Pathol. Oncol. Res. (2009) 15:241–250
DOI 10.1007/s12253-008-9109-x

ORIGINAL PAPER

Serum sFas and Tumor Tissue FasL Negatively Correlated with Survival in Egyptian Patients Suffering from Breast Ductal Carcinoma

Ashgan I. El-Sarha · Gehan M. Magour · Sameh M. Zaki · Mohamed Y. El-Sammak

Received: 30 May 2008 / Accepted: 2 October 2008 / Published online: 18 November 2008

Abstract Fas (CD95-APO-1), a member of tumor necrosis factor receptor super-family, exists in two forms, transmembrane and soluble (sFas). It had been suggested that circulating sFas levels and/or tissue FasL may reflect the severity of invasive breast ductal carcinoma. Few studies showed that neither DNA-index nor ploidy is an independent prognostic indicator, and there is no correlation with clinical outcome. The S-phase fraction (SPF) has been shown to be useful prognostic factor in both node-negative and node-positive tumors. The present work was done to find a correlation between sFas, tissue FasL, ploidy and SPF with prognostic factors and survival of breast ductal carcinoma patients. The present study included two groups; a patients group comprised 30 patients with breast ductal carcinoma and a control group that comprised 15 patients with benign breast swellings. Serum sFas was measured using commercially available ELISA kit and tissue FasL expression was studied using avidin–biotine immunohistochemical staining technique. Cell cycle studies were performed using flow cytometry. Serum sFas was significantly higher in breast ductal carcinoma group than in the benign breast swelling control group. A significant negative correlation between serum sFas and overall survival was found. Tissue FasL expression was directly correlated with distant metastasis and poor overall survival. A significant direct correlation was found between moderate and high SPF with worse pathologic parameters. Serum sFas level, tissue FasL immuno-expression and S-phase fraction are independent prognostic factors in breast ductal carcinoma cases.

Keywords sFas · FasL · Apoptosis · DNA ploidy · S-phase fraction

A. I. El-Sarha
Department of Pathology,
Medical Research Institute Teaching Hospital,
Alexandria University,
Alexandria, Egypt

G. M. Magour · M. Y. El-Sammak (✉)
Department of Chemical Pathology,
Medical Research Institute Teaching Hospital,
Alexandria University,
165 El Horreya Street, P.O. Box 21561, Alexandria, Egypt
e-mail: myelsammak@doctors.org.uk

S. M. Zaki
Department of Radiation Science,
Medical Research Institute Teaching Hospital,
Alexandria University,
Alexandria, Egypt

Introduction

Programmed cell death or apoptosis is a common form of cell death and is found during tumor regression and embryonic development. Apoptosis is characterized by changes in cellular morphology (e.g. nuclear condensation and membrane blebbing) and biochemically by rapid induction on DNA fragmentation. Fas/Fas ligand (FasL) system is a major regulator of apoptosis, induced by the immune system [1].

Fas (CD95-APO-1), a member of tumor necrosis factor (TNF)/nerve growth factor receptor super-family [2, 3], is a glycosylated 48KD surface protein and consist of 325 amino acids. Fas is a cell surface receptor protein that exists in two forms, transmembrane and soluble (sFas). The former induces apoptosis by ligation with FasL or agonistic anti-Fas antibody, whereas the latter inhibits Fas-mediated apoptosis by neutralizing its ligand [4]. Many organs express Fas; including liver, heart, kidney and ovary. However, Fas is highly expressed in activated mature

lymphocytes. It was reported that expression of Fas is enhanced by interferon-δ (INF-δ) and TNF [5].

On the other hand, FasL, a member of TNF family of membrane associated cytokine, is a protein of molecular weight 40 KD and consisting of 281 amino acids with the N-terminal region outside the cell [6]. After activation through T-cell receptor, cytotoxic lymphocytes express FasL which binds to transmembrane Fas on target cells, including cancer cells, to induce apoptosis. It has been suggested that circulating soluble Fas (sFas) contributes to tumor progression. Also it has been suggested that circulating sFas levels and/or tissue FasL may reflect the severity of invasive breast cancer [7].

Tumor cells and tumor infiltrating lymphocytes may express Fas and FasL in various proportions and their interplay may affect tumor behavior [5]. Down-regulation of Fas has been shown in some carcinomas including breast cancer [8], whereas FasL is sometimes over-expressed in many human tumors including breast cancer [9, 10, 12, 13]. FasL-expressing tumors have been reported to have a significantly worse prognosis [11–14]. It is hypothesized that these tumor cells can escape from immune surveillance via a counter attack on activated T cells and natural killer cells that express Fas [15]. Regarding anticancer therapy, some preclinical studies suggest that classic anticancer agents also require the death receptor Fas and its ligand (FasL) to induce cell death [16, 17].

The degree of DNA content abnormality is reported as the DNA-index which represents a ratio of G0–G1 peaks for the tumor cells and normal reference cells. Few studies showed that neither DNA-index nor ploidy is an independent prognostic indicator, and they have no correlation with clinical outcome [18, 19]. The S-phase fraction (SPF), a calculation of proliferation rate obtained by DNA analysis through flow cytometry, has been shown to be useful in both node-negative and node-positive tumors [20].

The aim of the present work was to evaluate the serum sFas level, tissue FasL immunostaining, DNA ploidy and SPF in Egyptian female patients suffering from breast ductal carcinoma and also to assess their correlation with conventional pathologic parameters and overall survival.

Subjects and Methods

The present study was approved by Medical Research Institute Ethical Committee. Female patients included in this study were admitted in the Cancer Research and Treatment Department in the Medical Research Institute, Alexandria University, Egypt. All patients and control subjects gave their written informed consent before participating in the study. The current study included two groups; a patients group comprised 30 cases with breast ductal carcinoma, and a control group, 15 cases, of matched age and socioeconomic status with benign breast swellings; nine patients had fibrocystic disease and six had fibroadenoma. All patients were randomly selected from a large sample of female patients presented with breast lumps. Subjects who are pregnant or receiving chemo-, radio- or hormonal therapy, contraceptives and steroid medication and those with liver, renal or heart disease were excluded from the present study. All cases were subjected to complete family history, physical examination of both breasts, axillae,, supraclavicular area, abdomen and pelvis, radiologic studies including chest X-ray, bilateral mammography, electrocardiogram (ECG), ECHO cardiography, abdominal ultrasound, bone scan and CT scan of brain especially in suspected cases, fine needle aspiration followed by surgical removal (lumpectomy or mastectomy), pathologic study and follow up to detect relapse free survival and patients outcome. Clinical staging of malignant cases was performed according to TNM staging system [21]. All subjects were not taking hormonal- or chemo-therapy at least 6 months before laboratory investigations. All malignant patients included in this study were followed up for 60 months.

