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Evaluating Gelsolin Gene Expression Among Iranian Breast Cancer Patients

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A B S T R A C T

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Background: Breast cancer is the most common type of cancer among women, and is among the five most prevalent cancer types among Iranian women. Advanced-stage breast cancer is often correlated with distant metastases. Alterations in gene expression affect general cytoskeleton changes during differentiation and oncogenesis, and can be considered an important factor in tumor progression. Gelsolin plays a significant role in actin assembly and has been introduced as a tumor activator. The aim of this study was to evaluate the expression of Gelsolin in breast cancer as well as its correlation with patients' clinical parameters.

Methods: In this study, 70 breast cancer patients, who had been referred to Cancer Institute, Imam Khomeini Hospital Complex for surgery were randomly selected. Normal and tumor tissues were prepared and stored at -80°C. Gelsolin gene expression was measured using real-time polymerase chain reaction (PCR).

Results: The results showed that Gelsolin gene expression had increased in 68.6% of the tumor samples. In addition, there was a significant association between increased levels of gene expression and tumor progression stages ($P < 0.05$). However, there was no significant association between increased levels of gene expression and other clinical findings, such as tumor grade, tumor size or patient age.

Conclusion: The results revealed that Gelsolin gene expression had increased in the tumor samples. Gelsolin overexpression also resulted in increased lymph node involvement in breast cancer. The expression of this gene also increased significantly during advanced stages of breast cancer; however, there was no significant relation between Gelsolin expression and tumor grade or tumor size.

Keywords: Breast cancer, Gelsolin expression, Iranian population, Tumor grade, Tumor size



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INTRODUCTION:

Despite considerable medical progresses, cancer remains one of the most important health issues of the day, and is the second cause of death worldwide, particularly in developed and developing countries¹. By 2020, it has been predicted that new incidents of cancer will be diagnosed in 15 million patients, and 60% of these will occur in developed countries^{2,3}. Breast cancer is among the top five most prevalent cancer types in Iran and is the most common cancer among Iranian women⁴. Breast cancer develops via the progressive branching system of ducts that originate at the nipple and end in one of many terminal ductal lobular units (TDLUs). These are the smallest functional units of the breast and over proliferation of epithelial cells covering the breast ducts can result in cancer^{5,6}. These cells can attack surrounding healthy tissues through the blood and lymphatics, leading to secondary metastasis⁷. Since breast cancer clinical trends differ for each patient, determining the patient's final perspective is impossible. Thus identification of factors that can predict patient prognosis would be beneficial for clinical and treatment strategy decisions⁸. According to recent statistics published by the Iranian Ministry of Health and Medical Education, the rate of breast cancer is 27 per 100,000 women⁹. It has also been shown that most Iranian women with breast cancer are younger compared to patients in western countries⁴.

To determine the stage of breast cancer, the TNM Classification of Malignant Tumors (TNM) system was utilized. In this system, tumor size (T), tumor spread to lymph nodes and lymph node involvement (N) and existence or lack of metastasis (M) are assessed. For instance, it has been revealed that increased tumor size and lymph node involvement are associated with poor prognosis¹⁰. Based on this system, breast cancer is classified into four stages: stages 1 to 3 are non-metastatic,

and stage 4 is usually accompanied by metastasis. This information is beneficial when choosing the appropriate treatment strategy¹¹.

Motility and invasion of cancer cells are essential for tumor cell metastasis, which requires cytoskeleton actin regulation^{12,13}. Cellular actin assembly is reported to be effective on cell migration, motility, attachment, tumor invasion and metastasis¹⁴. Gelsolin, which plays a significant role in actin assembly, is observed in almost all mammalian tissues. Actin assembly is mainly due to the growth and reforming of apoptotic cells¹⁵. This protein is coded by the GSN gene located on 9q33 and alterations in gene expression affect general cytoskeleton changes during differentiation and oncogenesis¹⁶. Gelsolin can act upstream of variable signaling cascades and affect the coordinated regulation of some signaling pathways. Moreover, studies have identified new areas in cancer signal transduction mediated by Gelsolin. Research has indicated the role of erbB-2/EGFR in cancer prognosis among positive tumor patients. Interactions between Gelsolin activation and erbB-2/EGFR (Erythroblastic Leukemia Viral Oncogene Homolog 2/ Epidermal Growth Factor Receptor) have been confirmed via clinical studies and multiple cell culture systems. Furthermore, activation of GTPase, Ras and Rac are crucial in order to mediate motility in downstream processes, and it should be mentioned that Gelsolin quantity regulates Rac expression¹⁷. Various studies have revealed that Gelsolin is not only a tumor suppressor but also a regulating cytoskeleton factor which controls cell motility and inhibits tumor growth. It has been indicated that the overexpression of Gelsolin can restrict metastasis via attachment to actin molecules. Variation in Gelsolin expression during differentiation and carcinogenesis also affects cytoskeleton changes^{12,18}. In other studies, Gelsolin has been introduced as a tumor activator; therefore, it is assumed that recognizing Gel-

