ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Evaluation the Diagnostic and Prognostic Value of Human Mammaglobin (MGB 1) Gene Expression in Iraqi Breast Cancer Patients

Jawdat N. Gaaib[1], Abdul Hussein M. AL-Faisal[2] , Khalid Tobal[3] and Nada Al-Alwan[4]

1. College of Applied Medical Science, Karbala University, Karbala, Iraq
2. Genetic Engineering and Biotechnology Institute, University of Baghdad, Baghdad, Iraq
3. Molecular Oncology Unit, Guys Hospital, Kings College, London, UK
4. National Cancer Research Center, Medical College, University of Baghdad, Baghdad, Iraq

Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 29 March 2014
Published Online: April 2014

Key words:
MGB 1, Breast Cancer, Benign tumor, Lymph nodes, Rt PCR, Expression.

Corresponding Author

Prof.Dr. Abdul Hussein M. AlFaisal

Abstract

Breast cancer is the most frequent carcinoma in females and the second most common cause of cancer related mortality in women. Early detection of breast cancer is widely reported to be one of the most effective ways leading to better prognosis and lower death rate. For marker discovery, the analysis of mRNA expression signatures in peripheral human blood has been widely used showing to be a promising technique. The human mammaglobin (MGB 1) is a novel gene that was diagnosed as a highly specific marker for primary breast cancer. The aim of the present study is to detect the expression levels of the human mammaglobin (MGB 1) mRNAs in the peripheral blood of breast cancer patients in comparison with benign tumors and healthy controls as a tool for screening and diagnosis the early stage breast cancers, and estimating the prognostic values of these levels in association with age, tumor size and lymph node status. The marker was determined in peripheral blood (PB) of 55 patients with Invasive Ductal Carcinoma and samples from 20 healthy donors, and 10 women with newly diagnosed benign breast tumors were served as control group using reverse transcriptase polymerase chain reaction (RT-PCR). Mammaglobin was detected in 30 (54.5%) of peripheral blood of breast cancer patients studied, 1(10%) of the benign tumors but not in any of healthy individuals. It showed statistically significant relations with size of the tumor, and Lymph node involvement. On the other hand, it was statistically non- significant for age of breast cancer patients. The present study results suggest that mammaglobin is a specific molecular marker for detection of breast cancer, discrimination between benign and malignant breast tumors, and it might be of value as a prognostic marker.

Introduction:

Cancer is one of the most important health problems of the current era and also a leading cause of death among populations. Cancer can simply be defined as a class of diseases or disorders that is characterized by uncontrolled division of cells and the ability of these abnormal cells to spread, either by direct growth into adjacent tissues through invasion, or by implantation into distant sites by metastasis (where cancer cells are transported through the bloodstream or lymphatic system (Blachford, 2002). Breast cancer is the most commonly diagnosed malignancy in women around the world, especially in the Western countries. It accounts for almost one fifth of deaths caused by cancer (Winer *et al*., 2001). Every year, one million new cases are reported worldwide, representing 18% of the total number of cancer in women. In Iraq it has been detected that the number of breast cancer cases are steadily rising since the 1991 war (Jaffer, 1999; Jasim, 2004).

Breast cancer is the malignant tumor that forms from the uncontrolled growth of abnormal breast cells. It usually affects tissues involved in milk production (Ductal and lobular tissues) (Madhavan *et al*., 2002). Its originated from the terminal ducto-lobular unit of breast tissue. Breast cancer that has not invaded the basement membrane and thus confined within the terminal ductolobular units is termed carcinoma in-situ. Mainly, there are two types of in-situ cancers; lobular carcinoma in-situ and ductal carcinoma in-situ (Atalay, 2004). Beside these common types of invasive breast cancers, there are other rare forms such as medullary, papillary, mucinous, tubular, apocrine and adenoid cystic carcinoma (Winer *et al*., 2001). As in the case of most of the cancers, staging of breast cancer takes into consideration the size of the tumor (T), the number and location of metastatic lymph nodes (N), and distant organ metastasis (M) (Greene *et al*., 2002). Previous studies have indicated that detection of circulating tumor cells (CTCs) in the peripheral blood can be used in staging and prognosis stratification for breast and colon cancer patients (Allen *et al*., 2010; Wülfing *et al*., 2006). To date, the most common CTCs detection method is quantitative real-time reverse transcription polymerase chain reaction (RT-PCR), a process that can detect mRNA expression levels of the genes coding for these tumor antigens (Ghossein *et al*., 1999). A high-quality detection marker is required for efficient quantitative real-time RT-PCR-mediated detection of CTCs. Therefore, identification of a good target marker is of the utmost importance for CTC detection. Several gene markers, such as MGB1, a member of a family of epithelial secretory proteins, the uteroglobulins, is considered to be a specific breast marker (Watson *et al*., 1996). MGB 1 has been studied as a marker for the molecular detection of CTCs in gastrointestinal cancers (Leelawat *et al*., 2006). Using different assays mostly in small patient cohorts, consisting of primary and metastatic breast cancer patients who have not been homogeneously treated (Silva *et al*., 2002; Reinholz *et al*., 2005). Therefore, its role as a prognostic marker in early breast cancer still remains unclear. The aim of the present study was to evaluate the diagnostic and prognostic values of the human mammaglobin (MGB 1) gene by comparing the levels of MGB 1 gene expression of breast cancer patients, benign breast tumors and healthy controls in relationship with certain clinical characteristics (tumor size and lymph node status).

Materials and Methods

Patients and clinical samples

Blood samples from 55 patients with different stages of newly diagnosed Invasive Ductal Carcinoma were provided by certain Iraqi hospitals (including National Center for Early Detection of Tumors and Al-Elweya Teaching Hospital) after patients underwent cytopathological (Fine needle aspiration FNA) and histopathological examination. Two control groups were used in this study, 10 samples of patients with benign breast tumors, and 20 samples from healthy donors. The required information about the patients and the histopathologic properties of the tumors were recorded from the patients' files. The samples preservation with TRIzol was done at the Genetic lab of National center for early detection of tumors in Baghdad Medical City. Out of 2ml of peripheral blood that drawn into EDTA tubes, 0.5 ml was preserved as whole blood after treating with trizol (sample which was centrifuged at 1,000 Xg for 5 min. at 4Cº followed by removing the supernatant and adding phosphate buffer saline (PBS) containing 5% Triton X-100 and vortexed to be homogenized then a 0.75 ml of trizol added to each sample in a ratio of 3 TRIzol :1Sample volume) then the samples were kept at -80Cº. Samples subjected to RNA extraction and molecular study by using Revers Transcription and Real Time PCR at Molecular Oncology Unit in Guy´s Hospital – Kings College/London-UK.

RNA extraction, reverse transcription and real-time RT-PCR assay

The total RNA of breast cancer, benign tumors and healthy control samples was extracted using the TRIzol® LS reagent(Life Technologies - Ambion CO.) following the protocol provided by the manufacturer. Total RNA was reversely transcribed using High-Capacity cDNA Reverse Transcription Kit. The procedure was carried out in a reaction volume of 20 µl following the protocol provided by the manufacturer (Applied Biosystem) cDNA was stored at -80 °C until use.

Expression of MGB 1 gene was analyzed using specific primers and probes (Table 1). Serial dilutions of primers and probes were used for preparing of standard curve. Standard curve were prepared for both the target and the endogenous control genes(Figure 3.1,3.2). The data generated from serial dilution of standard curve were excellent means which determined the overall performance of QPCR assay. In this assay, the housekeeping gene ABL was used as an internal control to normalize variations in integrity and the total amount of cDNA. Quantitative real-time PCR assays were performed in duplicate using TaqMan master mix (Applied Biosystem/ USA) in 20 µl reaction volume containing10 µl of master mix (TaqMan master mix), 1 µl of primer mixes , 5µl of RNase free water and 4µl of cDNA template on the 7900 HT Fast Real-time PCR system (Applied Biosystem/ USA). Real-Time PCR protocol was as follows; stage 1: 50 ºC for 2 minutes, stage 2: 95 ºC for 10 min and in a stage 3: in a two-

step cycle procedure (denaturation 95 ºC for 15 Sec. and annealing 60 ºC for 1 min) repeated for 50 cycles. Melting curve analysis was used to assess the specificity of the amplified products. The expression levels of MGB 1 gene from the cDNA were measured by quantitative real-time PCR using the relative quantification method ($2^{-\Delta\Delta Ct}$ method). The fold-change in gene expression was normalized to a housekeeping gene ABL and relative to a calibrator sample.

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to effect of difference factors in study parameters or percentage. The chi-square test at the comparative between percentage & least significant difference –LSD test to the comparative between means in this study.

Ethical use of data

Informed consent was obtained from all the study participants and the guidelines set by the ethics committee of our institute and hospitals were applied.

Results:

The patients' age range was 24-70 years and the median is 49 years with high frequency of patients in the range of 40-59 years. According to the family history, 50(90.91%) of patients were have negative family history which statistically high significance differences (X^2 =13.473 **, p<0.01) in comparison with patients that have positive family history. According to the lymph node status, the percentage of patients with multiple lymph nodes was higher than those with few or no lymph nodes which showed statistically high significant differences (p value 0.0017**p<0.001), (Table.2). In regard to the tumor size the highest percentage of patients showed the tumor size 2.0-2.9 cm. which showed statistically high significant differences (p value 0.0014**p<0.001), (Table.3). Out of 55 patients, 30 (54.5%) patients were MGB 1-positive while 25(45.4%) patients were MGB 1-negative. According to malignancy status the percentage of patients with high level of MGB 1 gene expression 22(40%) was significantly higher in compare with benign tumor patients and healthy controls that showed either low or no expression,(Figure.1). In correlation with age groups the present study showed statistically no significant differences in the levels of gene expression with age, (Figure 2). In correlation to the lymph node status the results of the present study showed that the highest percentage of MGB 1 positive patients 18(66%) were multiple for lymph node status that significantly different from percentage of MGB 1 positive patients with no or few lymph node status (p value 0.0019 **p<0.001), (Figure 3).

Table 1.Primers and Probes sequences

Primers and Probes used with RT-qPCR		
Primer	**Sequence**	**Melting temperature**
MGB 1-F	5′-TGCCATAGATGAATTGAAGGAATG-3′	47.2 Cº
MGB 1-R	5′-TGTCATATATTAATTGCATAAACACCTCA-3′	47.9 Cº
MGB 1-P	5′-TCTTAACCAAACGGATGAAACTCTGAGCAATG-3′	55.5 Cº
ABL-F	5′-TGGAGATAACACTCTAAGCATAACTAAAGGT-3′	49.9 Cº
ABL-R	5′-GATGTAGTTGCTTGGGACCCA-3′	47.3 Cº
ABL-P	5′-CCATTTTTGGTTTGGGCTTCACACCATT-3′	52.5 Cº

Table 2: distribution of patients according to lymph node status

Lymph node status	Patients	
	No.	%
No	9	16.36
Few	19	34.54
Multiple	27	49.1
Total	55	100
Chi-square value		11.092 **
P-value		0.0017

Table 3: Distribution of patients group according to tumor size

Tumor size (cm)	Patients	
	No.	%
1.0-1.9	14	25.45
2.0-2.9	19	34.55
3.0-3.9	18	32.73
4.0-4.9	4	7.27
Total	55	100
Chi-square value		11.267 **
P-value		0.0014

According to the tumor size the results showed that there was decreasing in the MGB 1 gene expression with increasing of tumor size since the highest percentage of MGB 1 positive patients 10(71.42%) were with tumor size 1.0-1.9 cm. which showed statistically high significant differences (p value 0.00038 **p<0.0001),(Figure 4).

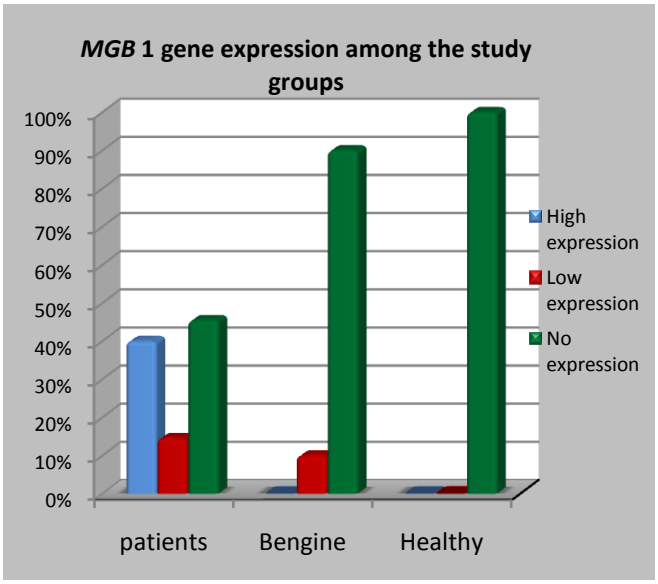

Figure1.Differences in *MGB 1* gene expression among the study groups

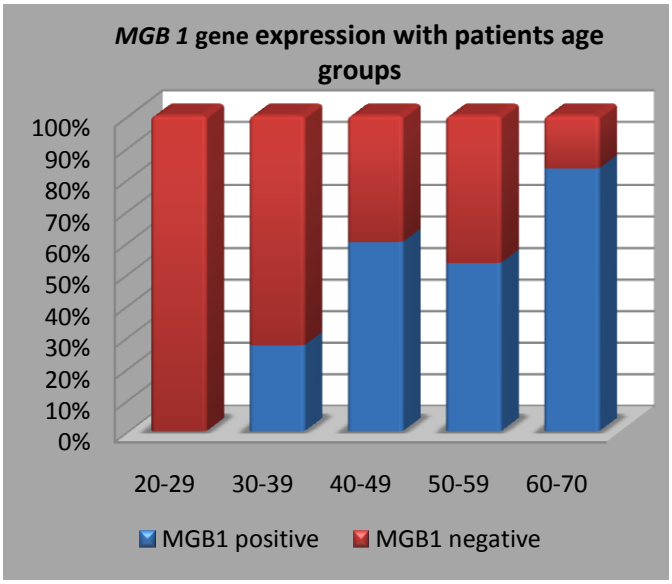

Figure 2.Differences in *MGB 1* gene expression with patient age groups

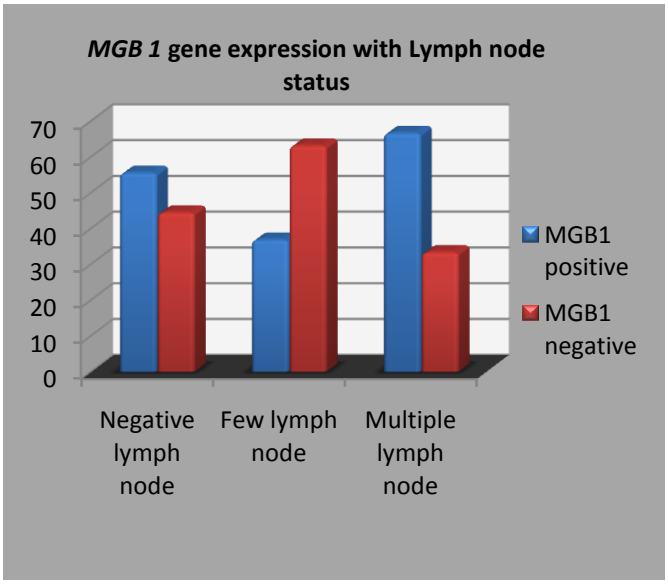

Figure 3. Correlation of *MGB 1* gene expression with lymph node status

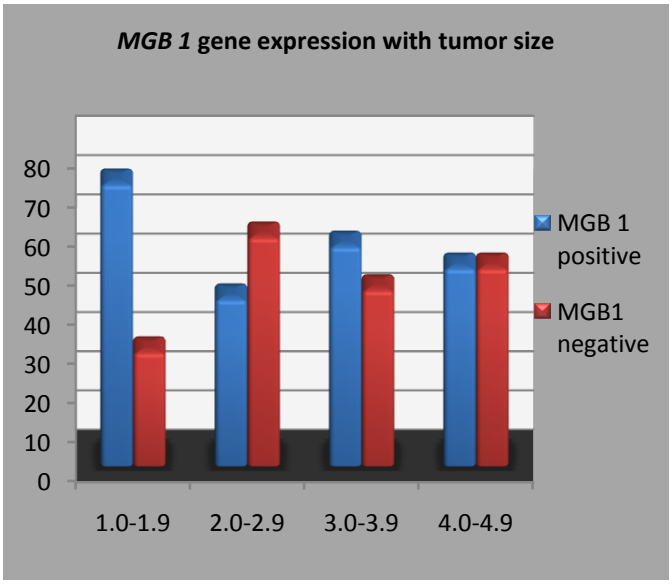

Figure 4.Correlation of *MGB 1* gene expression with tumor size

Discussion

The relationship between circulating tumor cells and the development of metastatic disease is not fully understood, but the ability to detect very small numbers of breast carcinoma cells in circulation could have both prognostic and therapeutic implications, as has already been shown for some hematologic malignancies (van Dongen *et al*., 1999). Some report suggested that MGB 1 mRNA detection by RT-PCR in peripheral blood is a promising marker for disseminated tumor cells of breast cancer origin (Zach *et al*, 1999). The present study examined the possibility of detecting mammaglobin mRNA in peripheral blood of breast cancer patients using qRT-PCR technique. MGB 1 expressing cells were detected by RT-PCR amplification in the peripheral blood of breast cancer patients but not in that of healthy individuals, which indicates high specificity of MGB 1 as a marker gene for cells derived from mammary glands. These results are comparable to that reported by studies of Kadry *et al*.,(2013); Bitisik *et al*.,(2010); Mikhitarian *et al*.,(2008); Zehentner *et al*.,(2004); Suchy *et al*.,(2000) ; Zach *et al*.,(1999), all those studies reported that the MGB 1 -positive percentages of breast cancer patients were significantly high, and the samples of healthy female donors were negative MGB 1 gene expression. The results were different from that reported by Silva et al.,(2002) who detected the MGB 1 gene expression in five normal breast tissues he studied.

The present study showed that MGB 1 gene mRNA detected in 1(10%) of benign breast tumor, this result is comparable to that of Silva *et al*.,(2002) who detected MGB 1 gene expression in one benign breast tumor, while its different from that reported by Kadry *et al*.,(2013) who didn't detect MGB 1 gene expression in any of 20 benign breast tumor he tested. The present study showed that more than half of breast cancer patients (30 -54.5%) were MGB 1-positive, these results have some similarity to that obtained by Zehentner *et al*.,(2004) who found that MGB 1 expression was positive in 51(61.7%) from 84 samples he tested. Cerveira *et al*.,(2004) showed that out of 54 samples he tested MGB 1 transcript detected in the peripheral blood of 22 (41%) these results different from that reported by other studies including Kadry *et al*.,(2013) who detected that 26% of peripheral blood of breast cancer patients studied were MGB 1-positive.

On the other hand low positivity was detected by Strati *et al*.,(2011) who found that out of 66 breast cancer patients 9 (13.6%) were positive for MGB 1, Ignatiadis, et al.,(2008) who showed that out of 175 patients, MGB 1 mRNA detected in only 14 (8%) of samples he tested and Mikhitarian *et al*.,(2008) showed that MGB 1 gene expression detected in only 7(4.0%) of 177 samples he tested. The identification of distribution according to the age groups of the present study showed that no significant correlation between MGB 1 gene expression levels and patients age groups which similar to that reported by other studies (Zehentner *et al*.,2004; Mercatali *et al*.,2005;

Bitisik *et al*.,2010). All these studies detected that mammaglobin mRNA transcription did not vary significantly with the age of breast cancer patients.

The lymph node status results of the present study showed that the highest percentage of MGB 1 positive patients 66%(18) were multiple for lymph node status, these results comparable to those of Labib *et al*.,(2007) who detected that MGB 1-positive cells were detected in 77.8% of patients with axillary lymph node. Grunewald *et al*.,(2000) who showed that MGB 1 mRNA expression was most frequent in patients with extensive axillary lymph node involvement. Other studies including Zehentner *et al*.,(2004) showed that detection of the mammaglobin transcript was only marginally associated with increased lymph node involvement. On the other hand, the present study results were different from results reported by other studies including Kadry *et al*.,(2013) who showed that it was statistically non- significant between MGB 1 mRNA expression and Lymph node involvement. Also Mikhitarian *et al*.,(2008) showed that no association between this marker positivity in peripheral blood (PBL) and status of axillary lymph nodes.

According to the tumor size the results showed that there was decreasing in the MGB 1 gene expression with increasing of tumor size since the highest percentage of MGB 1 positive patients were with tumor size 1.0-1.9 cm. The results of present study contradicting to those reported in other studies which showed either statistically non- significant between MGB 1 mRNA expression and tumor size (Zehentner *et al*., 2004; Roncella *et al*., 2006; El-Attar and Gaefar , 2007; Kadry *et al*., 2013). Others showed a significant association of MGB 1 gene expression with increase in tumor size (Ignatiadis *et al*., 2008).

In conclusion, the present study results of the analysis of mammaglobin expression level in breast cancer blood samples obtained from Iraqi breast cancer patients showed that mammaglobin expression can be consider as a promising diagnostic marker for breast cancer since it's have the ability to discriminate between malignant and benign ovarian tumors. It's also can be considered as potential prognostic marker since its overexpression associated with multiple lymph node status which reflect its role in breast cancer metastasis and its prognostic value which in turn can be target for breast cancer therapy and monitoring tumors metastasis.

REFERENCES:

- Allen J.E. and El-Deiry W.S. (2010).Circulating Tumor Cells and Colorectal Cancer. Curr Colorectal Cancer Rep.;6:212–220.
- Atalay M. C. .(2004). Multidrug resistance in locally advancing breast cancer. The graduate school of natural and applied science of the middlr east technical university.
- Bitisik O., Saip P., Saglam S., Derin D.and Dalay N. (2010).Mammaglobin and maspin transcripts in blood may reflect disease progression and the effect of therapy in breast cancer. Genet Mol Res.;9(1):97-106.
- Blachford, S. L .(2002). The Gale encyclopedia of genetic disorders. Gale group , Thomson learning .U.S.A. Vol.1.
- Botteri E., Sandri M.T., Bagnardi V., Munzone E., Zorzino L., Rotmensz N., Casadio C., Cassatella M.C., Esposito A., Curigliano G., et al. (2010).Modeling the relationship between circulating tumour cells number and prognosis of metastatic breast cancer. Breast Cancer Res Treat.;122:211–217.
- Cerveira N., Torres L., Rocha P., Bizarri S., Pereira D., Abreu J., Henrique R., Teixeira M. and Castedo S. (2004). Highly sensitive detection of the MGB1 transcript (Mammaglobin) in the peripheral blood of breast cancer patients. Int. J. Cancer: 108, 592–595 (2004).
- El-Attar N.I. and Gaefar H.A. (2007).Plasma mammaglobin messenger RNA in breast cancer patients as an addition to serum tumor. Egypt J Immunol.;14(2):111-21.
- Ghossein R.A., Carusone L.and Bhattacharya S. (1999).Review: polymerase chain reaction detection of micrometastases and circulating tumor cells: application to melanoma, prostate, and thyroid carcinomas. Diagn Mol Pathol.;8:165–175.
- Gradilone A., