Evaluation of Mammaglobin Transcription and its protein in Blood as a Marker For Diagnosis of Breast Cancer

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ABSTRACT

Breast cancer is the most common cancer and the second most common cause of cancer death among women in the Western world. As understanding of the pathophysiology of cancer increases, the role of tumor markers becomes more important in the management of cancer patients. An ideal tumor marker should be highly sensitive, specific, and reliable with high prognostic value, organ specific and it should correlate with tumor stages. Human mammaglobin (hMAM) gene was cloned in 1996, hMAM encodes a glycoprotein. The expression of hMAM was initially believed to be restricted to the adult mammary gland and breast cancer cell lines. The aim of the present study was to investigate the clinical reliability of each hMAM mRNA and its transcripts as a tumor marker in breast cancer patients to diagnose breast cancer. The subjects were selected from Oncology Department, Faculty of Medicine Menoufiya University before hormonal, chemotherapy or surgical treatment. Eighty six patients suffering from breast cancer and 59 subjects as a control group were enrolled in this study. The control group was subdivided into 11 apparent healthy subjects (18.6%) and 48 patients served as patient control group (81.4%) suffering from cancers rather than breast cancer. ***Results:*** *A highly significant increase in the mean serum level of hMAM was detected in the breast cancer patients compared to the control group. Regarding expression of hMAM mRNA, 50 females having breast cancer (73.5%) were positive, while it was negative in 100% of females in the control group. This gave 73.5% sensitivity, 100% specificity, 100% positive predictive value and 76% negative predictive value.* ***Conclusion:*** *The expression of hMAM is restricted to breast epithelial cells. So, hMAM is a promising marker of interest in breast cancer. Further studies are needed to evaluate its usage for screening and staging of breast cancer.*

INTRODUCTION

Breast cancer is the most common cancer and the second most common cause of cancer death among women in the Western world[(1)]. While at diagnosis up to 90% of the patients have disease limited to the breast and axillary lymph nodes, in nearly half the disease will relapse later on due to progression of clinically occult disease. Although tumor size and extent of axillary lymph node involvement are considered the two strongest predictors of breast cancer recurrence, the detection of bone marrow micro metastases by immunocytochemistry was also shown to correlate with prognosis and decline in disease-free survival[(2)]. Similarly, circulating tumor cells may lead to the development of metastases, and their detection in the peripheral blood of breast cancer patients may thus impact on a patient's prognosis[(3)].

In contrast to hematological malignancies, solid tumors rarely have specific diagnostic genetic changes. To overcome this limitation, tissue-specific markers have been widely evaluated as potential molecular targets for the detection of occult tumor cells[(4)]. A variety of tissue specific markers, including different cytokeratin transcripts, have been widely evaluated as targets for detection of occult breast cancer cells by reverse transcriptase polymerase chain reaction (RT-PCR), but have been shown to be non-specific[(5)]. In contrast to cytokeratins, human mammaglobin, a member of the uteroglobin gene family, was reported to be exclusively expressed in mammary epithelium and over-expressed in some breast cancers, making it a potentially useful RT-PCR target for breast cancer cell detection in hematopoietic products [(6)].

Various molecular markers have been proposed for detecting circulating breast cancer cells, including carcinoembryonic antigen (CEA), cytokeratin-19, β- hCG and Muc-1[(7)]. However, the sensitivity of these markers is influenced by tumor differentiation and the markers may also be expressed in non-breast tissue, including hematopoietic cells. Consequently, their diagnostic value is limited[(8)]. Human mammaglobin (hMAM) gene was cloned in 1996. It is a member of the uteroglobin family, it encodes a glycoprotein, but the cellular function of the gene product remains uncertain. The expression of hMAM was initially believed to be restricted to the adult mammary gland and breast cancer cell lines[(9)]. **Zach et al.[(10)]** reported that hMAM is over-expressed in 23% of primary breast tumors. Expression of hMAM mRNA among breast tumors did not correlate with histological type, tumor grade, tumor stage or hormone receptor status. Based on its breast cancer-associated expression, and breast tissue-restricted distribution, hMAM appears to be a promising candidate as a breast tumor marker[(11)]. Since the presence of circulating cancer cells may to some extent reflect the relative tumor load and possibly the capacity of tumor dissemination, hMAM mRNA expression in the peripheral blood could be a good prognostic factor and should be correlated to certain established clinicopathological

features for breast cancer[12]. Therefore, the aim of this study was to investigate the clinical reliability of each hMAM mRNA and its transcripts as a tumor marker in breast cancer patients to diagnose breast cancer.

SUBJECTS & METHODS

In this study, the patients were selected from Oncology Department, Faculty of Medicine, and Menoufiya University before hormonal chemotherapy or surgical treatment from September 2010 to December 2011. Eighty six female, with mean age 41.2±9.2 years, suffering from breast cancer were diagnosed by mammography (using GE-Senographe-DMR-Plus-Mammography-Machine, USA), breast ultrasound (using GE Logic p5, USA), MRI (using 1.5-T MR scanner, Hitache; Japan) and histological by needle biopsy. Fifty one patients (75%) were diagnosed as invasive ductal carcinoma, 5 patients (7.4%) were diagnosed invasive lobular cancer, 9 patients (13.2%) were diagnosed ductal carcinoma in situ and 3 patients (4.4%) were diagnosed mucinous carcinoma. The patients underwent chest x ray, abdomen and pelvis US, CT scan for chest & abdomen (using a helical scanner ; Somatom Plus 4, General Electric Medical Systems, Milwaukee, USA with 2-mm collimation and subsequent sagittal & coronal reformats) and bone scan (using Merdian9YS 8763, Denmark) for diagnosis of metastasis.

Control group:

This group included 59 subjects with mean age 38.4±10.3 years: 11 apparent healthy subjects (18.6%) and 48 patients (81.4%) served as patient control group subdivided as 12 patients (20.3%) having fibroadenoma [benign breast mass (BBM)], 13 patients (22%) having uterine cancer (endometrial adenocarcinoma), 13 patients (22%) having cancer ovary (ovarian clear cell carcinoma) and 10 patients (16.9%) having cancer colon (adenocarcinoma). The subjects in the control group underwent mammography and breast US. A written informed consent was obtained from all participants. The protocol was approved by the committee of ethics medical research of Faculty of Medicine, Menoufiya University.

US-guided Core Needle Biopsy:

Use of a high-frequency, correctly focused 10–12-MHz probe improved the resolution and contrast of the lesion. In addition, US techniques that optimize performance were essential. Sufficient gel was used, and the focal zone was placed just below the lesion. Adjustments in the dynamic range or post-processing gray scales improved contrast so that lesions were more visible. US-guided core needle biopsy performed with a 14-gauge spring loaded device was a low-cost, well-tolerated approach for the biopsy of lesions with a solid component that was expected to remain clearly visible throughout the procedure.

Paraffin-embedded samples were cut into 4-μm sections, stained with hematoxylin–eosin and E-cadherin. Immunoreactivity examined for diagnosis of different groups of studied patients.

