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Original Research

Clinicopathological significance and correlation of fatty acid synthase (FASN) and HER-2 expression in infiltrating duct carcinoma of the breast

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ABSTRACT

Objective: This study aimed to correlate the immunohistochemical expression of fatty acid synthase (FASN) and HER-2 with the clinicopathological variables in breast infiltrating duct carcinoma (IDC) to identify the impact of marker expression on tumor behavior and mammary carcinogenesis and to detect the correlations between the two markers. **Methods:** Immunohistochemical expressions of FASN and HER-2 were evaluated in 77 breast case including 10 normal breast, 12 ductal carcinoma *in situ* (DCIS) and 55 IDC and correlated with clinicopathological variables. The obtained data were statistically analyzed. **Results:** FASN was overexpressed in 91.7% and 70.9% of DCIS and IDC, respectively, with a significant difference from the normal ($P = 0.000$). In IDC, the expression of FASN was significantly more expressed in low-grade IDC ($P = 0.031$). FASN was significantly upregulated in larger tumors and lymph node metastasis ($P = 0.017$ and $P = 0.046$, respectively). The expression of FASN had a significant negative correlation with progesterone receptor (PR) ($P = 0.05$). HER-2 was overexpressed in 50% and 25.45% of DCIS and IDC, respectively, with a significant difference from the normal ($P = 0.026$). HER-2 was only expressed in high-grade DCIS ($P = 0.007$). In IDC, the expression of HER-2 was significantly up-regulated in larger tumors and lymph node metastasis ($P = 0.033$ and $P = 0.015$, respectively). The expression of HER-2 had a significant negative correlation with estrogen receptor (ER) and PR ($P = 0.001$ and $P = 0.023$, respectively). Correlation between the expression of FASN and HER-2 in IDC, revealed a significant positive correlation (Spearman correlation [r] = 0.374, $P = 0.005$). **Conclusions:** We concluded that overexpression of FASN and HER-2 may facilitate the early diagnosis of IDC. In IDC, their over expression might warn of a more aggressive course. Our correlation found that FAS expression is closely associated with over expression of HER-2 in IDC.

KEY WORDS: Breast, fatty acid synthase, HER-2, immunohistochemical, infiltrating duct carcinoma

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INTRODUCTION

Breast cancer happens at a high frequency among the women worldwide, accounting for 22.9% of all female malignancies. In Egypt, it is approximated to be the most common malignancy among females accounting for about 38% of total female cancer, about 12,600 newly reported cases in 2008 [1]. Early diagnosis and anticancer therapy increase patient survival. However, spread and metastasis are the most common cause of mortality in women with breast cancer [2]. Breast cancer is not a single disease. It has multiple histological varieties (ductal, lobular, etc.). According to the profiles of gene expression, breast cancer has been classified into luminal, basal-like, HER-2+ and normal breast-like subtypes. Even within each group, there is significant variation in the prognosis and response to therapy [3]. The biosynthetic enzyme fatty acid synthase (FASN) is the main enzyme needed for the anabolic conversion of dietary carbohydrates to fatty acids, and it works normally in cells that have high lipid metabolism. Under normal physiological conditions, any FASN increase is tightly controlled by a number of environmental, hormonal, and nutritional signals [4,5].

However, researches on human tissue detected that infiltrating carcinomas of the breast constitutively express higher levels of FASN in contrast to nontransformed mammary epithelial tissue [6,7]. Furthermore, the highest levels of FASN detected with the development of *in situ* carcinoma of the breast, suggesting a possible relationship between increased expression and increased risk of mammary carcinogenesis [8]. Others have then found that FASN was overexpressed in breast cancer of poor prognosis [6] and that its suppression was efficient to slow the growth mammary tumors in rodents [9]. Overexpression of FASN in several human malignancies and its association with worse prognosis both reinforce the hypothesis that FAS is

involved in the carcinogenesis, maintenance, and enhancement of the malignant phenotype [10].

