER2, by immunohistochemical testing, was determined according to the American Society of Clinical Oncology Guideline Recommendations (ASCO)[10].

2-6-Genetic analysis: (I) Sample collection: five ml of venous blood was collected from the patients and controls into a vacutainer tube with Ethylene Tetra-acetic acid (EDTA). samples were processed within hours, repetitive freezing/thawing cycles were avoided and was extracted from fresh or frozen material to ensure avoiding RNA degradation.

(II) RNA extraction using Gene JET Whole Blood Genomic RNA Purification Mini Kit(Catalog No. K0781).

(III) Complementary DNA formation using High-Capacity cDNA Reverse Transcription Kits with RNase Inhibitor(Applied Biosystems Part Number: 4374966).

(IV) Real-time PCR quantification: Real-timePCR quantification was done using: PowerUp™ Taqman SYBR™ Green Master Mix Gene Expression Assay (Applied Biosystems). The relative expression levels were analyzed using the $2^{-\Delta\Delta}$Ct method. All experiments were performed in triplicate and the averages were calculated, with House keeping gene used as the internal reference. The primers used were as follows: AK058003 forward, 5'-GGGAACAAAGATGGTTTCTACGT-3' and reverse, 5'- ACTGGTTCATAGTTAGGCTGGAT -3' . *β-actin* was used as an internal control. Forward (5'-CTCTTCCAGCCTTCCTTCCT-3') and reverse (5'-AGCACTCTGTTGGCGTACAG-3') primers of β-actin were applied for the normalization of real-time PCR. The conditions for PCR were as follows: 95˚C for 1 min then 95˚C for 15 sec, 60˚C for 30 sec, 72˚C for 15 sec, for a total of 40 cycles, followed by 72˚C for 5 min. The expression of the LncRNA AK058003 gene in a sample was determined by subtracting the cycle threshold of the reference gene from that of the target gene to obtain the normalized amount of the LncRNA AK058003 gene, then comparing this value to the calibrators' values.

2-7-Statistical analysis:

Data were collected, coded to facilitate data manipulation, and double entered into Microsoft Access and data analysis was performed using the Statistical Package of Social Science (SPSS) software version 22 in windows 7 (SPSS Inc., Chicago, IL, USA). Independent samples t test was done to compare quantitative data between two independent groups. Chi square test was used to compare between two of more than two qualitative groups. One-way ANOVA test was used to compare quantitative values between more than two independent groups of quantitative data. Descriptive statistics of quantitative variables was performed in the form of mean and standard deviation (SD). Description of qualitative variables was in the form of number (No.) and percent (%). The receiver operating characteristic (ROC) curve was done to test sensitivity, specificity, and cut off values. A p-value <0.05 was considered significant[11].

Results

This study was conducted on 27 breast cancer female patients. Their age ranged from 30 to 81 years with a mean value of 50.2±14.8. Twenty-seven age-matched healthy unrelated female volunteers were included as a control group ranging from 30 to 77 years with a mean value of 50.85±12.75 **(P=0.868).**

There was no statistical significance difference between breast cancer patients and controls regarding clinical history ,menstruation and type of contraception (P >0.05)**(Table 1).**

Table (1): *Comparison between Breast cancer patients and Control group regarding clinical history, menstruation and type of contraception*

Clinical History		Cases		Control		P value
		Count (27)	%	Count (27)	%	
Menopause	Positive	15	55.6%	14	51.8%	0.785
	Negative	12	44.4%	13	48.1%	
Contraception use	Positive	10	37%	7	26%	0.379
	Negative	17	63%	20	74%	
Lactation	Positive	24	88.9%	20	74%	0.161
	Negative	3	11.1%	7	26%	
Family history	Positive	3	11.1%	2	7.4%	0.639
	Negative	24	88.9%	25	92.6%	
Menstruation	Menopause	15	55.6%	14	51.8%	0.2
	Regular menses	12	44.4%	13	48.1%	
Type of Contraception	Negative	16	59.3%	20	74%	0.089
	Pills	7	25.9%	2	7.4%	
	IUD	2	7.4%	5	18.5%	
	Injection	2	7.4%	0	0%	