Laboratory Investigations

Venous blood samples were taken from each subject after an over night fast. One milliliter EDTA blood sample was used for complete blood picture. Four milliliters of blood were collected in plain tubes, centrifuged and analyzed for testing serum glucose, urea, creatinine, protein, albumin and liver enzymes; alanine and aspartate aminotransferases (ALT, AST), serum alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT), serum calcium (total and ionized) and phosphorus. These were measured using a Konelab Chemistry Analyzer (Thermo Electron Oy, Vantaa, Finland. https://www.thermo.com) [22]. Parathyroid hormone was measured using an immuno-radiometric assay "IRMA" technique (Kit from Scantibodies laboratory, Inc.) [23]. Serum soluble Fas (sFas) level was determined using a commercially available enzyme-linked immunosorbent assay "ELISA" kit (Bender Medsystems GmbH Campus Vienna, Austria (Cat.no:BMS245CE) [24]. The Assay has a sensitivity of 20 pg/ml and an intra-assay coefficient of variation (CV) of 4.5%.

Pathological Examination

All of the tissues, benign and malignant, had been fixed in 10% buffered formalin, processed, and embedded in paraffin according to the normal schedule used in the laboratory. Routinely prepared H&E sections were carefully

examined to diagnose each case (benign or malignant) and to grade ductal carcinoma cases.

Immunohistochemical Staining

From each paraffin block, 4 μm-thick sections were cut on coated slides and dried overnight at 37°C. The sections were deparaffinized in xylene and rehydrated through graded concentrations of ethanol to distilled water. Sections to be stained with anti-FasL antibodies were pretreated by digestion in 0.5% trypsin (pH 7.2) at 37°C for 30 min. Immunohistochemical staining was performed by using commercial Elite avidin–biotine complex "ABC" kits [25]. Blocking serum was applied for 15 min followed by overnight incubation with the diluted primary antibodies; anti-FasL (1:100, Santa Cruz Biotechnology, Inc, CA, USA), anti-ER and anti-PR (1:130 each, Biogenex, San Ramon, CA, USA), and anti-HER-2/neu (1:50, Dako, Carpintera, CA, USA). The sections were then incubated with the biotinylated secondary antibody and the peroxidase-labeled ABC solution for 30 min each. All of the dilutions were made in PBS (pH 7.2), and all of the incubations were performed in humid chambers at room temperature. Between each step in the staining procedures (except before incubation with the primary antibody), the slides were rinsed three times in PBS. Bound peroxidase was visualized in all of the slides with a 3-amino-9-ethylcarbazole solution as chromogenic substrate. Sections were then counterstained with Mayer hematoxylin and were mounted in aqueous mounting medium. Regarding assessment of FasL-immunostaining, it was noted that benign and malignant tumors displayed two kinds of immunoreactivity: (1) a well demarcated cell membrane staining prevalently accompanied by a granular cytoplasmic reactivity, or, (2) a diffuse cytoplasmic staining varying from moderate to strong. Cases with faint, uncertain cytoplasmic staining were regarded as negative. FasL immunostaining was scored, independent of membrane or cytoplasmic staining localization, as follows: negative, no expression on tumor cells; heterogeneous expression, 10–50% positive tumor cells; and homogeneous, >50% positive tumor cells [26]. Regarding estrogen and progesterone receptors, tumors were defined positive when 10% or more of the tumor cells showed unequivocal nuclear staining [25]. HER-2/neu was considered over-expressed only when at least 10% of the neoplastic cells displayed a distinct plasma membrane staining (++/+++score) [27].

Flow Cytometry

Cell cycle analysis was performed in the cells obtained from malignant tumor tissues. DNA flow cytometry was used as described elsewhere [28, 29]. Briefly, tumor samples were minced thoroughly with scissors. The nuclei were extracted at room temperature by incubation in acid pepsin (3,000 units/mg), dissolved in 100 ml of 0.9 NaCl containing 0.25% HCl, and carefully stirred for 20 min. After 30 s of sedimentation, 0.5 ml of the supernatant cell suspension was suspended in 1μg/ml 4′, 6-diamindino-2-phenylindole dissolved in tris-buffer (pH 7.8) for at least 30 min. Flow cytometry was carried out using a PAS II flow cytometer equipped with a high-pressure mercury lamp. A flow rate of about 100 counts/s was maintained by adjusting the vacuum. DNA histogram of at least 10,000 counts was blotted. The DNA index of aneuploid cells was expressed as the relative modal DNA value of the aberrant peak in relation to the diploid peak. The cell-cycle-phase distribution patterns of the diploid and aneuploid tumors were calculated using the Multicycle software package. Values of s-phase fraction were classified as low, medium and high values [20].

Therapeutic Procedures

Six cycles of FAC (5-flourouracil "500 mg/m^2", adriamycin "50 mg/m^2", and cyclophosphamide "500 mg/m^2") were considered to be the standard treatment in most patients repeated every 3 weeks if no contraindications to anthracyclines is present [30]. Patients with any contraindication for anthracyclin received six cycles of CMF (cyclophosphamide "600 mg/m^2", methotrexate "40 mg/m^2" and 5-fluorouracil "600 mg/m^2"). Patients were followed thoroughly every cycle, with clinical and radiological assessment after three cycles so as to continue on the same regimen or to shift to either navelbine "25 mg/m^2" day 1 and 8 and 5-fluorouracil "600 mg/m^2" day 1 and 8 or taxans based regimen. Adjuvant hormonal therapy "tamoxifen" only was administered whenever tumor was ER and/or PR positive. Loco-regional radiotherapy 50 Gy/5 weeks (5 fractions/week) was given as adjuvant therapy after modified radical mastectomy when indicated. Palliative radiotherapy "30 Gy × 10 fractions" was given to cases developed bone or brain metastases. Postoperatively, patient received only radiotherapy (55%) and/or chemotherapy (85%). Only patients with positive ER and/or PR continued hormonal therapy (60%).