Among the numerous oncogenes and their products, HER-2 is the most widely used in clinical oncology. HER-2 has shifted from a laboratory-based prognostic factor to be used as a target for specific therapy, such as treatment with trastuzumab [11]. HER-2 protein overexpression has been detected in 20-30% of human invasive breast carcinomas and is associated with a poor prognosis even with systemic chemotherapy [12-14]. The amplification and overexpression of HER-2 have been detected in up to 50% of ductal carcinoma *in situ* (DCIS) cases [15].

Many previous studies reported that FASN-dependent signaling regulates the expression, activity, and cellular localization of the HER-2 oncogene in breast and ovarian cancer cells. They concluded that pharmacological and small interference RNA-mediated suppression of FASN negative controls the expression of HER-2 at the transcriptional level, and detect the ets transcription factor PEA3 as a molecular mechanism through which FASN blockade transcriptionally represses HER-2 gene expression [16,17]. Menendez *et al*. [18] further proved that simultaneous targeting of FASN and HER-2 synergistically down regulates p185HER-2 and suppresses tumor cell proliferation by promoting apoptosis. The aim of this study was to correlate the immunohistochemical expression of FASN and HER-2 with the clinicopathological variables of infiltrating duct carcinoma (IDC) of the breast to identify the impact of marker expression on tumor behavior and mammary carcinogenesis and to detect the correlations between the two markers among the studied IDC.

MATERIALS AND METHODS

Patients and Clinical Data

Our research was performed at the Departments of Pathology and Surgery, Zagazig University Hospital, Egypt. We collected 25 cases of IDC from patients who underwent modified radical mastectomy between May 2015 and September 2016. We also used residual archival blocks of breast tissue with their related data from the archive of the pathology department in the period from 2011 to 2016. Finally, we chose 10 cases of benign breast disease (BBD) containing "normal" mammary glands adjacent to BBD, 12 cases of DCIS and 55 cases of IDC. All tissue samples were formalin-fixed and paraffin-embedded. We collected the clinical, pathological, and immunohistochemical (estrogen receptor [ER], progesterone receptor [PR] and Ki-67 expression) information from the medical records of the patients. All of the patients did not receive any chemotherapy or radiation before the surgical interference. Histological typing and grading have followed the World Health Organization classification and modified Bloom–Richardson grading [19]. The study was conducted with full local ethics approval. All collected blocks were cut at 4 microns and stained with ordinary H and E stain to confirm the diagnosis.

Immunohistochemical Staining

After cutting our blocks into 4μm, the sections were deparaffinized with xylene, rehydrated in graded alcohols, and placed in 0.5% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. Antigen retrieval was carried out by incubation in 0.01 M citrate buffer (pH 6.0) for 5 min in a pressure cooker. The sections were exposed to the primary antibody for 60 min at room temperature. We used streptavidin-biotin-peroxidase complex method for FASN (rabbit polyclonal antibody, Clone H-300, dilution 1:50, Dako, California, USA) and HER-2 (mouse monoclonal antibody, ready to use, clone e2-4001, catalog number MS-730-R7-A, Lab Vision, California, USA) by employing diaminobenzidine as the chromogen. Liver tissue and breast carcinoma were used as a positive control for FASN and HER-2, respectively, while negative controls for both markers were obtained by omitting the primary antibody.

Evaluation of Immunohistochemical Staining

Both intensity and extent of cytoplasmic FASN immunoreactivity were evaluated as follows: Staining intensity was graded as negative (0), weak (1+), moderate (2+), and strong (3+). The extent of stain was evaluated by determining the percentage of FASN stained cells with a range from 0% to 100%. Finally, the overexpression (the positive FAS expression) was defined as a tumor with positive staining (1+ to 3+) in 10% or more tumor cells [20]. Tissue expressions of membranous HER-2 immunoreactivity were similarly classified into four groups as follow: 0 (negative; no staining observed, or membrane staining in <10% of the tumor cells); 1 (negative; faint/barely perceptible focal membrane staining in >10% of tumor cells); 2 (positive; weak to moderate staining of the complete cell membrane in >10% of the tumor cells); or 3 (positive; strong staining of the complete membrane in >10% of the tumor cells) [21], finally, for statistical analysis the HER-2 expression was grouped into negative (0, 1) and positive or overexpressed (2, 3).