No statistical significance was found between breast cancer patients and controls regarding laboratory data(p>0.05)**(Table 2).**

Table (2): *Comparison between Breast cancer patients and Control group regarding laboratory data*

	Cases		Controls		P value
	Mean	SD	Mean	SD	
TLC (microlitre)	7.04	2.53	6.8	1.94	0.711
Hb(g/dl)	11.31	1.45	11.66	0.81	0.286
PL T/mcL	301.3	114.56	279.44	84,07	0.428
Cr(mg/dL)	0.92	0.29	0.84	0.18	0.212

There was a statistically significant difference between breast cancer patients and controls regarding LncRNAs AK058003 Gene expression level as the median level was 2.6 in patients compared to 0.88 in controls (P =0.025) **(Table 3).**

Table (3): *Comparison between Breast cancer patients and Control group regarding LncRNAs AK058003 gene expression.*

	Cases					Controls					P value
	Mean	SD	Median	Mini	Max	Mean	SD	Median	Mini	Max	
LncRNAs AK058003 gene	175.56	512.9	2.6	0.07	2136.38	1.698	2.33	0.88	0.11	8.35	**0.025**

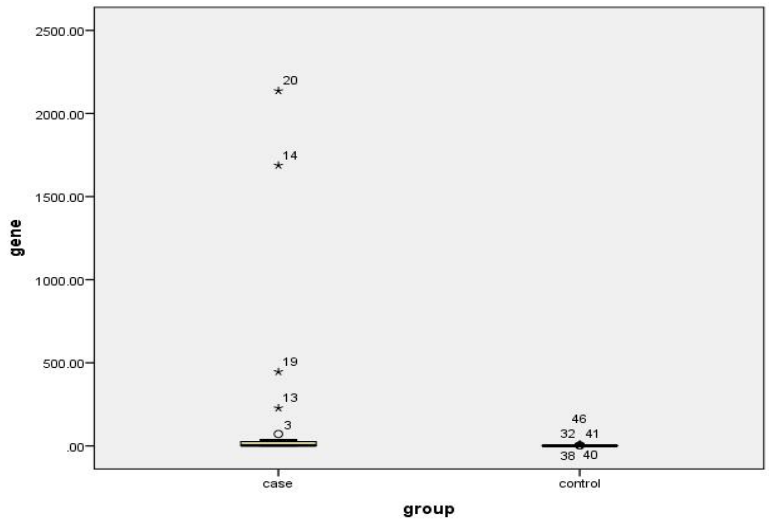

Figure (1): *Comparison between Breast cancer patients and Control garoup regarding LncRNAs AK058003 gene expression*

There was a statistical significance difference between LncRNAs AK058003 Gene expression level and family history (P- value=0.010) **(Table 4)**.

Table (4): *Relation between LncRNAs AK058003 gene and Clinical History of patients:*

		Gene				P value
		Mean	SD	Median	Range	
Menopause	**+ve**	35.36	113.97	2.45	0.07- 446.02	0.114
	-ve	350.81	738.24	16.9	0.09-2136.38	
Contraception	**+ve**	198.86	527.77	8.2	0.09-1687.83	0.860
	-ve	161.86	519.9	2.6	0.07-2136.38	
Family history	**+ve**	865.95	1121.08	446.02	15.5-2136.38	0.010
	-ve	89.26	343.76	2.5	0.07-1687.8	

No significance was found regarding LncRNAs AK058003 gene expression level and tumor markers of the breast; ER, PR, and HER2 as (p-value>0.05)**(Table 5).**

Table (5): *Comparison between LncRNAs AK058003 gene and tumor markers in patients group.*

		Gene				P value
		Mean	SD	Median	Range	
ER	+ve	232.1	588.9	8.2	0.07-2136.38	0.343
	-ve	14.01	25.86	2.7	0.21-71.55	
PR	+ve	232.03	588.94	8.2	0.07-2136.38	0.385
	-ve	16.25	27.57	4.91	0.21-71.55	
HER2	+ve	292.04	664.03	18.78	0.07-2136.38	0.376
	-ve	107.05	407.46	2.7	0.09-1687.8	

