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Original Article

Association of fatty acid synthase (FASN), ATP-citrate lyase (ACLY), and acyl-coenzyme A synthetase long-chain 4 (ACSL4) expression and human epidermal growth factor receptor 2 (HER2) status with metastasis and survival in breast cancer: a five-year follow-up

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Abstract

Background and purpose: Breast cancer is one of the leading causes of death among women worldwide, with rising incidence rates, particularly in rapidly developing countries such as Iran. This study aimed to investigate the relationship between lipid metabolism enzymes, fatty acid synthase (FASN), ATP-citrate lyase (ACLY), and acyl-CoA synthetase long-chain family member 4 (ACSL4), and patient survival, with a focus on their potential role in breast cancer metastasis. In addition, we evaluated the prognostic significance of human epidermal growth factor receptor 2 (HER-2) overexpression in breast cancer patients.
Experimental approach: A total of 52 breast cancer tissue samples were collected from patients at Ordibehesht Clinic in Isfahan, Iran. RNA was extracted and analyzed using qRT-PCR to quantify the expression of FASN, ACLY, and ACSL4. Kaplan-Meier survival curves and log-rank tests were applied to assess survival rates and metastasis.
Findings/Results: The Kaplan-Meier analysis showed an average time to metastasis of 36.18 months. No significant associations were found between metastasis and the expression levels of ACLY, FASN, or ACSL4. In contrast, HER-2 expression was significantly associated with metastasis, underscoring its potential as a critical prognostic marker. Other clinicopathological factors, including tumor grade, stage, size, and receptor status, were not significantly related to metastasis.
Conclusion and implications: Our study highlights the importance of HER-2 as a key prognostic marker in breast cancer and suggests that further research is required to clarify the mechanisms underlying its role in cancer progression.

Keywords: Breast neoplasms; Fatty acid synthase; ATP citrate lyase; Acyl-CoA synthetase; Receptor, ERBB-2; Neoplasm metastasis; Survival analysis.

INTRODUCTION

Breast cancer is a highly prevalent malignancy that affects women globally and ranks second to lung cancer in terms of female mortality. Despite advancements in therapeutic and diagnostic approaches, mortality of this disease continues to rise, especially in countries undergoing rapid demographic changes. Approximately 12% of women face the risk of invasive breast cancer during their lifetime, highlighting the importance of preventive programs and awareness campaigns (1,2).

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In Iran, breast cancer is the most commonly diagnosed cancer in women, comprising 24.4% of all malignancies (3). It is the fifth leading cause of mortality among Iranian women, following cancers of the stomach, blood, lung, bronchus, liver, and biliary passages. Notably, Iranian women are diagnosed with breast cancer at least ten years earlier than those in developed countries, emphasizing the urgency of this issue. Understanding the risk factors for breast cancer can play a pivotal role in the therapy and care of patients (1). The survival rate following cancer diagnosis and treatment is a critical metric used in therapeutic evaluations.

Breast cancer survival is influenced by various prognostic factors, which have been extensively studied in recent years. Several studies have identified a significant association between survival outcomes and prognostic factors, such as surgery, estrogen receptor (ER) status, education, histology, body mass index, human epidermal growth factor receptor 2 (HER2) receptor status, race, tumor size, tumor differentiation, age, grade, lymph node involvement, comorbidity index, and cancer stage. Among these, advanced cancer stages (stage 3 and stage 4), a comorbidity index of ≥ 3, poor tumor differentiation, and undifferentiated histology are considered the strongest negative prognostic factors, often associated with poor survival outcomes. Additionally, factors such as positive lymph nodes, older age, race, HER2 positivity, and overweight/obesity are also associated with reduced survival. However, their impact is generally less severe compared to the factors as mentioned above. However, heterogeneity has been observed across most studies (4,5).