Statistics

The results from the analysis of the continuous variable are expressed as a means ± standard deviation. Analysis of categorical data was performed using the x^2 or Fisher's exact test, Spearman correlation was performed to assess the correlation between FASN and HER-2. All statistical analyses were performed using SPSS software (version 19.0; SPSS, Chicago, IL) and $P \leq 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Patients and Their Clinicopathological Parameters

A total of 77 cases were included in this study, 10 (12.98%) cases were normal breast, 12 (15.58%) cases were DCIS, and 55 (71.42%) cases were IDC [Table 1]. Among the studied DCIS 5 cases were low-grade (GI/II) and 7 cases were high-grade (GIII) [Table 2]. The mean age of the studied 55 cases of IDC patients at initial surgery was 52.18 ± 10.52 years (range 28-66 years). 61.8% of the studied IDC were large (≥2 cm) in size. 76.3% of tumors were low-Grade (I and II) and 60% were stage I/II. Lymph node metastasis was positive in 58.2% of the cases. The positive rates of ER and PR were 63.6% and 54.5%, respectively. Ki-67 expression was high (≥20%) in 50.9% of cases [Table 3].

Table 1: Comparison of FASN and HER-2 expressions between Normal breast, DCIS, and IDC

Marker	Expression	Total *n*=77 (%)	Normal breast *n*=10 (%)	DCIS *n*=12 (%)	IDC *n*=55 (%)	*P* value
FASN	(+)	50 (64.9)	0 (0.0)	11 (22.0)	39 (78.0)	0.000
	(-)	27 (35.1)	10 (37.0)	1 (3.7)	16 (59.3)	
HER-2	(+)	20 (26.0)	0 (0.0)	6 (30.0)	14 (70.0)	0.026
	(-)	57 (74.0)	10 (17.5)	6 (10.5)	41 (72)	

FASN: Fatty acid synthase, DCIS: Ductal carcinoma *in situ*, IDC: Infiltrating duct carcinoma

Table 2: Correlation of FASN and HER-2 expressions in different grades of DCIS

Marker	Expression	Total *n*=12 (%)	DCIS		*P* value
			G I/II *n*=5 (%)	G III *n*=7 (%)	
FASN	(+)	11 (91.7)	5 (45.5)	6 (54.5)	0.583
	(-)	1 (8.3)	0 (0.0)	1 (100)	
HER-2	(+)	6 (50)	0 (0.0)	6 (100)	0.007
	(-)	6 (50)	5 (83.3)	1 (16.7)	

FASN: Fatty acid synthase, DCIS: Ductal carcinoma *in situ*, IDC: Infiltrating duct carcinoma

Table 3: Correlation of the clinicopathological parameters of the studied IDC cases with the results of FASN and HER-2 immunoreactivity

Variable	Number (%)	FASN		HER-2	
Age at surgery (y)		(+)	(-)	(+)	(-)
<55	25 (45.5)	19	6	6	19
≥55	30 (54.5)	20	10	8	22
P value		0.447		0.821	
Tumor size					
<2 cm	21 (38.2)	11	10	2	19
≥2 cm	34 (61.8)	28	6	12	22
P value		0.017		0.033	
Histological grade					
Low-grade (I and II)	42 (76.3)	33	9	9	33
High-grade (III)	13 (23.6)	6	7	5	8
P value		0.031		0.19	
Tumor stage					
I/II	33 (60)	24	9	7	26
III/IV	22 (40)	15	7	7	15
P value		0.716		0.376	
LN metastasis					
Positive	32 (58.2)	26	6	12	20
Negative	23 (41.8)	13	10	2	21
P value		0.046		0.015	
Oestrogen receptor					
Absent	20 (36.4)	13	7	10	10
Present	35 (63.6)	26	9	4	31
P value		0.465		0.001	
Progesterone receptor					
Absent	25 (45.5)	21	4	10	15
Present	30 (54.5)	18	12	4	26
P value		0.05		0.023	
Ki-67					
Expression≥20%	28 (50.9)	19	9	9	19
Expression<20%	27 (49.1)	20	7	5	22
P value		0.611		0.246	
Total (%)	55 (100)	39 (70.9)	16 (29.02)	14 (25.45)	41 (74.54)
		55 (100)		55 (100)	