solin as a tumor suppressor or activator depends significantly on the type of cancer¹⁹. This study focused on evaluation of Gelsolin gene expression and its correlation with clinical and demographic findings in Iranian breast cancer patients.

METHODS:

Population and tissue specimen:

In this study, 70 breast cancer patients who visited Imam Khomeini hospital between 2013 and 2014 were evaluated. Samples were obtained from Tumor Bank of Iran, Cancer Institute, Imam Khomeini Hospital Complex. Each sample was then stored at -80 °C for further procedures, which are outlined as follows.

RNA isolation and cDNA synthesis:

RNA extraction from each sample was carried out using a TriPure Isolation Reagent Kit according to the manufacturer's instructions (Roche, Germany). Analysis of RNA quantity was done spectrophotometrically and cDNA was synthesized via oligo dT and random hexamer primers using a RevertAid Kit (Thermo Fisher Scientific). RNA and primers were briefly incubated for 5 minutes at 65°C. After the mixture had cooled, the reverse transcription step was carried out using RevertAid enzyme, dNTP, buffers and RNAase inhibitor for 60 minutes at 42 °C, finishing with incubation at 65°C for 5 min. The Glyceraldehyde-3-phosphate de-

hydrogenase gene (GAPDH) was used as the house-keeping gene. Sequences of Gelsolin and GAPDH mRNAs were obtained from the NCBI database (their Gene ID in NCBI are 2934 and 2597, containing 42 and 10 exons respectively). Afterwards, desired primers were designed using Primer 3 software (**Table 1**). Quantitative Real Time-PCR was performed using RealQ Plus 2x Master Mix Green – (Ampliqon, Denmark). Quantitative Real-Time PCR reactions were performed in Rotogene Q (Qiagen, Hilden, Germany) in 20 µL of PCR master mix containing 10 µL of SYBR-Green QPCR Master Mix, 1 µL of primer, 1 µL of RT products and 8 µL of RNase free water. All tests were run in triplicate to minimize experimental error. Samples with Ct>37 were excluded from the analysis.

Statistical analysis:

Statistical analyses were carried out using SPSS 22.0. (SPSS, Chicago, IL, USA) and Prism 5.0 software. Data was expressed as the mean±SD of at least three independent experiments, and values of P<0.05 were considered statistically significant. After obtaining Ct from real-time PCR gene relations, the data was calculated via the $2^{-\Delta Ct}$ formula and assessed using clinical parameters among breast cancer patients. The normality of the data was evaluated using the Kolmogorov–Smirnov distribution and comparison between groups was performed using unpaired student's t test or ANO-

Table 1. Primer sequences for GSN and GAPDH target genes.

Primer Name	Sequence (5' → 3')	Size (bp)	Tm°C
<u>GSN Forward</u>	CCTGGGCTTGCTCTACCTTT	155	54.5
<u>GSN Reverse</u>	TGGAACCTTCGATTCTCCAG		53.8
<u>GAPDH Forward</u>	GAAGGTGAAGGTCGGAGTCA	109	53.6
<u>GAPDH Reverse</u>	AATGAAGGGGTCATTGATGG		53.3

VA ($P \leq 0.05$).

RESULTS:

Clinical and pathologic characteristic of patients:

The Imam Khomeini hospital tumor bank provided 70 tumor samples, as well as adjacent tissues as normal tissue samples. Patients' clinical and pathologic characteristics are presented in **Table 2**. The results for TNM stage I, II and III are 3, 38 and 29 respectively. 47% (n=33) of patients were less than 50 years old while 53% (n=37) of them were more than 50 years old. Based on histological grading, the number of patients with grade 2 (n=37) were more than grade 1 (n=9) or 3 (n=24).