Recent research has also highlighted the role of metabolic alterations, particularly in glucose and lipid metabolism, as emerging hallmarks of cancer cells. Although fatty acid metabolism has received less attention, findings indicate a reprogramming of lipid metabolism in cancer cells. Lipids serve as essential membrane constituents, undergo modifications related to protein synthesis, act as secondary messengers, and function as an energy source during nutritional deprivation. Various studies, including our previous research, have demonstrated a significant increase in the expression of key lipid synthesis enzymes such as fatty acid synthase (FASN), ATP citrate lyase (ACLY), and acyl-CoA synthetase long-chain family member 4 (ACSL4) in breast cancer tumor tissue compared to adjacent normal tissue (6-10). ACLY is a critical cytoplasmic tetramer enzyme and the first key enzyme involved in lipid synthesis, catalyzing the production of acetyl-CoA, a precursor for anabolic pathways (11). Up-regulated ACLY is commonly found in cancer cells and functions as an oncogene, influencing tumorigenesis and tumor growth (12). By regulating lipid synthesis and altering the metabolic patterns of tumor cells, ACLY can accelerate tumor growth and metastasis (13). FASN is an enzyme responsible for synthesizing long-chain fatty acids, primarily producing palmitate from acetyl-CoA and malonyl-CoA. Increased FASN expression has been linked to various carcinomas (14). The ACSL enzyme family catalyzes the activation of long-chain fatty acids into their corresponding acyl-CoA esters, a necessary step for the synthesis of triacylglycerols and phospholipids, or for entry into the β-oxidation pathway (15). This family includes five isoforms: ACSL1, 3, 4, 5, and 6. Elevated expression of ACSL4 has been observed in the invasive phenotype of breast cancer (15).

Our studies have also demonstrated a significant positive correlation between FASN expression and Ki-67, as well as a significant negative association between ACSL4 expression and Ki-67 (9). Ki-67, a nuclear protein marker of cellular proliferation, is expressed during the G1, S, G2, and M phases of the cell cycle. Cellular proliferation, the hallmark of cancer cells, is regulated by Ki-67, which plays vital roles in cell cycle regulation, ribosomal RNA processing, and DNA organization (16).

This protein is typically assessed through immunohistochemistry and is widely used for diagnosing malignant cell growth. Given its crucial role in cellular processes, ongoing research seeks to leverage Ki-67 for diagnosing and treating malignancy-related diseases. In clinical practice, routine evaluation of Ki-67 expression in breast cancer tissue helps formulate treatment strategies. Numerous

studies indicate that elevated Ki-67 levels are associated with poor prognosis, increased risk of disease recurrence, and reduced survival rates (16).

Considering the correlation of these enzymes with Ki-67, they may serve as potential predictive markers in breast cancer. Therefore, in the present study, we aimed to investigate the prognostic significance of HER2 expression and key lipid metabolism enzymes, including FASN, ACLY, and ACSL4, in relation to disease recurrence and five-year survival outcomes in patients with breast cancer.

MATERIALS AND METHODS

Human breast cancer specimens and clinicopathological data

A total of 52 fresh frozen breast cancer tissue samples were collected from patients undergoing surgery at Ordibehesht Clinic in Isfahan, Iran, between 2017 and 2018. Inclusion criteria were based on the pathological confirmation of breast cancer by an experienced pathologist. Clinicopathological variables, including tumor size, stage, grade, receptor status (ER, progesterone receptor (PR), and HER2), and Ki-67 index, were extracted from patients' medical and pathology records.

The expression levels of MYC, FASN, ACLY, and ACSL4 were analyzed at the mRNA level using quantitative real-time polymerase chain reaction (qRT-PCR). Cut-off values for each gene were defined based on the median expression level within the cohort.

The p53 status was evaluated in this study using immunohistochemistry (IHC). Under normal conditions, wild-type p53 protein is unstable, rapidly degraded, and present at undetectable levels by IHC, while mutant p53 exhibits conformational alterations that render it more stable and detectable by standard IHC techniques (17). Therefore, p53 status was reported as either p53-positive (mutant p53) or p53-negative (wild-type p53).