No association was found between LncRNAs AK058003 gene expression level in patients and pathological types of cancer breast (p-value =*0.577*)**(Table 6).**

Table (6): *Relation between LncRNAs AK058003 gene and pathological type.*

		Gene				*P value*
		Mean	*SD*	*Median*	*Range*	
Pathological types	***IDH***	*195.57*	*541.89*	*2.5*	*0.07-2136.38*	*0.577*
	Others types (papillary carcinoma, ductal carcinoma in situ, metastatic carcinoma)	*15.52*	*2.49*	*14.33*	*13.84-18.39*	

No relation was found between LncRNAs AK058003 gene expression level in patients and pathological grade, and TNM staging of cancer breast as (p-value>0.05)**(Table 7).**

Table (7): *Relation between LncRNAs AK058003 gene and pathological grade and TNM*

		Number	Median	Range	P-value
Pathological grade	Grade1	1	32.02	-	0.436
	Grade 2	16	15.47	0.09-2136.38	
	Grade 3	9	0.68	0.07-14.33	
T stage	T1	3	35.5	0.26-227.69	0.161
	T2	10	14.9	0.07-2136.38	
	T3	2	844.11	0.4-1687.83	
	T4	11	2.5	0.09-32.02	
N stage	N 0	5	19.9	13.8-2136.38	0.568
	N1	18	1.6	0.07-1687.8	
	N2	1	-	-	
	N3	2	43.5	15.47-71.55	
M stage	`M0	19	2.6	0.07-1687.8	0.147
	M1	8	14.9	0.26-2136.38	

ROC curve demonstrated that the sensitivity and specificity of AK058003 is 74.1% and 77.8% respectively at cut off value 0.42 (P= 0.025) which may be beneficial in diagnosis of breast cancer **(Table 8).**

Table (8): *Sensitivity and specificity of LncRNAs AK058003 gene for diagnosis of breast cancer cases*

	Sensitivity	Specificity	AUC	Cut off Point	P value	Confidence-interval
Gene	74.1%	77.8%	0.678	0.42	0.025	0.523-0.832

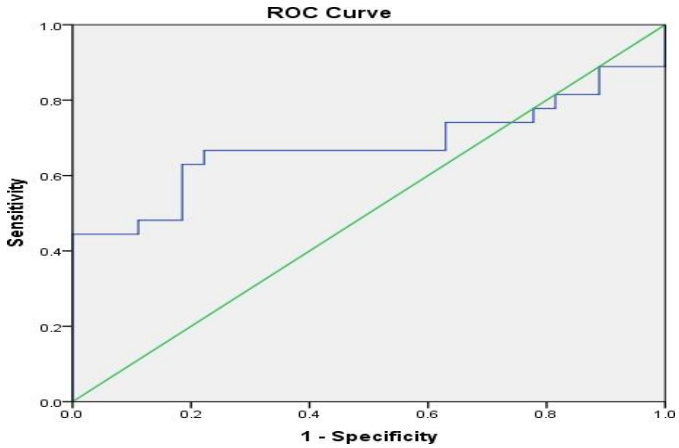

Figure (2): *Receiver Operating Characteristic curve for LncRNAs AK058003 gene as diagnostic marker of breast cancer cases*

Discussion:

Breast cancer is the second cancer to cause a high mortality rate [12]. It represents the disease that occurs due to changes in the DNA sequence of the cell's genome [13]. Breast cancers are caused by genetic variation and point mutation in tumor suppressor genes, which causes unmanaged cellular proliferation, failure of DNA repair, and other mutations in proto-oncogenes. still, a small percentage about 5-10% of all breast cancers are caused by a mutation in autosomal dominant genes [14].

LncRNAs are more than 200 nucleotides long and have no protein-coding purpose. Few studies have linked LncRNAs to cancer, and several of the altered LncRNAs can cause overexpression of surrounding protein-coding genes, which may add to tumor progression [15]. LncRNAs play a role in cancer progression via a range of mechanisms, which would include chromatin remodeling, genetic methylation, transcription factors, and DNA repair [16].