Levels of Gelsolin gene expression among normal and tumor tissues:

It was shown that Gelsolin gene expression increased 68.57% among tumor tissues in comparison with nor-

mal tissues. (**Fig.1**). Additionally, there was no significant relation between tumor size ($P = 0.8957$) and patient age ($P = 0.3934$).

The relation between levels of Gelsolin gene expression and lymph node involvement:

In this study, breast cancer patients were separated based on lymph node involvement. Results indicated that levels of Gelsolin expression were higher among subjects with no lymph node involvement. There was a significant relation between Gelsolin gene expression and lymph node involvement ($P = 0.0167$) (**Fig.2**).

The relation between levels of Gelsolin gene expression and disease stage:

Cancer patients were classified into four groups based on their disease stage. In this study, group IV was not included. Due to the small studied sample size, groups I and II were merged as one group and patients with stage III were regarded as an independent group. Based on the results, a significant relation between Gelsolin

Table 2. Clinical characteristics of 70 patients with breast cancer. (N= Regional lymph nodes)

Variable	Number	
Lymph node status	N0	32
	N	38
TNM staging	I	3
	II	38
	III	29
Histological grading	1	9
	2	37
	3	24
Tumor size	T1	5
	T2	47
	T3	18
Age	<50 years	37
	≥ 50 years	33

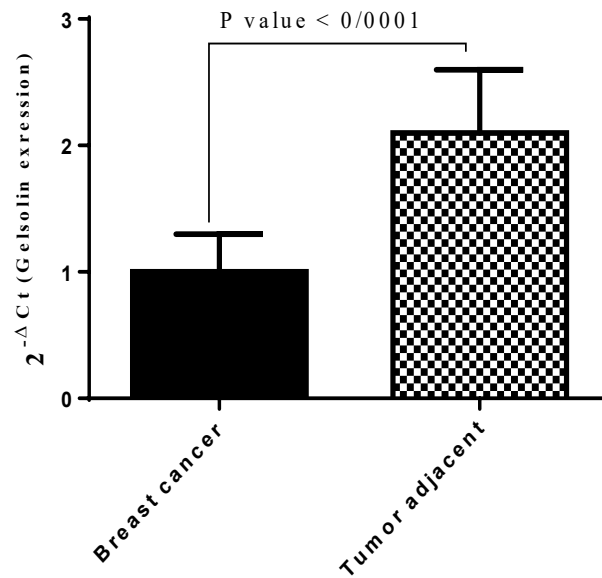


Figure 1. **Relative gene expression of GSN between control and patients group.** mRNA levels GSN were measured using real time PCR. GSN gene expression among tumor tissues is increased 68.57% in comparison with normal tissues. Data are represented as mean \pm SEM of at least three separate experiments.

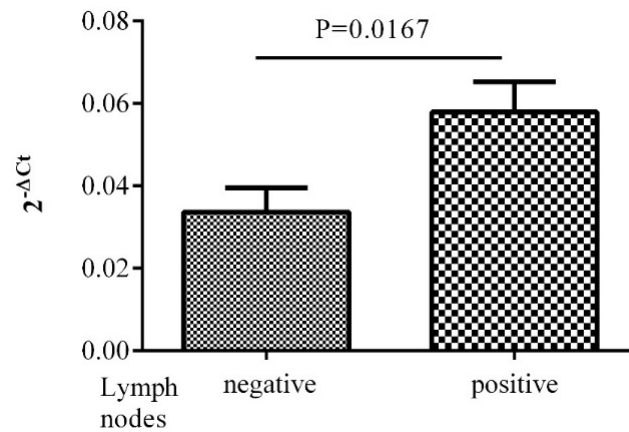


Figure 2. **GSN gene expression and lymph nodes involvement.** mRNA levels of GSN were measured using real time PCR between lymph nodes negative and lymph nodes positive. GSN gene expression among lymph nodes positive was 1.5 fold than lymph nodes negative. Data are represented as mean \pm SEM of at least three separate experiments

gene expression and disease stage was observed. ($P=0.0086$). (Fig.3)

The relation between Gelsolin expression levels and tumor grade:

According to pathological characteristics, tumors were classified into three grades in order to better determine

clinical approaches. The results showed no significant relation between levels of Gelsolin expression and tumor grade ($P = 0.7848$) (Fig.4).

The relation between Gelsolin levels and tumor size:

Tumor size as an indicator of tumor growth, is utilized

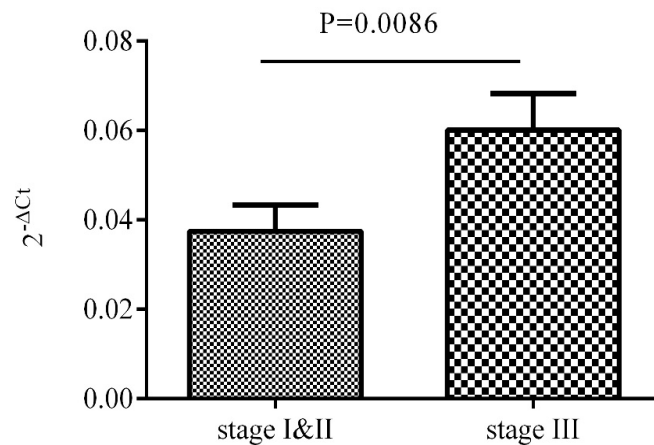


Figure 3. **GSN gene expression and disease stage.** mRNA levels of GSN were measured using real time PCR between stage I, II and III. Patients in stage III had higher expression GSN in comparison to stage I, II group. Data are represented as mean \pm SEM of at least three separate experiments

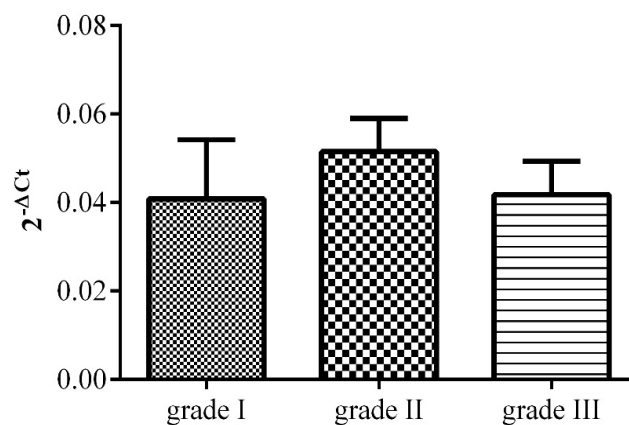


Figure 4. **GSN gene expression and tumor grade.** mRNA levels GSN were measured using real time PCR between three different grades. Data are represented as mean \pm SEM of at least three separate experiments

by pathologists for classification. For this study, tumors were classified into three sizes as follows: tumors smaller than 2 cm, tumors between 2 and 5 cm, and tumors larger than 5 cm. The ANOVA results demonstrated that there was no significant relation between

Gelsolin expression level and tumor size (**Fig.5**).

The relation between Gelsolin levels and patient age:

The effect of age on breast cancer patients depends on the family history of the disease and the individual's

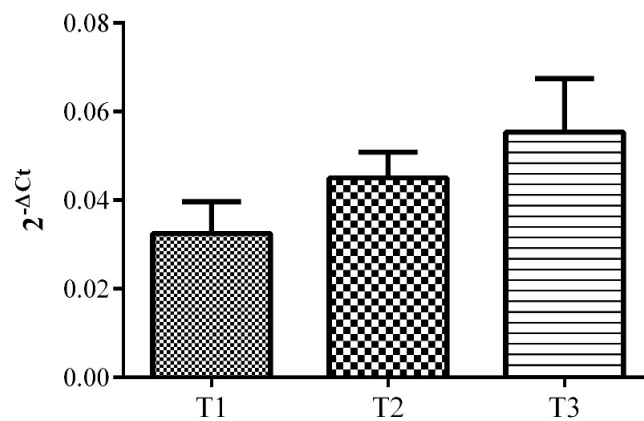


Figure 5. **GSN gene expression and tumor size.** mRNA levels GSN were measured using real time PCR between three different tumor size. Data are represented as mean \pm SEM of at least three separate experiments

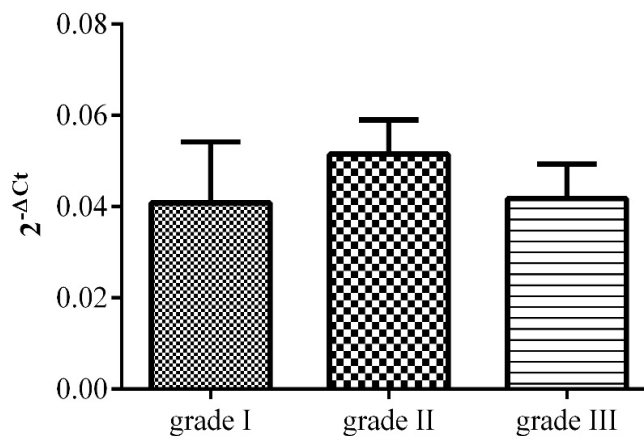


Figure 6. **GSN gene expression and patient's age.** mRNA levels GSN were measured using real time PCR between two different patient's age. Data are represented as mean \pm SEM of at least three separate experiments

genetic arrangement. The subjects' age ranged from 31 to 79 years in this study. The results revealed that there was no significant relation between Gelsolin expression levels and tumor size (**Fig.6**).

DISCUSSION:

Gelsolin is capable of disassembling, capping and assembling actin fibers and subsequently regulating the cytoskeleton dynamically. In addition to playing a role in certain important signaling pathways, it was also revealed that Gelsolin takes part in motility, apoptosis, proliferation, differentiation, epithelial-mesenchymal transition (EMT) and cancer phenotype indication. However, its action during malignancy initiation and progression is still unclear. Even though in most cancer types Gelsolin expression is inhibited, increased expression has been reported in gynecologic cancers. Therefore Gelsolin acts as both an activator and an inhibitor of apoptosis. This contradictory phenomenon has been confirmed in a variety of cancer types¹⁹⁻²¹. Gelsolin expression decreases among pre-malignancy tumors, while its expression rises among higher-grade tumors. Additionally, low levels of Gelsolin expression were also reported to be associated with worse prognosis in epidermal growth factor receptor-positive (EGFR+), erb-b2 receptor tyrosine kinase 2-positive (HER2+) breast cancer, which led to hypothesizing a role for Gelsolin in cell motility and invasion²². Since modifying Gelsolin's role in tumor progression is feasible; therefore, this protein might cooperate with other oncogenic factors to accelerate tumor progression²³. The results of our study showed overexpression of Gelsolin in nearly 68% of patients. In 2015, Runzhi Deng et al. investigated the effect of Gelsolin on the proliferation and motility of Tca 8113 oral carcinoma cells. Their results demonstrated that Gelsolin overexpression considerably enhanced the proliferation and

apoptosis of Tca8113 cells. They also indicated that the migration and invasion capability of Tca8113 cells was enhanced via Gelsolin overexpression. As a result, they concluded that higher levels of Gelsolin expression increase cellular growth and motility²⁰.

In this study, it was revealed that Gelsolin overexpression led to increased lymph node involvement in breast cancer. The expression of this gene was also greater during more progressed stages of breast cancer. Gelsolin is a key regulator of actin disassembly, so its overexpression may lead to cancer cells' motility, affecting the cancer progression trend. Subsequently, it is expected that Gelsolin expression increases along with disease stage. Therefore, an increase in gene expression may be an important factor for breast cancer cell invasion.

In compliance with our results, Wang-Yu Zhu et al. reported in 2012 that an increase in Gelsolin expression was related to lymph node metastasis and progression of disease stage²⁴. Also, Abedini et al., showed that in ovarian cancer samples high Gelsolin expression was associated with tumor progression, but there was no correlation between Gelsolin expression, age and tumor grade²¹.

Based on our results, there was no significant difference between Gelsolin expression, tumor grade or tumor size. Although Gelsolin expression was higher in younger patients, the difference was not significant, and could be attributed to small sample size. On the contrary, Dar-Bin Shieh et al. examined Gelsolin expression in different stages of oral carcinoma. They found that Gelsolin expression correlated with tumor size, and that expression increased considerably among younger patients²⁵.

CONCLUSION:

Based on our results, it is assumed that the Gelsolin

gene is probably an important factor in breast cancer as well as being a suitable biomarker for breast cancer clinical anticipation. Thus the pharmaceutical inhibitors of Gelsolin protein can hypothetically restrict the growth and progression of breast tumors. It should be noted that, even though the role of this gene in the invasive behavior of breast cancer cells has been clarified, more information can be obtained about the mechanism of metastasis and its control.

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