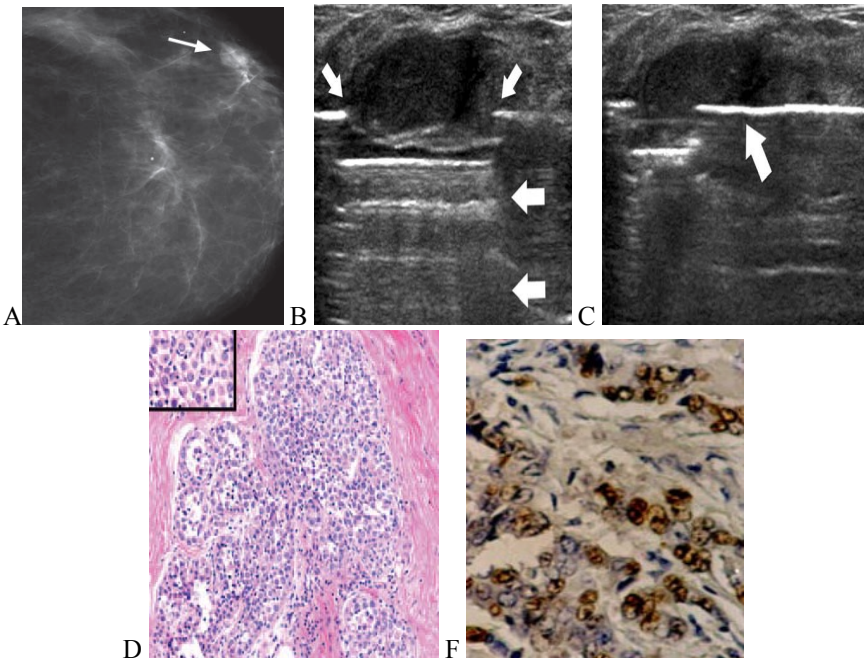

Figure (1) 50-year-old woman. **(A)** Mammogram shows round mass that corresponds to a palpable abnormality. **(B)** US image shows that the probe (small arrows) is posterior to the lesion, which is nevertheless within the limits of the probe aperture. The aperture can be clearly visualized owing to the discontinuity of the anterior wall of the probe and the ring-down artifact on the opposite side of the aperture (large arrows). **(C)** US image shows that the cutter (arrow) has been advanced through the lesion. **(D)** Magnification, _40 [background], _400 [inset]; H-E stain) of the subsequent biopsy specimen shows incidental carcinoma in situ. **(E)** Infiltrating ductal carcinoma showing positive staining (ER) (immunoperoxidase x300).

Immunohistochemical-study:

Streptavidin-biotin technique was used to investigate estrogen receptors (ER) and progesterone receptors (PR). Two slides from each case were deparafinized, hydrated and incubated in 3% hydrogen peroxide for 30 minutes to block the internal peroxidase activity. Antigen retrieval was done by microwave pretreatment for 10 minutes in 0.01 citrate buffer. For each case, one slide was incubated with mouse monoclonal antibody to ER at a dilution 1:50 (Dako Corporation). Second slide was incubated with mouse monoclonal antibody against PR (PgR636, 1:800; Dako Japan, Tokyo, Japan). Sections were then washed twice for 5 minutes with PBS and incubated for 10 minutes with biotinylated secondary antibody (Dako, Cytomation). The slides were washed twice for 5 minutes in phosphate buffer saline (PBS) and incubated for 10 minutes in performed avidin-biotin-peroxidase

complex (Dako, Cytomation). Chromogen development was accomplished by immersion of the sections in 2, 3-Diaminobenzidin tetrahydrochloride (BAB) (Dako, Cytomation) for 5 minutes. The nuclei were counter stained with hematoxylin, dehydrated, cleared and mounted. For negative controls, the primary antibody was omitted and replaced with PBS.

Laboratory investigations:

Under complete aseptic conditions, 5 ml of venous blood was collected by venipuncture in sterile plain vacuum tube from each subject, centrifuged at 3500 rpm for 15 min at 4^0C and the resulting serum was transferred into 1 ml aliquots and stored at -70^0C for later analyses. Assessments of serum CA_{15-3} was determined using VIDAS instrument, BioMerieux, France using the Enzyme Linked Fluorescent Assay (ELFA).

Serum mammaglobin protein level was measured by Total Mammaglobin A Cell-Based Fluorometric ELISA Kit, Immunoway, Sweden.

Detection of hMAM m RNA:

RNA extraction:

QIAmp RNA blood kit (QIAGEN Inc, USA) was used. The extracted RNA was diluted prior to its assay. The concentration and purity of RNA extracts were determined by measuring their absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) using a spectrophotometer. Pure RNA has an A_{260}/A_{280} ratio of 1.5-1.8. An absorbance of 1 µ unit at 260 nm corresponds to 40 µg. RNA/ml. The concentration of RNA stock was then determined (concentration of RNA stock = 40 RNA µg/ml x A_{260} x dilution factor), then the total yield was calculated by multiplying concentration by volume of stock in ml.

cDNA synthesis:

Total RNA was reverse transcribed into total cDNA using an oligo-dT primer (Promega, Madison, Wisconsin). Five µg of total RNA was incubated with 50 ng of oligo-dT primer at 70 °C for 10 minutes. 50µl. reaction was performed in 10 mM dNTPs (Pharmacia LKB Biotechnology, Uppsala, Sweden), 10 mM DTT, 1 X reverse transcriptase buffer (50 mM Tris HCl, 6 mM $MgCl_2$,40 mmol/1 KCl) final concentration, adding 20 U of ribonuclease inhibitor (RNAsin; Promega, Madison, Wisconsin) and 200 U Moloney murine leukemia virus reverse transcriptase (Superscript; GIBCO BRL, Gaithersburg, Maryland). The reaction mixture was incubated for one hour at 37°C. Reverse transcriptase was heat inactivated at 95 °C for five minutes. Amplification was performed in a thermal cycler (Perkin-Elmer 7500).

PCR amplification:

Two human genes were amplified with this protocol: B-actin and Mammaglobin. A second nested PCR was performed because the signal obtained from plasma RNA was weak. B-actin was used to verify the presence and integrity of serum RNA because storage can modify the results.

Two µl of each reaction product were analyzed by nested-PCR. The cDNA was amplified in 200 nmol/1 dNTPs, 1 x Taq buffer [50mmol/1 KCl, 10 mmol/1 Tris/HCl pH, I mmol/1 $MgCl_2$], adding 2,5 U of Taq DNA polymerase (Promega) and from

10 pmol of primers in a final volume of 50ul.

All nested PCRs were amplified using 1.2µl from the first PCR amplification in a final volume of 10µl in the presence of 0.3 units of Ampli Taq Gold DNA polymerase (Perkin-Elmer, Roche Molecular Systems, Inc., Branchburg, NJ), 1µl of 10 X PCR buffer, 200 µM deoxynucleoside triphosphate, 0.6 µM of each primer, and different concentrations of MgCl2, depending on the gene.

B-actin primers: (outer, sense) 5_CCAACCGCGAGAAGATGACC3, (outer, antisense) 5`_TGCCAATGGTGATGACCTGG3, (nested, sense) 5_GATCATGTTTGAGACCTTC3`, (nested, antisense) 5`_GTCAGGCAGCTCGTAG3`.
PCR conditions for outer primers were: 95°C for 9 min followed by 40 cycles of 95°C for 20 sec, 64°C for 15 sec, 72°C for 30 sec, and finally 72°C for 11 min. Nested PCR conditions were: 95°C for 9 min followed by 40 cycles of 95°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec and finally 72°C for 11 min.

Mammaglobin Primers: (outer, sense) 5`_CAGCGGCTTCCTTGATCCTTG 3` (outer, antisense) 5`_TAGCAGGTTTCAACAATTGTC 3` (nested, sense) 5`_AGCACTGCTACGCAGGCTCT3 ` (nested, antisense) 5`_ATAAGAAAGAGAAGGTGTGG 3`. PCR conditions for outer primers were: 95°C for 9 min followed by 43 cycles of 95°C for 3` sec, 55°C for 30 sec, 72°C for 60 sec, and finally 72°C for 11 min. Nested PCR conditions were: 95°C for 9 min followed by 40 cycles of 95°C for 30 sec, 61°C for 30 sec, 72°C for 30 sec and finally 72°C for 11 min [13].

Detection: The amplified products were analyzed by electrophoresis on 2% agarose gel for 55 min at 500V**.** The final 10µl of the PCR product was mixed with a 3µl volume of loading buffer (total volume, 13µl) (figure 2).

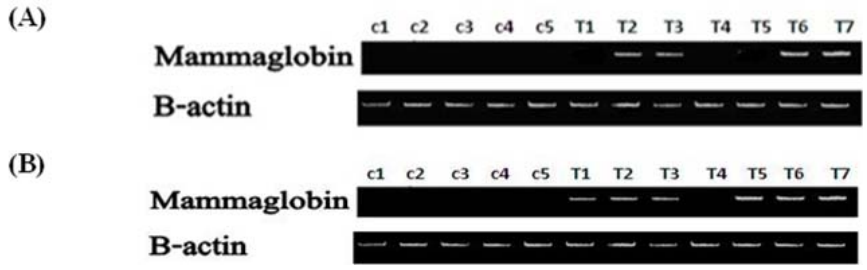

Figure (2): (A) First amplification PCR products of mammaglobin mRNA of controls (C) and patients (T) with breast cancer. The figure shows four patients (T2, T3, T6 and T7) with positive marker. (B) Second PCR amplification products of mammaglobin mRNA of the same controls (C) and patients (T) with breast cancer. The figure shows six patients (T1, T2, T3, T5, T6 and T7) with positive marker. B-actin shows positive markers in all controls and patients in both rounds.

Statistical Analysis:

Statistics were carried out using SPSS (Statistical Package for Social Science) program version 17.0 on IBM compatible computer. Descriptive statistics [percentage (%), mean (x) and standard deviation (SD)] were done. Analytic statistics as Chi-square test (χ^2) was used to study association between two qualitative variables, t- test was used to compare between two groups normally distributed having quantitative variables, Mann-Whitney test (nonparametric test) was used to compare between two groups not normally distributed having quantitative variables and Kruskal-Wallis test (nonparametric test) was used for comparison between more than two groups not normally distributed having quantitative variables. Receiver Operating Characteristic (ROC) curve was produced for the measured parameters to investigate the sensitivity, specificity, predictive values and the cut-off values of CA 15-3 and hMAM to differentiate breast cancer from other conditions. P value less than 0.05 was considered statistically significant.

RESULTS

Table (1) showed the characters of the studied groups regarding the radiological investigation. Mammography and MRI (breast) detected cancer breast in all cases group (68 patients). 11 females (18.6%) in the control group showed benign breast mass (BBM). Table (2) showed a highly significant increase in the mean serum levels of both CA $_{15-3}$ and hMAM in the case group compared to the control group. Regarding expression of hMAM mRNA, 50 females (73.5%) having breast cancer were positive. Table (3) showed the studied tumor markers in the control subgroups. All healthy and patient control subgroups were negative for expression of hMAM mRNA.

Figure (2) represented the ROC curve of both CA $_{15-3}$ and hMAM to differentiate breast cancer from other conditions.

At cut-off value >0.99µg/l; hMAM yielded a 82.4% sensitivity and a 64.4% specificity and at cut-off >21.85 IU/ml for CA $_{15-3}$ gave a sensitivity of 85.3% and a specificity of 66.1% to differentiate breast cancer from others as reported in table (4).

Table (5) showed that expression of hMAM mRNA was positive in 73.5% of breast cancer patients, while it was negative in 100% of females in the control group. This gave 73.5% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 76% negative predictive value (NPV). There was no significance difference between positive and negative expression of hMAM mRNA cases in the patients having breast cancer as regards tumor size and stage, clinical stage, lymph node stage and ER & PR receptors; but there is only a significant relationship which was observed in the mean serum level of hMAM as regards clinical and lymph node stages and distant metastasis (table 6).

Table 1: Radiological finding in the studied groups

		Studied groups			
		Cases N = 68		Controls N = 59	
		No	%	No	%
Mammography	BBM	0	0.0	11	18.6
	Free	0	0.0	48	83.4
	Breast cancer	68	100	0	0.0
Breast MRI	BBM	0	0.0	11	18.6
	Free	0	0.0	48	83.4
	Breast cancer	68	100	0	0.0
CT	No metastasis	55	80.9	00	00
	Metastasis in liver	5	7.4	00	00
	Metastasis in lung	8	11.8	00	00
Bone scan	Positive	11	16.2	00	00
	Negative	57	83.8	00	00

BBM: benign breast mass

Table 2: Statistical comparison between case and control groups as regards the studied tumor markers

		Studied groups				Test of significance	P Value
		Cases N = 68		Controls N = 59			
CA 15–3	X ± SD	248.89±360.42		17.31±14.54		7.6#	<0.001
hMAM (µg/l)	X ± SD	4.01 ±13.31		0.83 ±0.23		7.16#	<0.001
		No	**%**	**No**	**%**	χ^2	
hMAM mRNA	Positive	50	73.5	0	0.0	71.55	<0.001
	Negative	18	26.5	59	100		

P-value is highly significant at <0.001 # Mann-Whitney test was used

Table 3: The studied tumor markers in control subgroups.

	Control group									
	Healthy N = 11		BBM N = 12		Uterine cancer		Cancer ovary N = 13		Cancer colon N = 10	
CA 15–3 (IU/ml)	13.15±13.6		19.7±14.19		18.76±12.24		22.71±19.31		10.77±9.63	
hMAM (µg/L)	0.81 ±0.2		0.87±0.17		1.03±0.15		0.83 ±0.19		0.58 ±0.25	
	No	%	No	%	No	%	No	%	No	%
hMAM mRNA:										
Positive	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Negative	11	100	12	100	13	100	13	100	10	100

BBM: benign breast mass

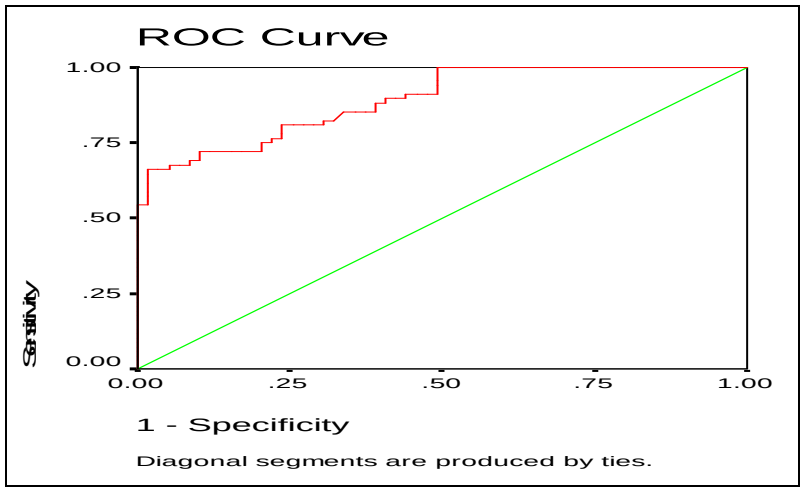

Figure 2: ROC curve of CA 15-3 and hMAM to differentiate breast cancer from other conditions

Table 4: ROC curve analysis of CA 15-3 and hMAM to differentiate breast cancer from other conditions

Variables	cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
CA 15-3 (IU/ml)	21.85	85.3%	66.1%	74.4%	79.6%	76.4%
hMAM (µg/L)	0.99	82.4%	64.4%	72.7%	76%	74%

PPV: positive predictive value NPV: negative predictive value

Table 5: Validity of hMAM mRNA to differentiate breast cancer from other conditions

	Studied groups					
	Cases N = 68		Control N = 59		Total N = 127	
	No	%	No	%	No	%
hMAM mRNA						
Positive	50	73.5	00	0.0	50	39.4
Negative	18	26.5	59	100	77	60.6

This test gave sensitivity= 73.5%, specificity = 100%,
positive predictive value = 100%,
negative predictive value = 76% and accuracy = 76.6%.

Table 6: Relation between each of hMAM and hMAM mRNA as regards pathological and radiological parameters.

	hMAM (µg/l)			hMAM mRNA				χ^2	P value
	Mean ± SD	Kruskal-Wallis test	P value	Positive N = 50		Negative N = 18			
				No	%	No	%		
Tumor size									
< 2 cm	2.15 ± 0.08			2	4.0	0	0.0		
2 – 5	2.51 ± 2.30	0.37	>0.05	13	26.0	6	33.3	1.05	>0.05
6 – 10	5.54± 18.73			25	50.0	9	50.0		
> 10	2.52 ± 1.84			10	20.0	3	16.7		
Tumor stage									
0	1.79 ± 1.05			6	12.0	3	16.7		
1	2.71 ± 2.39	0.40	>0.05	9	18.0	5	27.8	2.79	>0.05
2	5.38 ± 17.96			28	56.0	9	50.0		
3	2.53 ± 2.13			5	10.0	0	0.0		
4	2.34 ± 1.31			2	4.0	1	5.6		
Clinical stage									
I	2.20 ± 1.17			4	8.0	1	5.6		
II a	1.31 ± 0.54	21.68	<0.01	9	18.0	3	16.7		
II b	1.91 ± 1.42			8	16.0	6	33.3	5.50	>0.05
III a	1.76 ± 1.12			4	8.0	3	16.7		
III b	11.43± 33.04			8	16.0	3	16.7		
IV	4.28 ± 2.43			17	34.0	2	11.1		
Lymph node									
0	5.70 ± 21.07			16	32.0	11	61.1		
1	1.98 ± 1.30	14.17	<0.01	20	40.0	5	27.8	5.05	>0.05
2/3	4.27 ± 2.70			13	26.0	2	11.1		
3/4	5.4			1	2.0	0	0.0		
ER receptors									
UN	1.65±0.47	0.75	>0.05	2	4.0	0	0.0		
Positive	2.46 ± 2.06			43	86.0	17	94.4	1.12	>0.05
Negative	20.29± 44.45			5	10.0	1	5.6		
PR receptors									
UN	38.82± 62.52	4.75	>0.05	3	6.0	0	0.0	1.13	>0.05
Positive	2.53 ± 2.07			42	84.0	16	88.9		
Negative	1.38 ± 0.67			5	10.0	2	11.1		
Distant metastasis	4.56±2.31	4.79#	<0.001	17	34.0	1	5.6	5.5##	<0.05
Positive	3.81±15.5			33	66.0	17	94.4		
Negative									

ER: estrogen PR: progesterone P-value >0.05 isn't significant and p-value <0.01 is significant.

Mann-Whitney test was used. ## Fisher Exact test was used.

DISCUSSION

Breast cancer is a major problem among females allover the world. It is a heterogeneous disease with a varying propensity for spread. Despite all efforts done during the past years, the incidence of breast cancer mortality is still rising and represents the leading cause of death in women in their mid-life[14]. Clinical examination, mammography, fine needle aspiration biopsy and open surgical biopsy are established diagnostic methods in breast cancer and the final diagnosis of cancer should be based on histopathology [15]. In breast cancer, many tumor markers have been studied in the hope of finding a blood test for cancer but none of them has had such sensitivity and specificity that it could replace conventional diagnostic methods[16]. The objective of the present study was to investigate the detection of hMAM mRNA and its transcripts in peripheral blood of breast cancer patients to evaluate them as tumor markers in diagnosis of breast cancer.

In the current study, serum level of CA $_{15\text{-}3}$ was significantly higher in breast cancer patients compared to the control group. The present results agreed with those of **Sliwowska et al.**[17] as they stated that in the early diagnosis, treatment planning and follow-up of breast cancer, several tumor markers may be helpful especially CA $_{15\text{-}3}$. Also, **Duffy** [3] suggests that determination of CA $_{15\text{-}3}$ can provide real-time prognostic information in patients with breast cancer. This study showed that at cut-off value >21.85 IU/ml for CA $_{15\text{-}3}$ to diagnose breast cancer, it yielded 85.3% sensitivity and 66.1% specificity. However, the present result disagreed with that of **Malati**[2] as he reported that the diagnostic sensitivity of the CA $_{15\text{-}3}$ for breast carcinoma is low as its elevated levels are also observed in benign breast diseases, liver cirrhosis, acute and chronic hepatitis and metastatic cancers of pancreas, ovary, colorectal, lung, stomach and uterus. Also, **Sliwowska et al.**[1] found that the diagnostic sensitivity of the CA $_{15\text{-}3}$ was low (44%), while it had a high diagnostic specificity (90%).

In the present study, a highly significant increase in the mean serum level of hMAM was detected in the breast cancer patients than the controls. Also, expression of hMAM mRNA was found in 50 of the 58 breast cancer patients (73.5%), while it was negative in all healthy and patient control subgroups. At cut-off >0.99µg/l for hMAM gave 82.4% sensitivity, 64.4% specificity, 72.7% positive predictive value and 76% negative predictive value to diagnose breast cancer. While expression of hMAM mRNA gave 73.5%, 100%, 100%, 76% and 76.6% for sensitivity, specificity, PPV, NPV and accuracy respectively.

Lin and his colleagues[18] stated that hMAM is considered to be a promising candidate for a sensitive molecular marker for breast cancer. **El-Sharkawy and his co-workers**[19] found 79% of the breast cancer tumors had diffuse cytoplasmic hMAM expression. Also, **Zehentner et al.**[20] stated that mammaglobin tissue expression has been shown in ~80% of breast cancers in their study and it is much higher than previous results

as **Gruenewald et al.[21].** While **Wronski[12]** found the expression of hMAM mRNA in the peripheral blood was detected in 54% of cases. **Fleming and Watson[22]** stated that the frequency reported for hMAM expression in breast cancer patients varies from 20% to 95%. This difference in the detection rates between the studies may be attributed to the difference in the sensitivity of the technique as by nested PCR technique or by single round of PCR which is less sensitive than the former technique as explained by **El-Sharkawy et al.[19].** Also, the increased rate of detection in some studies as the current study may be related to the fact that the patients examined were untreated, whereas many of the women examined in other studies had undergone chemotherapy and the treatment may lower the number of detected circulating tumor cells as explained by **Zehentner et al.[20]**. Thr present results parallel those of **Bernsttein and his colleagues, [23]** as they stated that no hMAM mRNA positive cells were detected in the blood of the healthy control group or in patients with carcinomas other than breast cancer. Also, **Cerveira et al.[24]** stated that the expression of human mammaglobin mRNA in the peripheral blood was undetectable in other human cancers. This finding means that circulating hMAM mRNA is a specific marker for breast cancer.

Also, the present results confirm the findings of **Bitisik et al.[11]** as they revealed an overall sensitivity of hMAM mRNA was 64.6% and the specificity was very high (100%). **Ozyilkan et al.[15]** stated that specificity and PPV are more important in the detection of breast cancer. So, hMAM mRNA is more important in the detection of breast cancer.

In the current study, no significant correlation was observed between each of hMAM mRNA and hMAM with either of tumor size, tumor stage and estrogen & progesterone receptors while a statistical significant relation was observed between the same tumor markers with distant metastasis. A significant correlation was observed between hMAM only and each of clinical stage and lymph node. **Wronski,[12]** found that the expression of hMAM mRNA correlated with nodal status, tumor size and metastasis while he did not find any correlation with hormone receptor status, menopausal status, tumor grade or therapy in the group of breast cancer patients in his study. **Cerveira et al.[24]** and **El-Sharkawy et al.[19]** stated that there is a positive correlation between the expression of hMAM mRNA and breast cancer stage and the detection rates of it were going hand in hand with the progress of the disease. Moreover, our results arte comparable to those of **Lin et al.[18]** as they detected a significant correlation in frequency of positive RT-PCR between patients with a localized disease (29%) and patients with a metastatic disease (54%) and no correlation with primary tumor size, overall stage and estrogen receptors.

Zehentner et al.[20] and Bitisik et al.[11] stated that there is a lack of correlation between mammaglobin expression and clinical parameters.

Also, they stated that whereas expression of hMAM mRNA was frequently shown to be increased in patients with unfavorable prognostic factors (tumor size and disease stage), no significant difference could be confirmed and the detection of the mammaglobin transcript alone was only marginally associated with increased nodal involvement and not with other tumor or patient characteristics.

In conclusion: The expression of hMAM is restricted to breast epithelial cells. So, due to this exclusive expression in breast tissue, hMAM is a promising marker of interest in breast cancer. Further studies with larger series of patients is needed to evaluate its useful for screening and staging of breast cancer and its potential role in following patients after surgery and detecting recurrence or metastasis.

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تقييم كفاءة ماموجلوبين والبروتين الخاص به فى الدم كدلالة لتشخيص سرطان الثدى

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سرطان الثدي هو أكثر أنواع السرطان انتشارا، ويعد السبب الثاني الأكثر شيوعا للوفاة بالسرطان بين النساء في العالم. يزداد افراز دلالات الاورام فى عدد كبير من الخلايا السرطانية. وظهور هذه الدلالات وزيادة تركيزها فى الدم يتناسب مع نمو الاورام السرطانية. ينبغى ان يتوفر فى دلالات الاورام المثالية خصوصية وحساسية عالية وان تتناسب نسبته وتتطابق مع مراحل تطور الورم. ان تشخيص مرض سرطان الثدى يعتمد على الفحص السريرى والتصوير الاشعاعى للثدى واخذ عينة جراحية من الورم وتحليلها باثولوجيا. هناك العديد من دلالات الاورام تم دراستها على امل العثور على فحص بالدم لمرضى سرطان الثدى يساعد فى التشخيص المبكر ويعتمد على خصوصية و حساسية عالية. وقد استهدفت هذه الدراسة تقييم كفاءة چين ماموجلوبين والبروتين الخاص بة لتشخيص سرطان الثدى . وقد تم دراسة المرضى من قسم الأورام بكلية الطب، جامعة المنوفية قبل العلاج الهرموني، والعلاج الكيميائي أو الجراحي. تم دراسة ثمانية و ستون مريضة يعانون من سرطان الثدي و ٥٩ سيدة كمجموعة ضابطة في هذه الدراسة مقسمة إلى ١١ سيدة (١٨.٦٪) لا تعانى من اى امراض و ٤٨ مريضة (٨١.٤٪) يعانون من أمراض سرطان غير سرطان الثدي. ولقد اثبتت الدراسة زيادة احصائية كبيرة فى مستوى بروتين ماموجلوبين فى مجموعة مرضى سرطان الثدى عن المجموعة الاخرى. ولقد ثبت وجود چين ماموجلوبين فى ٥٠ من مرضى سرطان الثدى (٧٣.٥%) ، في حين كانت سلبية في ١٠٠٪ من الإناث في المجموعة الضابطة. مما سبق يتضح اهمية چين ماموجلوبين والبروتين الخاص بة كدلالة واعدة لتشخيص مرض سرطان الثدى ولكن هناك حاجة الى المزيد من الدراسات لتقييمه ودراسة اهميتة فى تحديد مراحل سرطان الثدى والكشف عن تكرار الورم.