According to research, LncRNA- AK058003 is found on the reverse strand of chromosome 10q22, which is less capable of protein-coding. LncRNA-AK058003 is thought to increase tumor progression by trying to target the SNCG (-synuclein gene). The uncontrolled LncRNA-AK058003 stimulates SNCG, to proliferate, invade, and distal spread BC [17].

Furthermore, ***Rajabi et al. (2022)*** [18]. reported that LncRNA-AK058003 expression was shown to promote breast cancer proliferation [18], invasion and metastasis by regulating SNCG expression of LncRNA-AK058003 was significantly higher in the tumour as compared to control group .

Previous research has revealed that LncRNAs play an important role in cancer occurrence. Further research has begun to focus on the regulatory function of LncRNAs. LncRNA expression levels facilitate novel drug targets and aid in the early detection of BC [19].

The present study aimed to investigate the association between LncRNA-AK058003 gene expression and the risk of BC in female patients.

Our results revealed that relative LncRNA-AK058003 expression levels were significantly higher in the blood samples of BC patient when

compared with controls **(P = 0.025).**

Also, several studies showed the association between LncRNA- AK058003 gene expression and Breast cancer female patients for example: ***Attar et al. (2021)*** [20] agreed with our study and demonstrated that there was a significant increase in the expression levels of LncRNA-AK058003 in BC blood specimens .

The present study suggested that sensitivity value of 74.1% and specificitiy of 77.8% at cut off 0.42 for LncRNA AK058003 might have possible implications as diagnostic markers for breast cancer.

Another study assessed the genetic marker value of LncRNA AK058003 gene and showed that a sensitivity and specificity of this gene were 75% and 69.1%, respectively in esophageal carcinoma which were consistent with the results of current research [21]**,** Another study showed that the LncRNA AK058003 gene was Upregulated in gastric cancer [22]**.**

In contrast to our research, another study revealed that LncRNA-AK058003 was a suppressor gene that inhibits HCC cell proliferation and metastasis [23]**.**

In this current study, there was no statistical difference between breast cancer patients and control groups as regards age (P value=0.868).

Similar to our result, ***Attar et al. (2021)*** [17] found no significant difference between the breast cancer group and control group as regards age . Also, ***Rajabi, and Mozdarani, (2022)***[24] found no significant association between the expression of LncRNA -AK058003 and patients' age.

In this study, there was no significant difference between females with breast cancer as regards the size of the tumor.

In agreement with our study, ***Attar et al. (2021)*** showed that the difference in the size of the tumor mass did not affect the expression of the gene [17].

In this present study, there was no significant difference between female patient groups as regards pathological type (P=0.960) and cancer grades **(P=0.436)**. In agreement with this current study, ***Attar et al. (2021)*** [20] found no statistical significant association as regards tumor grade **.** Also, ***Rajabi, and Mozdarani, (2022***) demonstrated that no significant association was observed between the expression of LncRNAs with patients' grade of the cancer [24]**.**

In this study, there was no statistical significant association as regards lymph node involvement and distant metastasis **(P < 0.05) .** Similar to these results, ***Attar et al. (2021)*** found no statistical significant association as regards lymph node involvement [20]**.**

Conversely to the present study, ***Rajabi et al. (2022)& He and Wang (2015)*** showed that there was a significant association as regards lymph node involvement [18, 25].

Attar et al. (2021), Rajabi and Mozdarani (2022) **,** found no statistical significant association as regards distant metastasis [20, 24]**.**

In contrast to this study**,** ***He and Wang (2015)***

showed that there was a significant association as regards metastasis [25] .

In this research, we found that the expression level of AK058003 was not significant relative to hormone receptors including ER, PR and HER2 with P value (p=0.343,p=0.385 and p=0.376 respectively).

Similar to these results ,another research observed that the expression levels of AK058003 gene was not significant relative to hormone receptors including ER, PR and HER2 [18].

Conclusion

In conclusion, the expression of LncRNA - AK058003 in blood samples were demonstrated to be significantly higher in BC patients . LncRNA-AK058003 gene expression may be associated for occurrence of BC. More studies should be done on larger population to achieve higher accuracy. Furthur assessment of other Lnc RNA genes are targeted to reach higher specificity in assessment of breast cancer. Investigations are needed to understand the molecular basis of LncRNA application in future.

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