FASN: Fatty acid synthase, IDC: Infiltrating duct carcinoma

Immunohistochemical Expression of FASN

FASN immunoreactivity was detected in the cytoplasm of malignant cells. FASN staining was overexpressed in 91.7% (11/12) and 70.9% (39/55) of the studied DCIS [Figure 1b and c] and IDC [Figure 2a-c], respectively, with a highly significant difference from the normal group which did not express any FASN positivity ($P = 0.000$) [Table 1]. FASN staining was strongly expressed in DCIS regardless of the grade ($P = 0.583$) [Table 2]. In the studied IDC cases, the expression of FASN was more strongly expressed in low-grade IDC when compared with high-grades with a significant relationship ($P = 0.031$). FASN was significantly up-regulated in the larger size tumors (tumor diameter >2 cm) and lymph node metastasis positive group ($P = 0.017$ and $P = 0.046$, respectively). The expression of FASN had also a significant negative correlation with a status of PR ($P = 0.05$). There was no significant correlation with age, clinical stage, ER and Ki-67 level ($P > 0.05$) [Table 3].

Immunohistochemical Expression of HER-2

In this study, membranous HER-2 immunoreactivity was expressed in 50% (6/12) and 25.45% (14/55) of the studied DCIS [Figure 1d] and IDC [Figure 2d], respectively, but not detected in all normal breast [Figure 1a], with a statistically significant difference ($P = 0.026$) [Table 1]. HER-2 was only expressed in high-grade DCIS with significant relationship ($P = 0.007$) [Table 2]. In the studied IDC, the expression of HER-2 was significantly up-regulated in the larger size tumors (tumor diameter >2 cm) and lymph node metastasis positive group ($P = 0.033$ and $P = 0.015$, respectively). The expression of HER-2 had a significant negative correlation with a status of ER and PR ($P = 0.001$ and $P = 0.023$, respectively). There was no significant correlation with age, clinical stage, and Ki-67 level ($P > 0.05$) [Table 3].

The Correlation Analysis between FASN and HER-2 Expressions among the Studied IDC Cases

Based on a correlation analysis between the results of immunohistochemical expression of the two markers among the studied IDC cases, a significant positive correlation was found between the expression of FASN and HER-2 proteins (Spearman correlation [r] = 0.374, $P = 0.005$), [Table 4].

DISCUSSION

FASN is a heterodimeric enzyme that facilitates the formations of long-chain fatty acids. The gene encoding FASN is located on the long arm of chromosome 17 (17q25). In some tumors, including breast cancer, the gene is overexpressed, possibly to satisfy the increase in membrane synthesis as a result of increased cell proliferation. [22,23]. It has been suggested that FAS plays an important function not only in tumor growth but also in tumor survival and drug resistance with de novo lipogenesis [24]. There may also be a regulatory impact of FASN on the expression of the close (17q21) HER-2 gene [18]

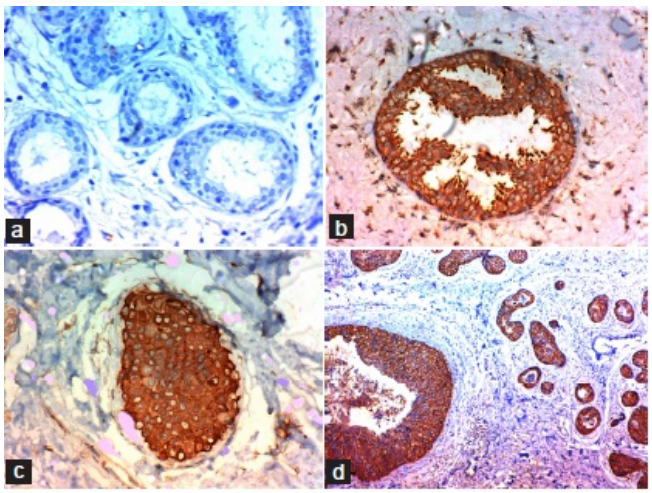

Figure 1: Fatty acid synthase (FASN) and HER-2 immunoreactivity in representative cases of normal breast and ductal carcinoma *in situ* (DCIS). (a) Normal breast shows negative HER-2 immunoreactivity (×400). (b) Strong cytoplasmic FASN staining in low-grade DCIS (×400). (c) Strong cytoplasmic FASN staining in high-grade DCIS (×400). (d) Diffuse membranous HER-2 staining in high-grade DCIS (×200)

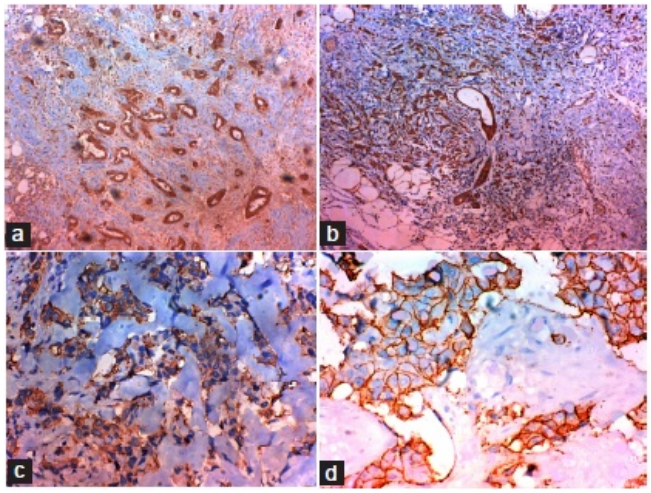

Figure 2: Fatty acid synthase (FASN) and HER-2 immunoreactivity in representative cases of infiltrating duct carcinoma (IDC). (a) Strong cytoplasmic FASN immunoreactivity in Grade I IDC (×200) (b) Strong and diffuse FAS staining in GII IDC (×100). (c) Weak FASN immunoreactivity in GIII IDC (×400). (d) Diffuse membranous HER-2 staining in GIII IDC (×400)

Table 4: The correlation analysis between FASN and HER-2 expression among the studied IDC cases

Marker expression	HER-2 *n* (%)		Total (%)
	(+)	(-)	
FASN *n* (%)			
(+)	14 (25.45)	25 (45.45)	39 (70.9)
(-)	0 (0)	16 (29.02)	16 (29.02)
Total	14 (25.45)	41 (74.54)	55 (100)

Spearman correlation (r)=0.374, *P*=0.005. FASN: Fatty acid synthase, DCIS: Ductal carcinoma *in situ*, IDC: Infiltrating duct carcinoma

and a study of Alli *et al*. [25] raises the possibility of cancer chemoprevention by suppression of FASN.

In this study, we examined FASN and HER-2 expression in a group of Egyptian breast cancer patients. The study included 55 cases of IDC, 12 cases of DCIS, and 10 normal tissues from BBD.

Our analysis detected FASN overexpression in 91.7% and 70.9% of the studied DCIS and IDC, respectively, and the expression was highly significantly different from the normal breast ($P = 0.000$), this is consistent with the result obtained from the previous related studies [26,27]. But in contrast to our finding as regards no FASN overexpression in any of the normal breast, Esslimani-Sahla *et al*. [28] detected low, diffuse and occasionally intense focal FAS staining in the cytoplasm of all normal epithelial mammary cells, this difference may be due to different methodology and different method of interpretation of the marker expression. But inconsistent with our finding, Esslimani-Sahla *et al*. [28] found that % of FASN positive cells was 2-4-fold higher in lesions than in adjacent normal cells and the increased FAS expression was significant in nonproliferative BBD, proliferative atypia, low-grade and high-grade DCIS, and continued to increase with increasing the risk of the lesions.

Therefore, we could infer that FASN may play role in early-stage carcinogenesis of mammary duct carcinoma and their overexpression may assist the early diagnosis of IDC.

In this study, we found that FAS expression was not correlated with the grade of DCIS, and thus similar to the finding of Yang *et al*. [26] and Esslimani-Sahla *et al*. [28] who found that FAS level in the low-grade DCIS was not significantly different from that in the high-grade. However, this is different from the finding of Milgraum *et al*. [8] who found increased FAS expression in high-grade DCIS but not in low-grade DCIS. The reason for the discrepancy in FASN expression in low-grade DCIS may be due to a different mode of quantification of marker expression.

In this study, correlations of FASN expression with the clinicopathological features of the studied IDC cases revealed significant up regulation of FASN in the larger size tumor group ($P = 0.017$), which is similar to many previous related studies [6,26,27]. FASN expression also more strongly expressed in low-grade IDC when compared with high-grades IDC with a statistically significant relationship ($P = 0.031$), this is similar to previous related study [26]. FASN was also significantly up-regulated in the lymph node metastasis positive group ($P = 0.046$), this is consisting with the finding of Zhou *et al*. [27] and also in agreement with the study of Alo *et al*. [6] who found significant FASN expression with peritumoral lymphatic vessel invasion.

In this study, a significant negative correlation between FASN and PR was found. This finding is similar to previous related studies [6,26]. A similar negative correlation between FASN and PR in DCIS cases was detected by Esslimani-Sahla *et al*. [28].

In this study, HER-2 expressed in 50% and 25.45% of the studied DCIS and IDC, respectively, which is nearly similar to

previous related studies [15,21,29]. This finding was statistically significantly different from the finding in the normal group that did not show any overexpression of HER-2 ($P = 0.026$). This is consistent with the finding of Allred *et al*. [29] who did not detect any overexpression of HER-2 in any of the examined hyperplastic or dysplastic lesions. In this study, we found that HER-2 was only significantly expressed in high-grade DCIS in comparison with the low-grade DCIS ($P = 0.007$), this is similar to the finding of Esslimani-Sahla *et al*. [28]. Park *et al*. [21] found that HER-2 amplification was significantly increased in the high-grade intraductal component of IDC, which is consistent with our finding, but in contrast to ours, he found no significant change in HER-2 amplification according to the grade of pure DCIS.

These results supported the hypothesis that HER-2 plays a more important role in initiation and carcinogenesis than in progression of ductal carcinomas.

In this study, correlations of HER-2 expression with the clinicopathological features of the studied IDC cases revealed that up regulation of HER-2 was significantly associated with several poor prognostic features including larger size of the tumor ($P = 0.033$) and lymph node metastasis ($P = 0.015$), which is consistent with many previous related studies [30,31]. The expression of HER-2 had a significant negative correlation with a status of ER and PR ($P = 0.001$ and $P = 0.023$, respectively), which is consistent with many previous studies [31-34].

In this study, a significant positive correlation was detected between the expression of FASN and HER-2 among the studied IDC cases (Spearman correlation [r] = 0.374, $P = 0.005$). This finding is consistent with Yang *et al*. [26] who concluded that FAS expression is closely associated with HER-2 gene amplification in IDC ($r = 0.44$, $P < 0.01$). Kumar-Sinha *et al*. [35] characterized a molecular connection between the HER-2 oncogene and FAS in human breast cancer cells that is implicated in tumorigenesis. Many recent studies try to get benefit from the relationship between the two genes in cancer chemotherapy resistance [36-38].

It is concluded that overexpression of FASN and HER-2 may facilitate the early diagnosis of IDC. We concluded that both are prognostic marker that up regulated with poor prognostic features including larger size of the tumor and lymph node metastasis. Hence, their over expression in IDC might warn of a more aggressive course. Our result found that FAS expression is closely associated with over expression of HER-2 in IDC. Hence, more studies on larger cohorts of Egyptian women, to get benefit from the expression and the relation between these two genes in breast cancer diagnosis, prognosis, and chemotherapy are deserved.

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