Gene Expression of Maspin and Multidrug Resistance mRNAs In Egyptian Breast Cancer

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ABSTRACT

Breast cancer is considered to be one of the most common cancers affecting women in Western countries as well as in Egypt. In the present study, the expression of MDR-1 (multidrug resistance) gene and maspin (mammary serine protease inhibitor) gene were detected in breast cancer tissue as well as in benign breast diseases using PCR techniques. Serum C-erbB-2 and TGF-β1 levels were also measured by ELISA techniques. Study groups comprised 19 healthy women; 20 benign breast disease patients and 41 breast cancer patients. Relation between different prognostic association and correlation between different studied parameters were assessed. In the present work, the mean serum level of C-erbB-2 was significantly elevated while that of TGF-β1 was significantly decreased in breast cancer patients as compared to normal healthy and benign groups. Significant negative correlation between serum C-erbB-2 and serum TGF-β1 has been observed in the breast cancer patients. Statistical analysis by the Chi-square test showed that MDR-1gene was present more frequently in breast cancer group (51.2%) than in benign group (11%), while maspin gene was present more frequently in benign group (77.8%) than in breast cancer group (26.8%). Moreover, maspin gene expression showed an inverse relationship to histological grades and clinical stages of breast cancer; this indicates that the expression of maspin in breast tumor cells limits their growth and metastasis. Our results showed that serum levels of C-erbB-2 were significantly higher in MDR-1 positive and maspin-negative tumors while serum levels of TGF-β1 were significantly higher in maspin positive tumors only. In addition, we have found that the possibility of maspin positive tumors was more significantly frequent in MDR-1 negative tumors in breast cancer patients. Evaluation of these factors may improve the ability to identify and select breast cancer patients at high risk for poor prognosis and aggressive treatment and also it may have important implication concerning the overall biology of the breast.

RIASSUNTO

Espressione dei geni della maspina e della resistenzaultipla ai farmaci nel carcinoma della mammella in Egitto

Il carcinoma della mammella è considerato uno dei più comuni carcinomi che colpiscono le donne, nei paesi occidentali come pure in Egitto. Nel presente studio si sono verificate le espressioni dei geni MDR-1 (resistenza multipla ai farmaci) e maspina (inibitore della proteasi serinica mammaria) nel tessuto canceroso e nel tessuto da patologia benigna della mammella, utilizzando tecniche PCR. Con tecniche ELISA si sono pure misurate le concentrazioni di C-erbB-2 e TGF-β1 nel siero. Nello studio erano inclusi i seguenti gruppi: 19 donne sane; 20 pazienti con patologia benigna della mammella; 41 portatori di carcinoma mammario. Sono state valutate le relazioni tra differenti associazioni prognostiche di parametri, e le correlazioni tra i differenti parametri. Le concentrazioni medie di C-erbB-2 e di TGF-β1 del siero erano, rispettivamente, aumentate e diminuite in presenza di carcinoma, in confronto ai normali sani ed ai gruppi benigni. Si osservava anche una correlazione negativa significativa tra C-erbB-2 e TGF-β1 nei portatori di carcinoma mammario. La analisi statistica (Chi-quadro) mostrava che il gene MDR-1 era presente con maggior frequenza nel gruppo del carcinoma (51,2%) rispetto al gruppo della patologia benigna (11%), mentre il gene della maspina era presente con frequenza più elevata nel gruppo della patologia benigna (77,8%) che nel gruppo del carcinoma (26,8%). Inoltre, l'espressione del gene della maspina mostrava una relazione inversa con il grado istologico e lo stadio clinico del carcinoma; ciò indica che l'espressione della maspina nel carcinoma mammario ne limita l'accrescimento e la metastizzazione. Veniva anche evidenziato che nel siero le concentrazioni di C-erbB-2 erano più elevate nei tumori MDR-1-positivi e maspina-negativi, mentre le concentrazioni di TGF- 1 del siero risultavano significativamente innalzate solo nei tumori maspina-positivi. E' stato anche evidenziato che l'evento di tumore maspina-positivo era significativamente più frequente nei tumori MDR-1 negativi, nei pazienti affetti da carcinoma mammario. La valutazione di questi fattori può migliorare la capacità di identificare e selezionare i pazienti di carcinoma mammario a più alto rischio, ai fini di una prognosi più sfavorevole e di trattamento aggressivo. Può anche avere importanti implicazioni in relazione alla biologia globale della mammella.