After a five-year follow-up period, clinical outcome data were collected, including information on chemotherapy, radiotherapy, recurrence, and overall survival. All patients provided written informed consent, and the study protocol was approved by the Ethics Committee of Khomein University of Medical Sciences under the Ethical code: (IR.KHOMEIN.REC.1402.011),

RNA extraction and qRT-PCR

Total RNA was extracted from fresh frozen breast cancer samples using the BioFACT™ Total RNA Prep Kit (Ver. 2.0, BioFACT, Daejeon, Korea) according to the manufacturer's instructions. The quality and quantity of RNA were assessed by gel electrophoresis and NanoDrop spectrophotometry (OD at 260/280 nm). Complementary DNA (cDNA) was synthesized using the BioFACT™ RT-Kit (BioFACT, Daejeon, Korea) with 1 µL oligo dT primer, 1 µL random hexamer primer, 9 µL RNA, and 9 µL master mix, incubated at 50 °C for 60 min, followed by 95 °C for 5 min.

qRT-PCR was performed using the BioFACT™ 2X Real-Time PCR Master Mix on an ABI StepOnePlus system (Applied Biosystems, USA). β-actin was used as an internal control. The PCR protocol consisted of enzyme activation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing, and extension. The annealing temperature was set at 60 °C for FASN, ACLY, and ACSL4 primers, while for lipin primers, the annealing temperature was 58 °C (Table 1). The extension step was performed at 72 °C for 30 s.

Table 1. The sequences of the primers used in the study.

Genes	Forward sequences	Reverse sequences	Product length
FASN	5′-ACCATCCTGCCCAAGACT-3′	5′-ACCTTCCCACTCACTACCA-3′	107
ACLY	5′-TGCTCGATTATGCACTGGAAGT-3′	5′-ATGAACCCCATACTCCTTCCCAG-3′	202
ACSL4	5′-AGAATACCTGGACTGGGACCGAAG -3	5′-TGCTGGACTGGTCAGAGAGTGTAA-3′	148
MYC	5′-GCGACTCTGAGGAGGAACA-3′	5′-CTGCGTAGTTGTGCTGATGT-3′	183
β-Actin	5′-GTTGTCGACGACGAGCG-3′	5′-GCACAGAGCCTCGCCTT-3′	93

Melting curve analysis was conducted from 60 °C to 95 °C at 0.3 °C increments per 5 s to confirm the specificity of amplification. The threshold cycle (Ct) was determined as the fractional cycle number at which fluorescence surpassed the background. Relative expression levels were calculated using the –ΔCt method (ΔCt = Ct target – Ct β-actin). Fold changes were determined using the $2^{-\Delta\Delta Ct}$ method, comparing tumor samples with corresponding controls (18). PCR efficiency was verified by standard curve analysis with serial cDNA dilutions.

Assessment of patient survival rate

The evaluation of patient survival rates in breast cancer research is a crucial factor in understanding the effectiveness of treatment approaches and the overall prognosis of patients. In this study, we utilized a rigorous methodology to accurately assess the survival rates of breast cancer patients.

Data on patient demographics, clinical characteristics, tumor histology, treatment modalities, and follow-up information were collected during five years from Ordibehesht Clinic in Isfahan, Iran. The data collection process adhered to strict ethical guidelines and patient confidentiality protocols. The Kaplan-Meier method estimates the survival function, which is the probability of "surviving" (i.e., the probability that the event has not yet occurred) beyond a certain time point. This curve is a step function in which the estimated survival probability drops vertically whenever one or more outcome events occur, with a horizontal time interval between events. Plotting several Kaplan-Meier curves allows for a visual comparison of estimated survival probabilities between treatment or exposure groups; the curves can formally be compared with a log-rank test. The null hypothesis tested by the log-rank test is that the survival curves are identical over time; it thus compares the entire curves rather than the survival probability at a specific time point.

Statistical analysis

Statistical analyses were performed using R 4.0.5. The Chi-square test and Fisher's exact test, where appropriate, and the t-test were conducted to compare variables. For the analysis of time-to-event data, the Kaplan-Meier estimator and the log-rank test (19) were applied. *P*-values < 0.05 were considered statistically significant.

RESULTS

Survival analysis

Follow-up data were successfully obtained for 44 out of the initial 52 patients. Kaplan-Meier survival analysis was performed over 48 months to evaluate metastasis-free survival. During follow-up, 11 patients developed metastasis, while the remaining patients were censored.

Kaplan-Meier curves were generated for multiple clinicopathological and molecular variables, including age, tumor size, grade, stage, ER, PR, Ki-67, HER2, FASN, ACLY, and ACSL4. Log-rank tests were applied in univariate analyses to compare metastasis-free survival between subgroups.

The results showed that HER2 overexpression was significantly associated with shorter metastasis-free survival, as illustrated in Fig. 1. In contrast, no significant survival differences were observed for age, tumor size, grade, stage, ER, PR, Ki-67, FASN, ACLY, or ACSL4 (Fig. 2).

The mean time to metastasis was 36.18 ± 5.07 months (95% CI: 26.24-46.12), and the median was 36 months (95% CI: 24.13-47.87).

The relationship between metastasis and breast cancer clinicopathological characteristics

Statistical analysis using the log-rank test indicated that there was no significant difference in metastasis among breast cancer patients based on age, tumor grade, stage, or tumor size (Table 2).

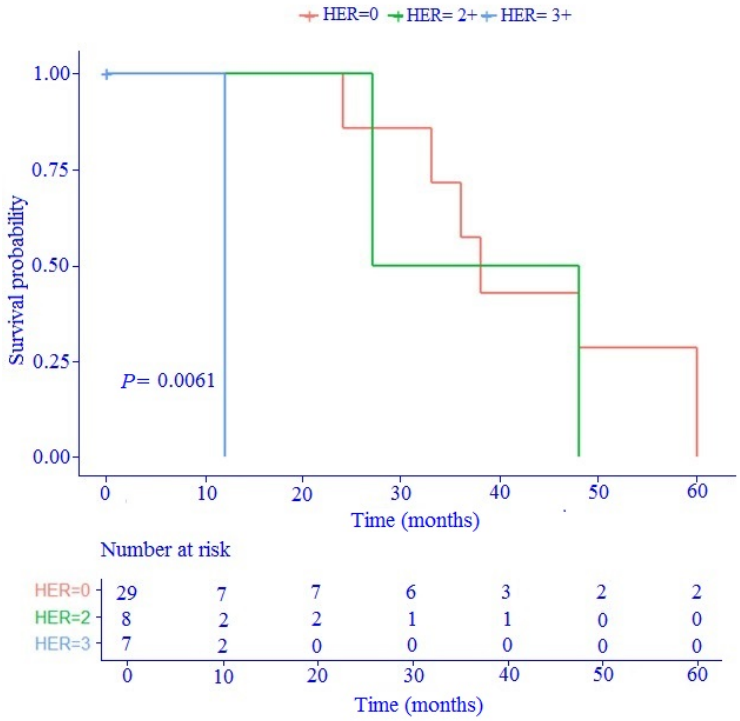
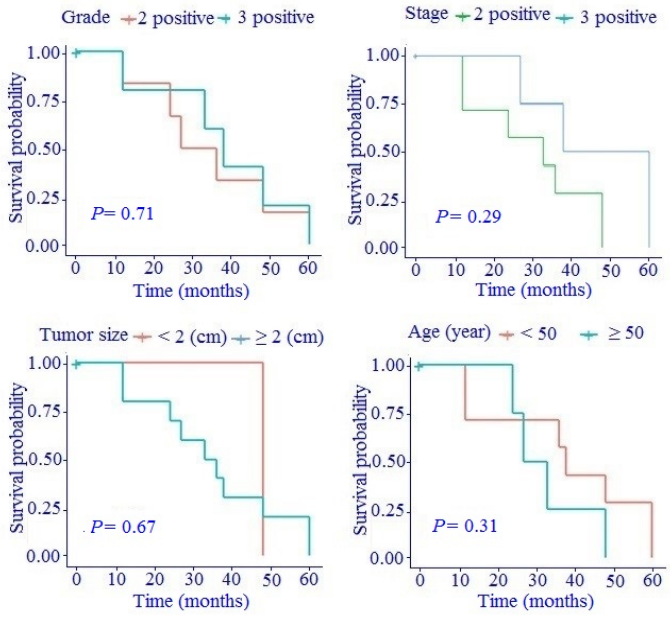


Fig. 1. Kaplan-Meier curve of metastasis-free survival according to HER-2 expression levels. Patients with HER-2 overexpression (3+) showed a significantly shorter metastasis-free survival compared to those with HER-2 negative (0) and HER-2 equivocal (2+) tumors (log-rank test, P = 0.0061). The number at risk at different time points is presented below the plot. HER, Human epidermal growth factor receptor.



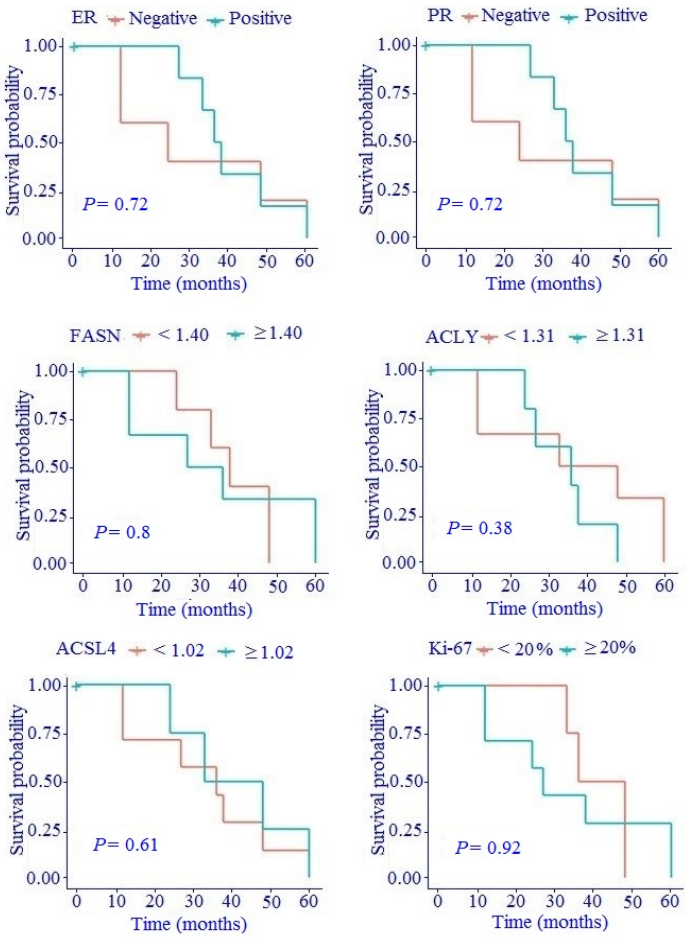


Fig. 2. Kaplan-Meier survival curves for metastasis-free survival according to clinicopathological characteristics and gene expression levels. ER, Estrogen receptor; PR, progesterone receptor; FASN, fatty acid synthase; ACLY, ATP citrate lyase; ACSL, acyl-CoA synthetase long chain.

The relationship between metastasis and expression of p53, Ki-67, ER, PR, HER, and MYC

We used the log-rank test to evaluate the relationship between metastasis and various molecular markers. Analysis of the Kaplan-Meier data set revealed a strong correlation between metastasis and HER expression in breast cancer patients. However, no statistically significant relationship was found between metastasis and the expression of ER, PR, p53, MYC, or Ki-67 (proliferation index) (Table 2).

Relationship between metastasis and ACLY mRNA expression

The relative transcriptional level of the ACLY gene was analyzed in 52 human breast cancer tissues using qRT-PCR to evaluate its potential role in breast cancer metastasis. Since the ACLY gene expression data were not normally distributed, the median expression level was used as the cutoff threshold to classify patients, and the results were divided into two groups: below 1.31 and above 1.31. Analysis using the log-rank test revealed no significant association between ACLY mRNA expression and metastasis (Table 2).

Table 2. Log-rank test results for the association of clinicopathological characteristics and gene expression levels with metastasis-free survival in breast cancer patients.

Factors	N#	Observed	Expected	*P* value
Age				
< 50 years	24	7	8.3	0.30
≥ 50 years	20	4	2.7	
Grade (n = 42)				
1	6	0	0.00	0.999
2	22	5	4.83	
3	16	5	5.17	
Stage (n = 42)				
1	4	0	0.0	0.100
2	30	7	4.8	
3	10	4	6.2	
Tumor size (n = 42)				
< 2 cm	8	1	1.43	0.700
≥ 2 cm	34	10	9.57	
Estrogen receptor (n = 43)				
Negative	10	4	4	0.999
Positive	33	6	6	
Progesterone receptor (n = 42)				
Negative	13	4	4	0.999
Positive	29	6	6	
Human epidermal growth factor receptor (n = 43)				
Negative	29	7	8.22	**0.010***
Positive (2+)	8	2	1.68	
Positive (3+)	7	1	0.10	
P53 (n = 37)				
Negative	18	3	2.44	0.600
Positive	19	7	7.56	
Ki67 (n = 43)				
< 20	24	4	3.82	0.900
≥ 20	20	6	6.18	
MYC (n = 40)				
< 1.75	20	5	4.64	0.600
≥ 1.75	20	6	6.36	
ATP-citrate lyase (n = 41)				
< 1.31	23	6	7.21	0.400
≥ 1.31	18	5	3.79	
Acyl-CoA synthetase long-chain family member 4 (n = 40)				
< 1.02	17	7	6.29	0.600
≥ 1.02	23	4	4.71	
Fatty acid synthase (n = 43)				
< 1.40	24	4	3.82	0.800
≥ 1.40	19	6	6.18	

*$P < 0.05$ indicates significant differences between the observed and expected values; #, gene expression analysis was performed on tissue samples from all 52 patients; however, not all of them could be followed during the 5-year follow-up period. Therefore, in the tables, the sample size (n) reflects only those patients for whom complete follow-up and outcome data were available.

Relationship between metastasis and FSAN mRNA expression

The relative transcriptional level of the FASN gene was examined in 52 human breast cancer tissue samples using qRT-PCR to assess its potential impact on breast cancer metastasis. Since the FASN gene expression data were not normally distributed, the median expression level was used as the cutoff threshold to classify patients, and the results were divided into two groups: below 1.40 and above 1.40. Analysis using the log-rank test revealed no statistically significant association between FASN mRNA expression levels and the incidence of metastasis in these breast cancer patients (Table 2).

Relationship between metastasis and ACSL4 mRNA expression

The expression levels of the ACSL4 gene were analyzed in 52 human breast cancer tissue samples using qRT-PCR to assess its potential impact on breast cancer metastasis. Since the ACSL4 gene expression data were not normally distributed, the median expression level was used as the cutoff threshold to classify patients, and the results were divided into two groups: below 1.02 and above 1.02. The log-rank test revealed no significant association between ACSL4 mRNA expression and metastasis (Table 2).

DISCUSSION

Dysregulation in fatty acid metabolism has been implicated in several types of cancers, including breast cancer (20,21). This suggests that increased fatty acid metabolism promotes tumor progression in various types of cancers (20). Hence, in this study, some factors that, according to previous research, seem to influence the survival of breast cancer patients were examined. Enzymes related to lipid metabolism, including FASN, ACLY, and ACSL4, are among these factors.

FASN activity is upregulated to support *de novo* fatty acid synthesis from excess pyruvate in cancer cells, particularly those exhibiting the Warburg effect. This process is essential for maintaining cell membrane production during proliferation, and increased FASN expression has been linked to various carcinomas. Similarly, upregulated ACLY is commonly found in cancer cells and functions as an oncogene, influencing tumorigenesis and tumor growth (12). By regulating lipid synthesis and altering the metabolic patterns of tumor cells, ACLY can accelerate tumor growth and metastasis (13,22). ACSL4, one of the ACSL isoforms, has also been shown to be overexpressed in various types of cancer (23). However, in the analysis of the relationship between the expression of genes of these enzymes and survival-related data, including treatment (chemotherapy, radiotherapy, or both), recurrence, and mortality of patients after 4 years of follow-up, no significant association was observed. Interestingly, our results regarding lipid metabolism enzymes diverge from several previous studies. For instance, Flavin *et al*. demonstrated that FASN overexpression was significantly associated with poor prognosis and increased tumor aggressiveness in breast cancer (24), findings were not confirmed in our cohort. Similarly, overexpression of ACLY and ACSL4 has been reported by Sun Xi *et al*. and Uchihara D. *et al.* to correlate with enhanced tumor growth and metastatic potential, in contrast to the lack of significant association observed in our study (25,26). Several biological explanations could underlie this lack of significance. First, mRNA levels may not fully reflect protein activity due to post-transcriptional and post-translational modifications. Second, compensatory metabolic pathways, such as glycolysis and glutamine metabolism, may diminish the impact of lipid biosynthetic enzymes on cancer progression. In addition, the tumor microenvironment and host-related factors such as obesity, diet, and genetic variability may have influenced lipid metabolism in ways not captured by our analyses. It should also be noted that while immunohistochemistry was performed on 10 samples per group to partially validate qRT-PCR results and showed consistent findings, comprehensive protein-level validation across all cases was not available.

Our results also showed no significant difference in the occurrence of metastasis in patients with respect to clinicopathological factors such as histological grade, stage, tumor size, and ER or PR expression. However, the log-rank test revealed a significant difference in metastasis among patients with varying HER levels.

HER-2 is a tyrosine kinase protein encoded by the ERBB2 oncogene, located on chromosome 17q21 (27). Under normal physiological conditions, this oncogene remains inactive, functioning as a standard component of the cellular genome that regulates cell growth, differentiation, and division. However, when activated, typically through gene amplification, aberrant transcriptional regulation, or mRNA overexpression, HER-2 expression can become excessive, leading to oncogenic transformation and tumorigenesis (27).

Extensive research has established a strong association between HER-2 overexpression and the pathological characteristics and biological behavior of various tumors, notably colorectal, gastric, ovarian, and breast cancers (28,29). Ma *et al.* corroborated these findings, demonstrating that peptide fragments of HER-2, following enzymatic digestion, closely resemble those of the epidermal growth factor receptor (EGFR). This similarity can lead to sustained activation of EGFR protein kinase, promoting uncontrolled cellular proliferation and tumor development, even in the absence of ligand binding (29).

The prognostic significance of HER-2/neu in breast cancer has been widely debated. While our results, along with some other studies, suggest that HER-2/neu overexpression is a crucial prognostic marker indicating poorer outcomes, other research has not consistently found a correlation between HER-2/neu status and prognosis (30,31). For example, Halon *et al.* reported that survival analyses did not confirm the prognostic relevance of HER-2 overexpression in patients with gastric cancer (32).

Further evidence from immunohistochemical evaluations of 396 breast cancer specimens revealed that HER-2/neu overexpression was present in 18% of invasive cases and was associated with a poor prognosis. Ilija *et al*. also highlighted rare but significant differences in HER-2 status between primary breast tumors and axillary lymph node metastases, which could influence therapeutic strategies. While these findings suggest that determining HER-2 status in lymph node metastases is beneficial, the potential impact of minor HER-2 variations across different lymph nodes remains unclear and requires larger studies to confirm (33).

Additionally, Wan *et al.* found that the co-expression of HER-2 with 14-3-3ζ promotes the development of invasive ductal carcinoma in the breast and facilitates lymph node metastasis. Their research suggests that the simultaneous overexpression of HER-2 and 14-3-3ζ significantly increases the risk of invasion and metastasis in breast cancer, indicating that detecting both biomarkers could be critical for guiding clinical treatment decisions and predicting patient outcomes (34).

Our study further reinforces the prognostic value of HER-2 overexpression in breast cancer, with survival curves calculated using the Kaplan-Meier method showing a significant correlation between HER-2 overexpression and decreased survival in 54 breast cancer patients. Similarly, Han *et al*. identified HER-2 as a potent predictive marker for lymph node metastasis in patients with undifferentiated early gastric cancer, linking HER-2 overexpression to increased lymphovascular invasion and metastasis (35).

In line with this, Sujarittanakarn *et al.* observed discordances in ER, PR, and HER-2 status between primary tumors and concurrent axillary lymph node metastases in 10.1% to 20.2% of cases, suggesting that retesting biomarkers in node-positive breast cancer patients is beneficial, particularly for those with negative hormone receptor and/or HER-2 status in the primary tumor but positive results in the lymph nodes (36). Correspondingly, LaBoy *et al.* reported that patients with HER-2-positive tumors face a higher risk of cancer recurrence and metastasis, regardless of the tumor's morphological features (37). However, although our findings and many others support HER-2 as a negative prognostic marker, it is important to acknowledge contradictory reports. For example, Halon *et al*. and others found no significant correlation between HER-2 status and overall survival in certain cancer cohorts (30,32). Such inconsistencies highlight the heterogeneity of breast cancer biology and suggest that the prognostic value of HER-2 may vary across patient subgroups and clinical settings.

It is important to note that the relatively small sample size (n = 52) represents a major limitation of the present study. The limited number of cases reduces the statistical power to detect significant associations, particularly for subgroup analyses. Some of the borderline results, such as the association between HER-2 expression and survival, might have reached statistical significance in a larger cohort. Therefore, the findings should be interpreted with caution and validated in larger, multi-center cohorts.

Together, these findings and our results underscore the importance of HER-2 as a

critical prognostic marker in breast cancer. Importantly, the lack of significant associations observed for FASN, ACLY, and ACSL4 in our cohort study is most likely attributable to the limited sample size and insufficient statistical power, rather than a true absence of effect. Accordingly, these negative findings should be regarded as hypothesis-generating, highlighting the need for larger, multi-center studies to more definitively elucidate the prognostic role of these lipid metabolism enzymes in breast cancer.

Several limitations of this study should be acknowledged. First, the relatively small cohort size (n = 52, reduced to n = 44 for survival analysis) substantially limits the statistical power, particularly for detecting modest associations. This constraint likely explains the lack of significant associations observed for FASN, ACLY, and ACSL4, and these negative findings should therefore be interpreted as hypothesis-generating rather than conclusive. Second, treatment-related information, especially regarding the administration of HER2-targeted therapies such as trastuzumab, was not available. This represents a critical confounding factor, as disparities in access to anti-HER2 therapy could strongly influence survival outcomes. As a result, we cannot determine whether the poorer survival observed in HER2-positive patients is attributable to the intrinsic biology of HER2 overexpression or to differences in treatment. Third, although partial validation at the protein level was attempted by IHC in a subset of samples, complete validation across the entire cohort was not feasible. Finally, as a single-center study conducted in Isfahan, Iran, the generalizability of these findings to broader populations may be limited.

CONCLUSION

In conclusion, our results support HER2 overexpression as an important prognostic marker in breast cancer, consistent with previous reports. However, the absence of treatment data and the small sample size limit the strength of these findings, particularly for survival analysis. The lack of significant associations observed for lipid metabolism enzymes should not be interpreted as evidence of no effect, but rather as preliminary observations requiring confirmation. Future large-scale, multi-center studies with comprehensive clinical and treatment data are warranted to more definitively clarify the prognostic role of both HER2 and lipid metabolism enzymes in breast cancer.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

M. Pourfarzam and R. Azizi supervised the study. N. Dinarvand designed the experiments. N. Dinarvand, M. Emadi, and M. Karbalaei Hashemiyan analyzed the data and wrote the first draft of the paper. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

Ethical approval

This study was approved by the Ethics Committees of Isfahan University of Medical Sciences (396510) and Khomein University of Medical Sciences (IR.KHOMEIN.REC.1402.011).Informed consent was taken from all patients.

Data availability

Data available upon request from the corresponding author.

AI declaration

To improve readability and language, ChatGPT was used. After using this tool, the author(s) reviewed and edited the content and take full responsibility for the content of the publication.

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