Statistical Analysis

Comparison among the groups was conducted using ANOVA test for normally distributed variables. The Nonparametric Mann–Whitney *U* test was used for variables with non Gaussian distribution. Normally distributed variables were expressed as Mean ± SD while variables with non-Gaussian distribution were expressed as median, range, and 25 to 75 percentiles. For correlation studies, Pearson correlation test was used. *P* value of <0.05 was considered statistically significant. The commercial statisti-

cal software package used was SPSS 11.0 (SPSS, Inc., Chicago, IL).

Correlations with some biochemical and histopathological parameters were analyzed using the chi square "χ^2" test. Correlations with overall survival were analyzed using the Kaplan–Meier methods, and comparison of study groups was performed using the log-rank test.

Results

The median age of cancer patient at time of presentation was 50 years (23–70). Tumor size was classified according to the TNM staging system [21] into; four cases T1, 15 cases T2, 7 cases T3 and four cases T4. Twenty cases (67%) were associated with axillary lymph node involvement at initial surgery. All cases were free from distant metastasis at initial diagnosis. Twenty one cases (70%) were positive to ER. PR positivity was found in 18 cases (60%). Her-2/neu showed positivity in only nine cases (27%). Kaplan–Meier graphs (Fig. 2) showed that there was a significant statistical difference in survival rate in favor of Fas-negative cases.

Patients were followed up for 60 months. During this period of observation seven cases (23%) showed distant metastasis; two cases (7%) to liver, two cases (7%)to lung, one case (3%) to brain, one case (3%) to brain and liver, and one case (3%) to liver and bone. One case (3%) showed local recurrence at 13 months of the follow up, while 12 cases (40%) died of the disease.

Laboratory Results

Table 1 illustrates some biochemical data of the studied groups. There was no significant age difference between the two groups enrolled in this study. Serum alkaline phosphatase was found to be significantly higher in breast cancer patients compared to patients with benign breast swellings ($P < 0.05$). Serum sFas was significantly higher in breast ductal carcinoma group than in the benign control group ($P < 0.05$). The median range of sFas in patients with breast ductal carcinomas and those with benign breast lesions were 1133 pg/ml (167–3555 pg/ml) and 400 pg/ml (133–650 pg/ml) respectively (Fig. 1). Follow up data proved that patients with high serum sFas had a poor survival compared with those with low serum levels (Fig. 2a). Statistically, it was found that there was a significant negative correlation between serum sFas and survival (r: −0.405 & $P < 0.05$).

FasL Expression

Thirteen out of the included 30 ductal carcinoma cases showed positive FasL immunostaining (Table 2, Fig. 3).

Table 1 Clinical and biochemical criteria of the studied groups

Parameter	Controls (n=15)	Patients (n=30)
Glucose (mg/dl)	80.1±2.4	92.3±4.1
Calcium		
Total (mg/dl)	9.14±0.4	9.2±1.4
Ionized (mg/dl)	4.03±0.21	4.7±0.21
Phosphorus (mg/dl)	3.0±0.5	3.2±0.5
PTH (pg/ml)	21.01±0.63	21.2±0.61
Cholesterol (mg/dl)	206.0±67.1	210.4±55.1
TG (mg/dl)	101.5±49.9	132.6±79.9
SAP (U/L)	82.1±11.3	154.1±135.5[a]
AST (U/L)	25.9±39.9	24.5±24.51
ALT (U/L)	24.9±62.7	24.4±16.6
Protein (g/dl)	6.75±0.62	6.9±0.9
Albumin (g/dl)	4.05±0.3	3.8±0.5
Urea (mg/dl)	36.7±21.5	36.4±18.13
Creatinine (mg/dl)	0.9 7±0.16	0.95±0.3

Data are presented as mean ± SD. Control group include patients with benign breast masses (mainly fibroadenoma). Patients group include patients with different types of breast cancer

[a] Significant difference versus the control group

Regarding correlation with prognostic pathologic parameters, we found that Fas L immunostaining is manifested in cases with more than three positive nodes than in node-negative or less than three positive node cases (10/17 "58.8%" vs. 3/13 "23.1%" respectively). Fas L positivity is directly correlated with cases with distant metastases (5/

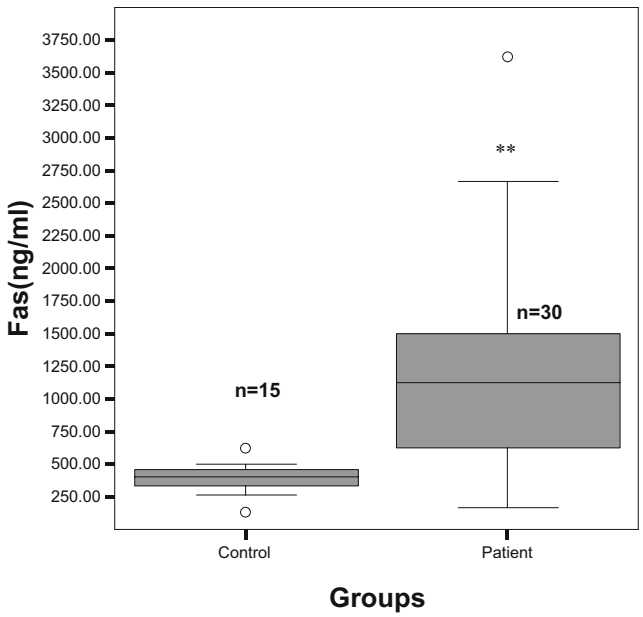

Fig. 1 Serum sFas levels in the studied groups: the *box* plot represents the interquartile range from 25th to the 75th percentiles. The whiskers below and above represents 10th–90th percentiles. The *line across each box* represents the median value. n = number of patients included in each group. *Circle* = outliers (values larger than the upper quartile plus 1.5 times the interquartile range)

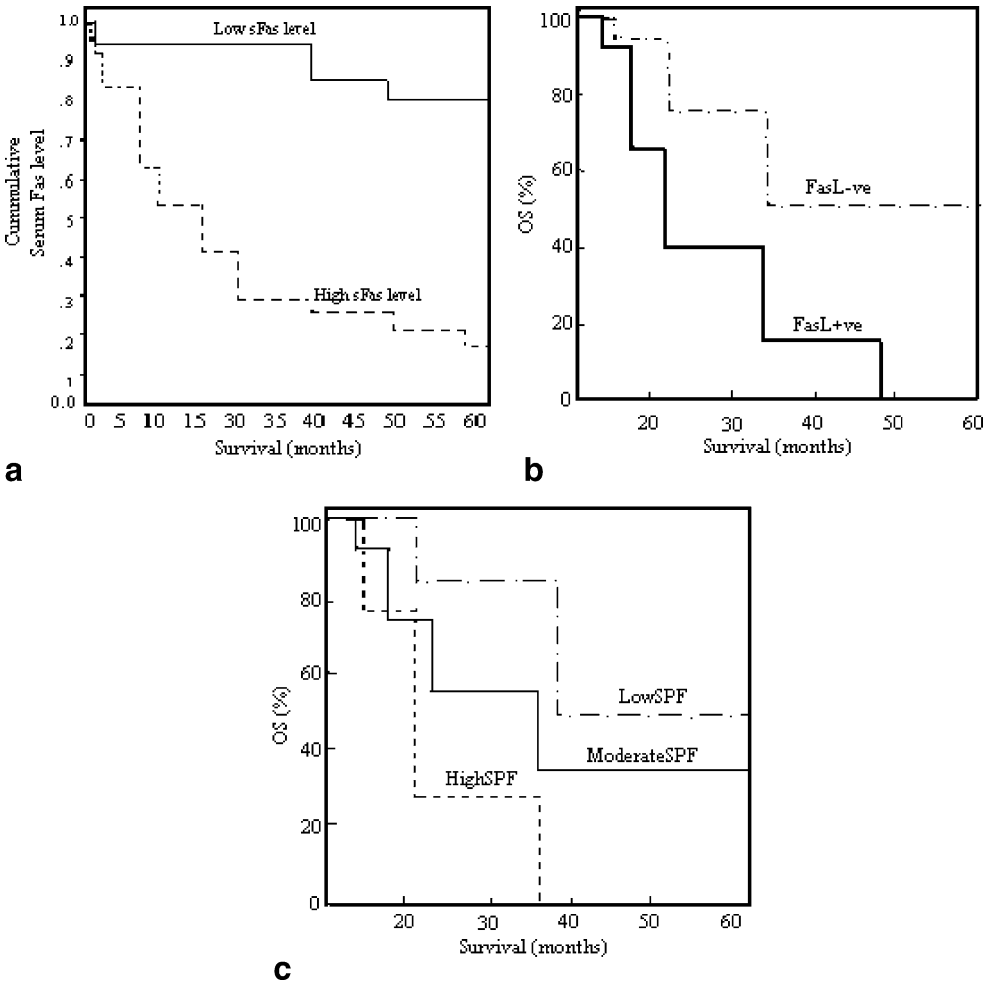

Fig. 2 Kaplan–Meier graphs showing: **a** correlation between Serum sFas Level and overall survival. **b** Correlation between FasL immunostaining reaction and overall survival (*OS*). **c** Correlation between S-phase fraction (*SPF*) and overall survival (*OS*)

8 “62.5%” cases with distant metastases vs. 8/22 “36.4%” cases without metastases). Regarding nuclear grade, it was found that the four grade I tumors were negatively stained with Fas L in contrast to grade II and III tumors which showed positive staining in 50% of cases (13/26).

There was a significant reversible relationship between FasL immunostaining and ER staining ($P = 0.020$) and PR staining ($P = 0.008$) in contrast to HER-2 reaction that showed a significant direct correlation with FasL immunostaining ($P = 0.009$).

Follow up data proved that patients with positive FasL immunostaining had a poor survival compared with negative staining (Fig. 2b).

DNA Ploidy

Eight out of the included 30 ductal carcinoma cases were aneuploid; six of them (75%) were with more than three positive nodes and five of them (62.5%) with nuclear grade III (Table 2, Fig. 4). Statistically, a significant correlation only with high nuclear grade ($P = 0.001$) was found. However, there was no detectable relationship between aneuploidy and other pathologic parameters; tumor size, distant metastases, hormonal status and HER-2 positivity.

S-phase Fraction: (Table 2, Fig. 4)

Nineteen out of the included thirty ductal carcinoma cases (63.3%) were proved to have moderate or high SPF values. It was found that there were direct correlations between moderate and high SPF with worse pathologic parameters: tumor size (9/11(81.8%) of large size tumors vs. 10/19 “52.6%” of small size tumors), nodal status (13/17 “76.5%” of cases with more than three positive nodes vs. 6/13 “46.2%” of cases with less than three positive nodes), nuclear grade (all seven grade III cases, 12/19 “63.2%” of grade II cases and non of grade I tumors vs. 11/22 “50%” of cases with no distant metastases) distant metastases (all cases “8” with distant metastases), negative hormonal reaction (all ER- and 11/12 of PR-negative cases vs. 10/21 of ER- and 8/18 of PR-positive cases) and positive HER-2 reaction (all positive cases vs. 11/22 negative cases). Statistical study showed that there were significant correlations between moderate and high SPF with high

Table 2 Correlation of FasL immunostaining, ploidy and S-phase fraction with prognostic pathologic parameters

	FasL expression			Ploidy			SPF			
	+ve	−ve	Test	D	A	Test	Low	Mod.	High	Test
Size										
≤2 cm (19)	8	11	χ^2=0.032	13	6	χ^2=0.639	9	9	1	FET=3.962
>2 cm (11)	5	6	p=0.858	9	2	p=0.672[a]	2	6	3	MCp=0.116
Nodal status										
0 (10)	2	8	FET=3.940	8	2	FET=1.459	6	4	0	FET=4.976
1–3 (3)	1	2	MCp=0.168	3	0	MCp=0.586	1	2	0	MCp=0.273
>3 (17)	10	7		11	6		4	9	4	
Distant metastasis										
−ve (22)	8	14	χ^2=1.632	15	7	χ^2=1.120	11	9	2	FET=7.042*
+ve (8)	5	3	p=0.242[a]	7	1	p=0.391[a]	0	6	2	MCp=0.026
Nuclear grade										
I (4)	0	4	FET=3.481	14	0	FET=12.421*	4	0	0	FET=12.742*
II (19)	10	9	MCp=0.180	6	3	MCp=0.001	7	11	1	MCp=0.003
III (7)	3	4		2	5		0	4	3	
ER										
+ve (21)	6	15	χ^2=6.212*	16	5	χ^2=0.292	11	9	1	FET=9.498*
−ve (9)	7	2	p=0.020[a]	6	3	p=0.666[a]	0	6	3	MCp=0.006
PR										
+ve (18)	4	14	χ^2=8.167*	14	4	χ^2=0.455	10	7	1	FET=7.531*
−ve (12)	9	3	p=0.008[a]	8	4	p=0.678[a]	1	8	3	MCp=0.024
HER-2										
+ve (8)	7	1	χ^2=8.666*	6	2	χ^2=0.015	0	5	3	FET=8.875*
−ve (22)	6	16	p=0.009[a]	16	6	p=1.000[a]	11	10	1	MCp=0.007

χ^2 chi square test, *FET* Fisher exact test, *MCp* p for Monte Carlo test
*$p \leq 0.05$
[a] p for FET was applied

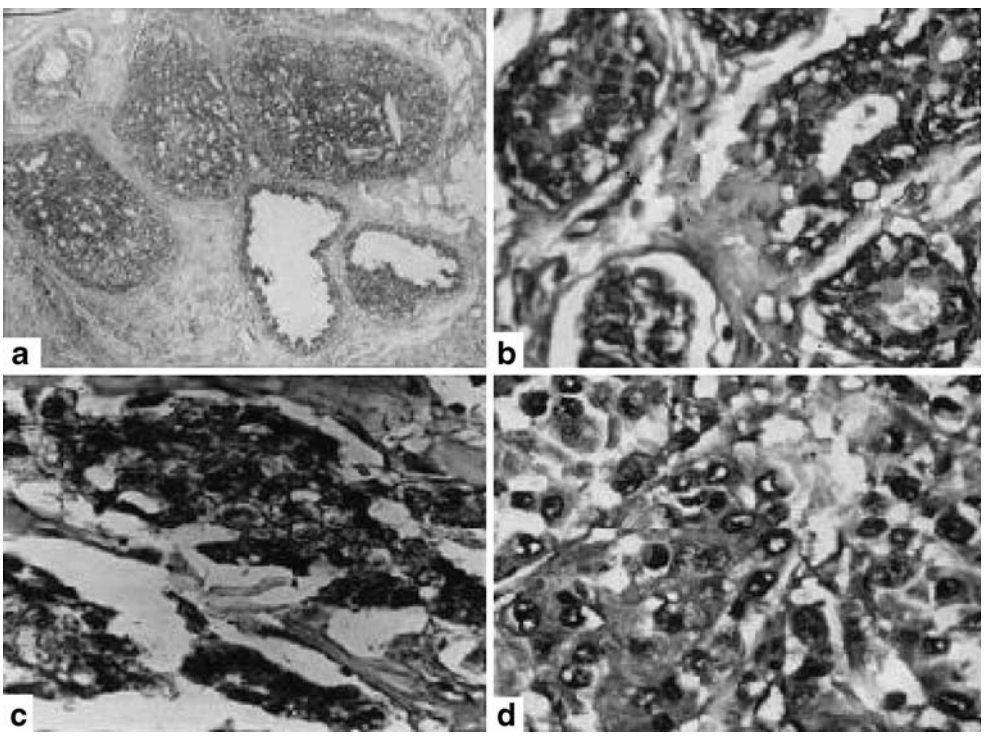

Fig. 3 FasL immunohistochemical staining reaction in breast ductal carcinoma: **a** intraductal carcinoma showing strong reaction to Fas L immunostain ×10, **b** invasive ductal carcinoma, grade II, showing membranous and cytoplasmic reaction to Fas L immunostain ×40, **c** invasive ductal carcinoma, grade III, showing membranous and cytoplasmic reaction to Fas L immunostain ×40, **d** invasive ductal carcinoma, grade III, showing only cytoplasmic reaction to Fas L immunostain ×40

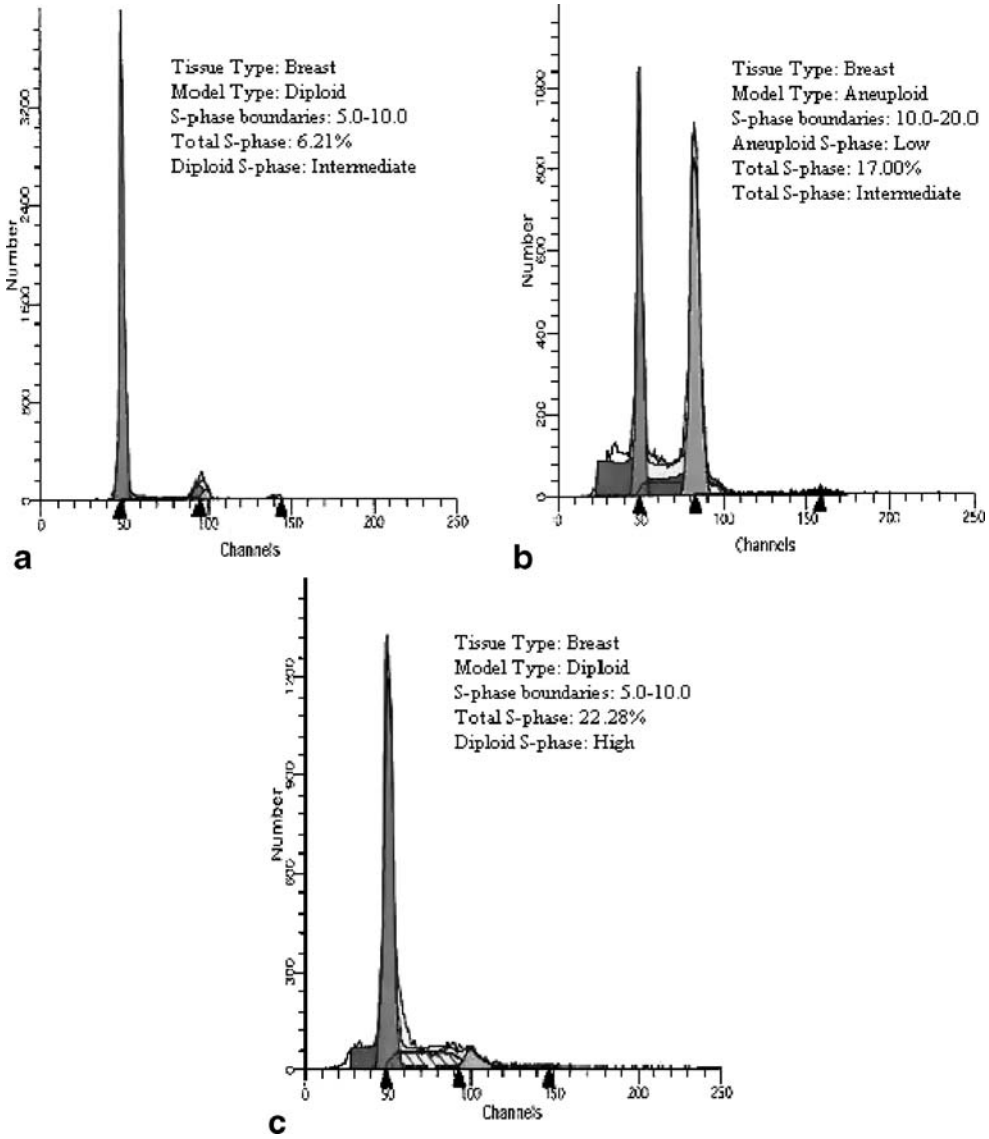

Fig. 4 Flow cytometric analysis of three breast ductal carcinoma cases: **a** diploid case with intermediate SPF. **b** Aneuploid case with low SPF. **c** Diploid case with high SPF

nuclear grade ($P = 0.003$), distant metastases ($P = 0.026$), negative hormonal reaction ($P = 0.006$ for ER & $P = 0.024$ for PR) and HER-2 positivity ($P = 0.003$).

Follow up data proved that patients with moderate and high SPF had a poor survival compared to those with low SPF (Fig. 2c).

Discussion

Breast cancer is the commonest cause of cancer death in women worldwide [31] and invasive ductal carcinoma is the most frequent type; constituting up to 75% of cases [32]. Over the last several years, researches on the role of apoptosis in malignancy in general and in breast cancer in particular had increased [12, 14, 33, 34]. Apoptotic markers are now being investigated to have a role in detecting the progression of cancer and its response to various chemotherapeutic agents [35].

Fas/FasL system, a major regulator of apoptosis, is involved in cancer cell death induced by the immune system and anticancer drugs. Fas receptor and Fas Ligand are both expressed in breast cancer cells and in spite of that it was found that these cells are resistant to apoptosis [36].

It was reported that circulating serum Fas inhibits apoptosis by neutralizing its ligand [37], however little is known about its role in breast cancer. In the present study, the median value of serum Fas was significantly higher in breast cancer patients' group (1,133 pg/ml "167–3,555 pg/ml") than breast benign tumor control group (400 pg/ml "133–650 pg/ml"). These findings are in accordance with Sheen-Chen et al. [36] who reported high circulating soluble Fas levels in breast cancer patients than controls. Survival analysis demonstrated that patients with high soluble Fas

levels had a worse prognosis than those with low levels, confirming the fact that circulating soluble Fas levels was an independent prognostic indicator in overall and disease free survival. It was suggested that cancer cells regulate or stimulate soluble Fas production to protect themselves from Fas mediated apoptosis [37]. Djerbi et al. [37] reported that increased soluble Fas causes resistance to treatment by inhibiting Fas mediated apoptosis in cancer cells. This clarifies the correlation between high serum soluble Fas levels and poor prognosis due to drug resistance.

It has been reported that breast carcinoma is one of the malignant conditions in which alkaline phosphatase was frequently elevated [38]; the increase was attributed to the occurrence of bone and/or liver metastasis. In the present study, the mean value of ALP was significantly increased in breast cancer patients than control benign breast tumor group (Table 1). These findings are in accordance with other studies [38] who claimed that ALP is elevated in breast cancer patients but not altered in benign breast tumors. Thus ALP measurements can be an effective base line screening for relapses and follow up in breast cancer patients [38].

In the present study, there was no significant statistical difference in total and ionized calcium in cancer and benign breast tumor groups. It has been suggested that PTH is normal in serum of majority of patients with malignancy not associated with hypercalcaemia [39]. In the present work, PTH showed no significant statistical difference between cancer and benign breast tumor groups (Table 1). Phosphatase ions have been reported to influence the secretion of PTH; however its effect can be explained by the decrease in free calcium. In the present work, no statistical difference was found in phosphate in cancer and benign breast tumor groups.

Previous studies have reported that Fas-expressing tumors are prone to develop apoptosis and that tissue-FasL immuno-expression can be an independent predictor for survival in lung cancer [40], hepatocellular carcinoma [41], esophageal cancer [42], and gastric cancer patients [43]. It was also proved that Fas was more commonly expressed in early-stage and well-differentiated tumors than in advanced and poorly differentiated tumors, respectively.

It was also found that many tumors express FasL [14] and it was proved that FasL expression had a significant association with lymph node metastasis and bone metastasis in breast cancer, gastric cancer and colorectal cancer [11, 13, 44, 45]. Furthermore, FasL immuno-expression was found to be associated with a poor prognosis in ovarian cancer [12]. Moreover, Sjöström et al. [46] reported a significant association of FasL and bcl-2 with the overall survival in univariate and multivariate analyses in breast cancer. In the present study, it was found that FasL immuno-expression was manifested in breast ductal carcinomas with high grades (II&III), with axillary lymph node involvement, in hormone receptor negativity and Her2 positivity. Statistically, there was a significant correlation between FasL immuno-expression and hormone receptor negativity ($P = 0.020$ & $P = 0.008$) and Her2 positivity ($P = 0.009$) (Table 2).

In the present work, it was found that DNA aneuploidy was evident in tumors with high grades (II&III), lymph node positivity (more than three positive nodes), hormone receptor negativity and Her2 positivity. However, a direct significant correlation was found only with high nuclear grade ($P = 0.001$). However, there were direct significant correlations between SPF with most of worse prognostic variables of breast ductal carcinomas (nuclear grade; $P = 0.003$, distant metastasis; $P = 0.026$, hormone receptor negativity; $P = 0.006$ & 0.024 and Her2 positivity; $P = 0.007$). These results were in accordance with those observed by Chassevent et al. [20] who reported that the frequency of aneuploidy and high s-phase fraction increased significantly with pathologic tumor size, histological node involvement, nuclear grade and hormone receptor negativity. Regarding the prognostic significance, it was confirmed that the ploidy status has no prognostic impact in the overall population with breast cancer or in subgroups defined by lymph node status, histological grade and histological type [20, 47, 48]. However, among the aneuploid subclasses, near triploidy and hypodiploidy tended to be associated with a poor 5-year prognosis [20, 49, 50]. In relation to SPF, it was proved that patients with medium and high s-phase fraction values had a clearly lower 5-year disease free survival than patients with lower values [20, 51]. It was also reported that in node-negative patients, high s-phase fraction values distinguished patients with a higher risk of recurrence [44, 52, 53]. Chassevent et al. [20] found that node-negative patients with high SPF values had disease free survival rates similar to those of node-positive patients, suggesting the need for additional therapy.

It may be concluded that serum sFas, FasL over-expression and s-phase fraction could be used as independent prognostic factors in breast ductal carcinoma cases. Future work may evaluate possible genetic mutation in the Fas gene and whether it may increase serum sFas levels or tissue FasL and whether they correlate with the patients' prognosis.

References

1. Reed JC (2000) Mechanisms of apoptosis. Am J Pathol 156:1415–1430
2. Saas P, Walker PR, Hahne M et al (1997) Fas ligand expression by astrocytoma in vivo: maintaining immune privilege in the brain? J Clin Invest 99:1173–1178

3. Schneioer P, Bodmer JL, Holler N et al (1997) Characterization of Fas (Apo-1, CD95)-Fas ligand interaction. J Biol Chem 272:18827–18833
4. Griffith TS, Ferguson TA (1997) The role of FasL induced apoptosis in immune privilege. Immunol Today 18:2240–2244
5. Müschen M, Moers C, Warskulat U, Even J, Niederacher D, Beckmann MW (2000) CD95 ligand expression as a mechanism of immune escape in breast cancer. Immunology 99(1):69–77
6. Sheen-Chen SM, Chen HS, Eng HL, Chen WJ (2003) Circulating soluble Fas in patients with breast cancer. World J Surg 27(1):10–13, Jan
7. Suda T, Takahashi T, Golstein P, Nagata S (1993) Molecular cloning and expression of the Fas ligand: a novel member of the tumor necrosis factor family. Cell 75:1169–1186
8. Keane MM, Ettenberg SA, Lowrey GA, Russell EK, Lipkowitz S (1996) Fas expression and function in normal and malignant breast cell lines. Cancer Res 56:4791–4798
9. Harrnring C, Reimer T, Jeschke U, Makovitzky J, Kruger K, Gerber B, Kabelitz D, Friese K (2000) Expression of the apoptosis-inducing ligands FasL and TRAIL in malignant and benign human breast tumors. Histochem Cell Biol 113:189–194
10. Mullauer L, Mosberger I, Grusch M, Rudas M, Chott A (2000) Fas ligand is expressed in normal breast epithelial cells and is frequently upregulated in breast cancer. J Pathol 190:20–30
11. Mann B, Gratchev A, Bohm C et al (1999) FasL is more frequently expressed in liver metastases of colorectal cancer than in matched primary carcinomas. Br J Cancer 79:1262–1269
12. Munakata S, Enomoto T, Tsujimoto M et al (2000) Expression of Fas ligand and other apoptosis-related genes and their prognostic significance in epithelial ovarian neoplasms. Br J Cancer 82:1446–1452
13. Nagashima H, Mori M, Sadanaga N et al (2001) Expression of Fas ligand in gastric carcinoma relates to lymph node metastasis. Int J Oncol 18:1157–1162
14. Munakata S, Watanabe O, Ohashi K, Morino H (2005) Expression of Fas ligand and bcl-2 in cervical carcinoma and their prognostic significance. Am J Clin Pathol 123(6):879–885
15. Gatierrez LS, Eliza M, Niven-Fairchild T, Naftolin F, Mor G (1999) The Fas/Fas-ligand system: a mechanism for immune evasion in human breast carcinomas. Breast Cancer Res Treat 54 (3):245–253
16. Robert I (2002) New concepts for the study of anticancer drug resistance [French]. Bull. Cancer 89:17–22
17. Hortobagyi GN (2001) Adjuvant systemic therapy for early breast cancer: progress and controversies. Clin Cancer Res 7:1839–1842
18. Ross JS, Linette GP, Stec J, Ross MS, Anwar S, Boguniewicz A (2003) DNA ploidy and cell cycle analysis in breast cancer. Am J Clin Pathol 120(Suppl):S72–S84, (ISSN: 0002-9173)
19. Chávez-Uribe EM, Viñuela JE, Cameselle-Teijeiro J, Forteza J, Puñal JA, Otero IL, Puente-Dominguez JL (2002) DNA ploidy and cytonuclear area of peritumoral and paratumoral samples of mastectomy specimens: a useful prognostic marker? Eur J Surg 168(1):37–41
20. Chassevent A, Jourdan ML, Romain S et al (2001) S-phase fraction and DNA ploidy in 633 T1T2 breast cancers: a standardized flow cytometric study. Clin Cancer Res 7(4):909–917, (ISSN: 1078-0432)
21. Greene FL, Page DL, Fleming ID et al (2002) Breast IN: American joint committee on cancer, AJCC cancer staging manual, 6th edn. Springer, New York, p 171
22. Burtis CA, Ashwood ER, Bruns DE (2006) Teitz text book of clinical chemistry and molecular diagnostics, 4th edn. Elsevier Saunders, St Louis, pp 870–871, 797–803, 582–590, 546–549, 604–611, 1892–1904 & 1698
23. Bouillon R, Coopman SW, Degroote DEH, Eliard PH (1990) Immunoradiometric assay of parathyrin polyclonal and monoclonal region specific antibodies. Clin Chem 36 (2):271–276
24. Chen J, Zhou T, Liu C, Shapiro JP, Brauuer MJ, Kieler MC (1994) Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 263:1759–1762
25. Chamness GC, McGuire WL (1979) Methods for analyzing steroid receptors in human breast cancer. In: McGuire WL (ed) Breast cancer. Advances in research and treatment. Plenum, New York, p 149
26. Bębenek M, Duś D, Koźlak J (2006) Fas and Fas ligand as prognostic factors in human breast carcinoma. Med Sci Monit 12 (11):CR457–CR461
27. Almasri NM, Al Hamad M (2005) Immunohistochemical evaluation of human epidermal growth factor receptor 2 and estrogen and progesterone receptors in breast carcinoma in Jordan. Breast Cancer Res 7:R598–R604
28. Feichter GE, Müller A, Kaufmann M et al (1988) Correlation of DNA flow cytometric results and other prognostic factors in primary breast cancer. Int J Cancer 41:823–828
29. Feichter GE, Kaufmann M, Müller A et al (1989) DNA index and cell cycle analysis of primary breast cancer and synchronous axillary lymph node metastases. Breast Cancer Res Treat 13:17–22
30. Konrey GG, Pegram MD, Beryt M et al (1999) Therapeutic advantage of chemotherapy drugs in combination with herceptin against human breast cancer cells with HER-2/neu overexpression. Breast Cancer Res Treat 57:114
31. Kev TJ, Verkasalo PK, Banks E (2001) Epidemiology in breast cancer. Lancet Oncol 2(3):133–140
32. Reeves GK, Beral V, Green J, Gathan T, Bull D (2006) Hormonal therapy of menopause and breast-cancer risk by histological type: a cohort study and meta-analysis. Lancet Oncol 7(11):910–918
33. Jager JJ, Jansen RL, Arendo JW (2002) Clinical relevance of apoptotic markers in breast cancer is not yet clear. Apoptosis 7:361–365
34. Zhang L, Levi E, Majumder P, Yu Y, Aboukameel A, Du J (2007) Transactivator of transcription-tagged cell cycle and apoptosis regulatory protein-1 peptides suppress the growth of human breast cancer cells in vitro and in vivo. Mol Cancer Ther 6:1661–1672
35. Motyl T, Gajkowska B, Zarzy J, Gajewska M, Lamparska P (2006) Apoptosis and autophagy in mammary gland remodeling and breast cancer chemotherapy. J Physiol Pharmacol 7:17–32
36. Sheen-Chen SM, Chen HS, Eng HL, Chenw J (2003) Circulating soluble Fas in patients with breast cancer. World J Surg 27:10–13
37. Djerbi M, Screpanti Y, Catrini A et al (1999) The inhibitor of death receptor signaling. FLICE inhibitory protein defines a new class of tumor progression factors. J Exp Med 190:1025–1031
38. Nemesansky E, Tasnadi K, Juhasz P (1971) Diagnostic value of alkaline phosphatase isoenzyme in cancer patients. Cancer 27:1388–1397
39. Kao P, Cmklee GG, Taylor R, Heath H (1990) Parathyroid hormone related peptide in plasma of patients with hypercalcaemia and malignant lesions. Mayo Clin Proc 65:1399–1407
40. Koomagi R, Volm M (1999) Expression of Fas (CD95/APO-1) and Fas ligand in lung cancer, its prognostic and predictive relevance. Int J Cancer 84:239–243
41. Nagao M, Nakajima Y, Hisanaga M et al (1999) The alteration of Fas receptor and ligand system in hepatocellular carcinoma: how do hepatoma cells escape from the host immune surveillance in vivo? Hepatology 30:413–421
42. Shibakita M, Tachibana M, Dahr DK et al (1999) Prognostic significance of Fas and Fas ligand expression in human esophageal cancer. Clin Cancer Res 5:2464–2469

43. Ohno S, Tachibana M, Shibakita M et al (2000) Prognostic significance of Fas and Fas ligand system-associated apoptosis in gastric cancer. Ann Surg Oncol 7:750–757
44. Mottolese M, Buglioni S, Bracalenti C et al (2000) Prognostic relevance of altered Fas (CD95)-system in human breast cancer. Int J Cancer 89:127–132
45. Bebenek M, Duś D, Koźlak J (2007) Fas/Fas-ligand expressions in primary breast cancer are significant predictors of its skeletal spread. Anticancer Res 27(1A):215–218
46. Sjöström J, Blomqvist C, Boguslawski K et al (2002) The predictive value of bcl-2, bcl-xL, bag-1, Fas, and FasL for chemotherapy response in advanced breast cancer. Clin Cancer Res 8:811–816
47. Kute TE, Quadri Y, Muss H et al (1995) Flow cytometry in node-positive breast cancer: cancer and leukemia group B protocol 8869. Cytometry 22:297–306
48. Bryant J, Fisher B, Gunduz N et al (1998) S-phase fraction combined with other patient and tumor characteristics for the prognosis of node-negative, estrogen-receptor positive breast cancer. Breast Cancer Res Treat 51:239–253
49. Toikkanen S, Pylkkanen L, Joensuu H (1997) Invasive lobular carcinoma of the breast has better short- and long-term survival then invasive ductal carcinoma. Br J Cancer 76:1234–1240
50. Remvikos Y, Magdelenat H, Dutrillaux B (1995) Genetic evolution of breast cancers. III. Age-dependent variations in the correlations between biological indicators of prognosis. Breast Cancer Res Treat 34:25–33
51. Leonardi E, Cristofori A, Caffo O, Dalla Palma P (1997) Cytometric DNA analysis and prognostic biomarkers in breast carcinoma. Expression of P53 product in the different ploidy classes. Anal Cell Pathol 15:31–45
52. Dettmar P, Harbeck N, Thomssen C et al (1997) Prognostic impact of proliferation-associated factors MIB1 (Ki67) and s-phase fraction in node-negative breast cancer. Br J Cancer 75:1525–1533
53. Rudolph P, Alm P, Heidebrech H et al (1999) Immunologic proliferation marker Ki-S2 as prognostic indicator for lymph node-negative breast cancer. Natl Cancer Inst (Bethesda) 91:271–278