INTRODUCTION

Oncogenes or cancer-causing genes are derived from proto-oncogenes, which promote normal growth and differentiation (1).

Proto-oncogenes represent a family of normal cellular genes that were identified on the basis of their similarity to genetic sequence with known tumorgenic or transforming potential (2). Proto-oncogenes may become oncogenic by influences that alter their behaviour in situ, thereby converting them into cellular oncogenes (C-oncs) (3).

The oncogene C-erbB-2 is located on chromosome 17 at q 21 and codes for a 185 KD oncoprotein. Overexpression of this oncogene correlates with poor prognosis and associated with a decreased disease free survival and overall survival in breast carcinoma (4).

Transforming growth factor-β1 mRNA is expressed in 84% of primary breast tumors. TGF -β1 have been implicated in the genesis of breast cancer. TGF-β1 is a homodimeric peptide with molecular mass of 25 KD. It is particularly interesting because elevated tumor levels have been reported to both reduced and enhanced breast cancer growth (5).

TGF-β1 is known to play an important role as a negative growth factor in epithelial tissue. Evidence in favour of such a rule came from several studies which demonstrated that TGF-β1 serves as suppressor of malignant proliferation and loss of TGF-β1 is an important event in the progression of malignancy (6).

The successful treatment of breast cancer using antineoplastic agents is limited by the occurrence of drug resistance. The development of cross-resistance to many natural anticancer drugs, termed multidrug resistance (MDR), is one of the major reasons why cancer chemotherapy ultimately fails (7). This type of MDR is often associated with overexpression of the MDR-1 gene product (8).

A strong correlation has been reported between the increased levels of P-glycoprotein (P-gp), encoded by MDR1 gene, and the prognosis in advanced breast cancer. The role of proteolytic enzymes in tumor metastasis and invasion is well established. Protease inhibitors exert an inhibitory effect on growth promoting proteases as they have the potential to inhibit the proteolytic cascade involved in tumor metastasis, invasion, and to inhibit proteolytic reactions which promote cell growth (9).

Maspin is a 42 KD member of the serpin family of protease inhibitors that has been shown to function as a tumor suppressor in human breast epithelium (10,11).

The aim of this study is to evaluate the expression of C-erbB-2, TGF-β1 in serum samples of breast cancer patients as compared to both benign and control groups, also to evaluate the expression of MDR-1 and maspin genes in breast cancer tissues as well as in benign breast diseases using PCR techniques. Relationship between different prognostic factors and studied parameters is also performed. Another target for this work is to reveal if it is possible to obtain a relationship between different studied parameters.

SUBJECTS AND METHODS

I- subjects studied:

The study population consisted of 80 individuals classified into three groups:

Group (A): Forty one breast cancer patients came from the different governorates of Egypt and admitted to the (NCI) National Cancer Institute, Cairo University, the age of patients varied from 16 to 72 years with a mean of 41 years. For all breast cancer patients' full history and clinical data were recorded.

Pathological assessment of the tissue removed was done to determine the type of the tumor and its grade according to Bloom and Richardson (12). The size of the tumor and the number of lymph nodes were also determined.

According to menopausal status 12 of patients were pre-menopausal, while 29 of them were post-menopausal. According to American Joint Committee of Cancer (AJCC) (13) the patients were classified into the following stages: stage II (17 cases), stage III (16 cases) and stage IV (8 cases).

Regarding the pathological grading (12), the patients were classified into two grades: 23 patients with grade II and 18 patients with grade III. As for axillary lymph node metastasis, we had two groups of patients, 10 patients had no lymph node metastasis and 31 patients had lymph node metastasis.

Group (B): Twenty patients with benign breast diseases were included in this study. Their ages ranged from 15 to 40 years with a mean of 33 years. Full history and clinical data were recorded for all patients. They underwent operative biopsy and histopathological examination of all biopsiesrevealed, two cases with fibroadenosis, 17 cases with fibroadenoma and one case with breast abscess.

Group (C): Nineteen normal healthy women were included in this work as control. Their ages ranged from 20 to 55 years with mean of 39 years.

II- samples:

a) Serum samples:

Blood samples were taken from cancer patients before receiving any line of treatment, as well as from patients with benign tumors and control. Sera were separated and divided into several aliquots and stored at - 80°C until assayed.

b) Tissue samples:

Breast tissue samples (both benign and malignant) were obtained directly from the operating theatre, after surgical removal of the tumor mass. Immediately, the tissue sample was washed by ice cold saline. Fat, necrotic tissues and skin were rapidly dissected from the tissue of interest. The remaining tumor tissue was divided into two parts, one part for pathological examination and the other part was immediately stored in liquid nitrogen for expression of MDR-1 & Maspin genes by PCR techniques.

III- Methods:

*Quantitative determination of serum activated human transforming growth factor beta-1 (TGF-β1) using kit pro-

vided from Quantikine® (R&D systems U.K). This assay employs the quantitative sandwich enzyme immunoassay technique.Samples were assayed according to the manufacturers instructions.

*Quantitative determination of serum C-erbB-2 (HER2/neu): using kit provided from Oncogene Research Products, USA. The oncogene research products neu ELISA is a sandwich enzyme immunoassay which utilized a mouse monoclonal antibody for capture and a rabbit polyclonal serum for the detection of human neu protein.Samples were assayed according to the manufacturers instructions.

* Molecular Biology Methods

1-RNA Extraction:

Tissue homogenization in lysis buffer was done. Then total RNA was extracted from the homogenate using SV total RNA extraction kit provided from Promega Corporation, Madison, WI, USA. The amount of RNA was quantitated by reading the Optical Density (OD) at wave length of 260 nm by using spectrophotometer.

II- cDNA synthesis and PCR of Maspin gene:

The RT-PCR method here is the one step method, that means one tube-two enzymes. The kit was provided from Promega Corporation, Madison, WI, USA. The RT-PCR amplification protocol was formed of: Buffer (5x), $MgSO_4$ (25mM), dNTPs (10μM), primer (P1, 10μM), primer (P2, 10 M),Taq (modified), RT(enzyme) and RNA extract in a total volume of 50μL. The oligonucleotide primer sequences used for amplification of maspin gene were designed from the coding sequence of the human maspin cDNA (Gene Bank accession number U04313): P1 5\ TCAAGCGGCTCTACGTAGAC 3\ Sense and P2 5\ CCTCCACATCCTTGGG TAGT 3\ Antisense

The PCR cycling condition was as follows: 35 cycles of 95°C for 1min, 55°C for 1 min and 72°C for 2 min. To increase the sensitivity of the RT-PCR assay of maspin, 2.5 μL of the first amplified product was subjected to nested PCR under the same cycling condition. The sequences of the nested oligonucleotide primers were as follows: P3 5\ GAT CTC ACA GAT GG CC ACTT 3\internal sense. P4 5\ GCACT GG TTT GG T GT CT GTC 3\internal antisense. The expected length of the nested PCR product was 175 bp (Figure 1).

III- cDNA synthesis and PCR of MDR-1 gene:

The RNA was reverse transcribed by using random hexamer primers, 5x buffer, dNTPs and RT enzyme. The total volume of 20μL was introduced to PCR apparatus for 15 min at 42°C. Then the following was added for PCR mix: 10x buffer, $MgCl_2$ dNTPs, Taq polymerase enzyme and cDNA in a total volume of 50μL.

The PCR cycling condition was: 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min. The primer sequences used for amplification of MDR1 were : P5 5\ CCCATCATTGCAATAGCAGG 3\ Sense and P6 5\ GTTCAAACTTCTGCTCCTAG 3\ Antisense

Ten L of the PCR product was separated electrophoretically in a 1.5 agarose gel stained with ethidium bromide and observed under UV light. The expected length of the PCR product was 167 bp (Figure 2).

RESULTS

In breast cancer group, the mean serum level of C-erbB-2 was significantly increased while the mean serum level of TGF-β1 was significantly decreased as compared to control or benign group (Table 1).

The mean serum level of either C-erbB-2 or TGF-β1 failed to reveal any significant difference regarding the different histological grades as well as different stages of the tumor (Table 1).

According to lymph node involvement, the mean serum level of TGF-β1 showed significant elevation in patients with lymph node involvement in comparison to patients without lymph node involvement, while no significant difference was observed in serum C-erbB-2. Meanwhile, no significant difference was detected between pre-and postmenopausal groups of breast cancer patients (Table 1).

As regards the tumor size, significant negative correlation has been observed between serum TGF-β1 level and size of the tumor($r = -0.309$, $p < 0.05$) , while no significant correlation has been detected in case of C-erbB-2. However, significant positive correlation has been observed between serum C-erbB-2 level and age of breast cancer patients($r = 0.309$, $p< 0.05$), while no significant correlation has been observed in case of TGF-β1. Significant negative correlation between serum C-erbB-2 and serum TGF-β1 has been observed in the breast cancer patients ($r = -0.340$, $p< 0.05$).

Statistical analysis by the Chi-square test showed that MDR-1 gene was present more frequently in the breast cancer group (51.2%) than in benign group (11%), while maspin gene was present more frequently in the benign group (77.8%) than in breast cancer group (26.8%) (Table 2).

Our results showed that the expression of maspin gene was dramatically affected by the histological grade of the tumor. The frequency of Maspin-positive tumors was distinctly lower in grade III category as compared to grade II. On the other hand, frequency of MDR-positive tumors was not affected by the grade of the tumor. At the same time, the stage of the tumor did not affect the frequency of positivety of MDR-1 gene, while the frequency of Maspin gene was significantly decreased in stages III and IV in relation to stage II (Table 2)($P<0.005$).

At the same time, Table (2) shows the frequency of expression of MDR-1 and Maspin gene in relation to LN involvement and menopausal status. The frequency of maspin gene was significantly decreased in malignant tumors with positive lymph nodel involvement as compared to malignant tumor without lymph node involvement, while the frequency of MDR-1 gene was not affected by lymph node involvement. Moreover, according to meno-

pausal status in breast cancer group, the frequency of MDR-1 positive tumors was significantly higher in post-menopausal than in pre-menopausal groups, while the frequency of Maspin-positive tumors was significantly lower in post-menopausal group.

The present study indicates that the possibility of maspin-positive tumors were more significantly frequent in MDR-1 negative tumors in breast cancer patients (Table 3).

The association between serum levels of either C-erbB-2 or TGF-β1 and expression of MDR and maspin genes showed that higher serum levels of C-erbB-2 were significantly higher in MDR-1 positive and Maspin-negative tumors while the serum levels of TGF-β1 was significantly higher in Maspin-positive tumors only (Table 4).

DISCUSSION

Breast cancer is the most common malignancy among women not only in Western countries but also all over the world and all women are at risk of developing breast cancer (14). In the past 15 years the study of oncogenes has advanced our understanding of molecular mechanisms leading to cancer. The application of molecular biology techniques has led to discovery of both dominantly acting transforming genes and tumor suppressor genes (1).

In the present work, the mean serum level of C-erbB-2 was significantly elevated in breast cancer patients as compared to normal healthy and benign groups. These findings were in agreement with those reported by Bankfalvi et al., (15) who concluded that there is a significant

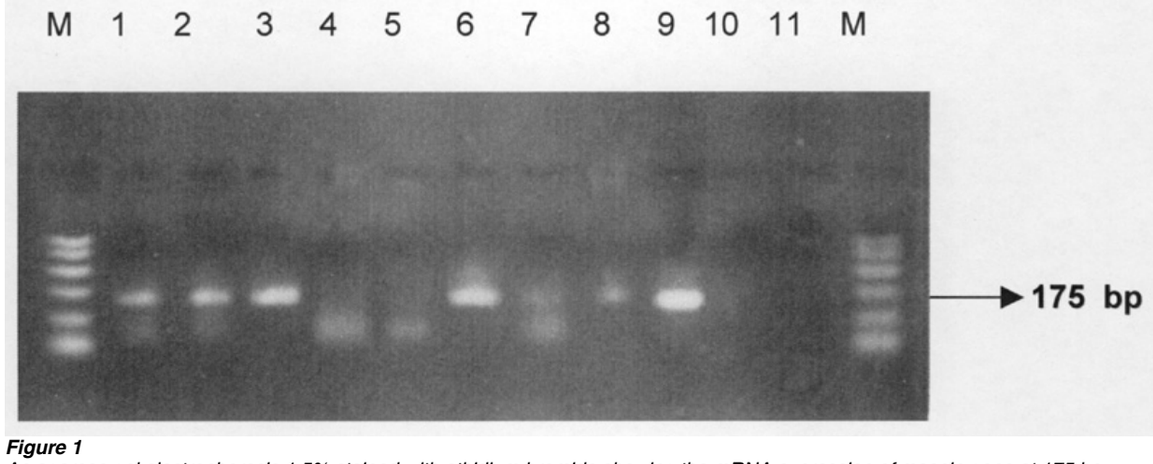

Figure 1
An agarose gel electrophoresis 1.5% stained with ethidium bromide showing the mRNA expression of maspin gene at 175 bp.
M : Molecular marker (1000, 750, 500, 300, 150, 50)
Lanes 1- 3, 6-9: Positive case.
Lanes 4, 5, 10: Negative cases.
Lane 11 : Negative control.

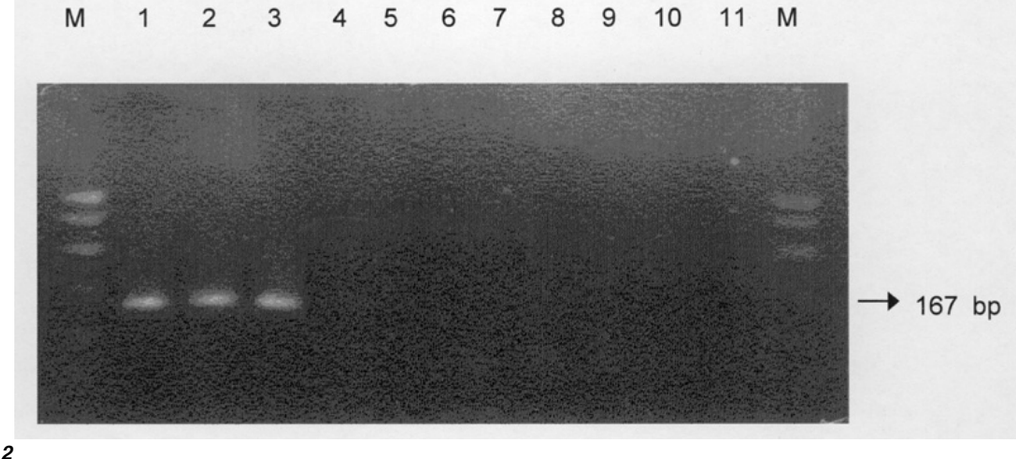

Figure 2
An agarose gel electrophoresis 1.5% stained with ethidium bromide showing the expression of MDR-1 gene at 167 bp.
M : Molecular marker (1000, 750, 500, 300, 150, 50)
Lanes 1- 3: Positive case.
Lanes 4- 9: Negative cases.
Lane 10 : Negative control.

overexpression of C-erbB-2 protein associated with the progression of breast cancer.

Overexpression of the proto-oncogene C-erbB-2 in breast cancer and certain other tumors appears to be a central mechanism that may be partly responsible for cellular progression of the neoplastic phenotype. The overexpression of C-erbB-2 results in reduced sensitivity to cytotoxic effects of tumor necrosis factor (TNF) and reduced sensitivity to immune effector killing (16).

Our study didn't show any significant correlation between expression of C-erbB-2 level and histological grades, clinical stages, tumor lymph node involvement, menopausal status and different histological types of breast cancer patients. These data are in agreement with that obtained by Fusco et al., (17). However, a significant positive correlation between C-erbB-2 level and age of breast cancer patients has been detected. This is consistent with the results obtained by Tandon et al., (18) and Heatlyl et al., (19). On the other hand, Friedmann et al.(20) and Fusco et al.(17) found no such correlation.

C-erbB-2 expression was found to be highly expressed in post-menopausal breast cancer patients compared to pre-menopausal patients, which is in agreement with that reported by Looi and Cheah (21).

Growth factors may play an important role in tumor growth by their influence on tumor cell proliferation (22). Expression of growth inhibiting factors, such as TGF-β1, in breast cancer patients has been reported by Pierce et al., (5) and Marcus (23).

In this study, the mean serum level of TGF-β1 was significantly decreased in breast cancer group as compared to control or benign group. This fits well with other studies showing that TGF-β1 is one of the only known endogenous inhibitors of cell growth and more highly expressed in normal cells (23,24). This explains the role of TGF-β1 as a suppresser of malignant progression and the loss of TGF- 1 is an important event in the progression of malignancy (25).

However, some studies found that TGF-β1 is significantly increased in breast cancer, which may be due to the general absence of TGF-β1 receptors (22).

In the present study; we have found that the mean serum level of TGF-β1 was significantly decreased in breast cancer patients without lymph node involvement than in patients with positive lymph node involvement. This finding goes with that reported by Walker and Dearing (26).

In the present work there was no significant relationship between serum TGF-β1 and histological grades, clinical stages, menopausal status, age or histological types of breast cancer patients. Similar observations were reported by Feng et al.,(27).

Table 1
Serum C-erbB-2 and TGF- 1 levels in control, benign and malignant groups, this last further divided into subgroups according to the grade, the stage, the lymph node involvement (LN) and the menopausal status

Group	No. of cases	C-erbB-2 [HNU/mL] Mean ± SE	TGF- 1 [ng/mL] Mean ± SE
CONTROL	19	35.5 ± 1.86	49.0 ± 2.37
BENIGN	20	34.5 ± 2.96	42.0 ± 3.42
MALIGNANT	41	59.9*± 5.89	26.5* ± 1.24
Grade II	23	60.4 ± 9.39	28.4 ± 1.79
Grade III	18	59.3 ± 6.35	24.2 ± 1.55
Stage II	20	36.9± 10.18	28.2 ± 1.96
Stage III	16	60.2 ± 8.19	23.7 ± 1.75
Stage IV	5	63.1 ± 2.80	31.0 ± 2.65
LN Positive	32	61.3± 6.71	28.4 ± 1.79
LN Negative	9	55.1± 12.95	20.6°± 1.93
Pre-menopausal	12	42.7 ± 4.62	25.0 ± 3.46
Post-menopausal	29	67.1 ± 7.77	27.2 ± 1.05

* Difference from both control and benign group statistically highly significant ($p < 0.001$)
° Difference from the positive LN group statistically significant ($p < 0.05$)
HNU/m/L: Human Neu Units per ml

Table 2
Frequency of expression of the MDR-1 and the Maspin genes, by PCR, in benign and malignant groups, this last further divided into subgroups according to the grade, the stage, the lymph node involvement (LN) and the menopausal status
1

Group	No. of cases	Frequency of MDR-1 + Number (%)	Frequency of Maspin + Number (%)
BENIGN	18	2(11.1)	14 (77.8)
MALIGNANT	41	21 (51.2)*	11 (26.8)*
Grade II	23	12 (52.2)	8 (34.8)
Grade III	9	4 (44.4)	3 (16.6)*
Stage II	20	9 (45.0)	7 (35.0)
Stage III	16	9 (56.3)	2 (12.5)*
Stage IV	5	3 (60.0)	1 (20.0)*
LN Positive	32	17 (53.1)	7 (21.9)*
LN Negative	9	4 (44.4)	4 (44.4)
Pre-menopausal	12	3 (25.0)	6 (50.0)
Post-menopausal	29	18 (62.1)*	5 (17.2)*

* Significantly different from the benign group ($p < 0.001$)

Table 3
Relation between MDR and Maspin gene expression in breast cancer patients

Patient status	MDR-1+	MDR-1 -	Total
Maspin +	6(24%)	19(76%)*	25
Maspin -	17 (50%)	17 (50%)	34
Total	23	36	59

Highly Significant ($p < 0.001$)

Our results showed a significant negative correlation between serum TGF-β1 level and the tumor size, which shows that TGF-β1 has been strongly implicated in the control of tumor growth. Our results are in agreement with Reiss and Barcellos (28) who suggested that TGF-β1 acts as a tumor suppressor and inhibits the out growth of carcinoma in situ via its antiproliferative functions. On the contrary, some authors found that tumor size was not correlated to the serum level of TGF-β1 (27). Multidrug resistance (MDR) is a unique phenomenon in cancer patients and is commonly associated with overexpression of human MDR gene (MDR-1). Multidrug resistance of cancer is one of the major problems in cancer chemotherapy that is frequently associated with the expression of P-glycoprotein (Pgp) encoded by MDR-1 genes (29).

The current study showed that the frequency of MDR-1 expression was significantly elevated in malignant breast cancer patients compared to patients with benign breast diseases. This result is comparable with that of Correnti et al., (30) and Zhang et al., (31), indicating that normal breast cells have little or no expression of MDR-1 gene. Our results showed that no differences in histological grade, stage, lymph node involvement and histological type were observed between MDR-1 positive and MDR-1 negative expression. These findings are in agreement with Liu et al., (32) and Punyammalee et al., (29).

However, the current study showed that the frequency of MDR1- positive cases was significantly elevated in post-menopausal breast cancer patients in relation to pre-menopausal breast cancer patients.

Maspin is a relatively novel serine protease inhibitor with tumor suppressor function in breast cancer (33,34). The present study showed that maspin gene was more frequently expressed in patients with benign breast diseases in comparison to breast cancer patients. These findings can be explained on basis that maspin is a tumor suppressor gene and usually highly expressed in myoepitelial cells of human breast tissue and is significantly down regulated in breast cancer cells (35). The intracellular proteinase cascades normally function to delete cells through apoptotic mechanism; however, in cancer, tumor cells have acquired ability to evade these mechanisms either through the expression of survival factors or the down regulation of apoptosis inducers (9). Recently, it was proved that the complex-molecular mechanisms of maspin that may suppress breast tumor progression not only at the step of the invasion but also by regulating tumor cell apoptosis (36).

In the present study, expression of maspin gene was related to histological grades of breast cancer. The incidence of maspin-positive tumor was significantly decreased in grade III in comparison to grade II tumors. This observation denotes that decreased expression of maspin gene was associated with higher aggressiveness of the tumor. These results are consistent with those reported by Hojo et al., (35) who found that there was a significant correlation between maspin-positive tumor specimens and low pathological grade of malignancy. These results suggest that maspin production in myoepithelial cells could down regulate the malignant phenotype of breast cancer.

In the present work, maspin gene expression showed an inverse relationship to clinical stages of breast cancer, where maspin-positive tumors were significantly decreased in stages III and IV in relation to stage II this indicates that the expression of maspin in breast tumor cells limits their growth and metastasis, which is in agreement with the studies of Zhang et al., (37) and Streuli,(38).

The relationship of maspin expression with lymph nodal involvement was also assessed in the current study. Maspin expression was significantly decreased in malignant tumors with positive lymph node involvement. Our finding is in accordance with that of Maass et al., (39). This observation strongly supports the biological role of maspin as a tumor suppressor gene, inhibiting tumor invasion and metastasis.

In the current study, we have found that maspin gene expression was significantly decreased in invasive duct carcinoma as compared to other histological types of breast cancer, however, other investigators failed to find such relationship (40).

Our present data showed that the incidence of both MDR-1negative/maspin-positives was significantly elevated in breast cancer patients, which are consistent in part with those reported by Kim et al., (41) who found that Pgp, the glycoprotein encoded by MDR-1 gene is involved in the transport of some protease inhibitors in vitro. Moreover, recent studies reported a strong correlation between increased Pgp levels and the poor prognosis in breast cancer (42). Association of high maspin gene expression with absence of MDR-1 may confirm a less aggressive nature of the tumor, less drug resistance, less tumor progression and good prognosis.

The present study indicated that serum C-erbB-2 level was negatively correlated with serum TGF-β1 level. A possible explanation for this finding is that in the process of tumor progression there is a significant overexpression of C-erbB-2 protein while TGF-β1, which is one of the

Table 4
Relative incidence of MDR-1 and maspin gene expression in breast cancer patients in relation to serum C-erbB-2 and TGF-β1 levels

Parameters	MDR		Maspin	
	MDR-1 +	MDR-1 -	Maspin+	Maspin-
C-erbB-2 (Mean + SE)	72.4 ± 9.75*	39.3 ± 1.83	35.7 ± 2.89	64.3 ± 6.73**
TGF- β1 (Mean + SE)	29.2 ± 2.15	32.6 ± 2.28	38 ± 3.19***	26.3 ± 0.95

* Highly significant form MDR- ($p < 0.001$)
** Highly significant from Maspin + ($p < 0.001$)
*** Significant from Maspin - ($p < 0.05$)

endogenous inhibitors of cell growth, is down regulated.

In the current study, the association between serum levels of either C-erbB-2 or TGF-β1 and the incidence of MDR-1 and maspin genes expression showed that the increase in serum C-erbB-2 results in up-regulation of MDR-1 gene and down regulation of maspin gene. It has been demonstrated that there is a direct connection between oncogenic activation and MDR-1 gene expression gene (43).

Our results are in agreement with Schneider et al., (44) who found that highly statistically significant co- expression of P-glycoprotein and C-erbB-2 took place in the subgroup of aggressive, locally advanced mammary carcinomas. Our observation is consistent with Liu et al., (32) who found that human breast cancer cells that have been transfected with oncogene C-erbB-2 complementary DNA express high levels of the transmembrane glycoprotein p185 (C-erbB-2) and exhibit increased resistance to the chemotherapeutic agent paclitaxel via p175 (MDR-1) expression. Moreover, Hojo et al., (35) focused on identifying the correlation between maspin expression and C-erbB-2 expression. They found that maspin-positive tumors showed down- regulation of C-erbB-2 expression.

Lastly,our results showed also that the serum level of TGF-β1 was significantly higher in maspin-positive tumors. This finding can be explained on the bases that maspin acts as a tumor suppressor gene and has been shown to inhibit cell motility, invasion and metastasis by regulating tumor cell apoptosis (36) and TGF-B1 also inhibits breast cancer progression by inhibition of epithelial proliferation and induction of apoptosis (45,46) and co-expression of both maspin gene and TGF-β1 is the cause of good prognosis.

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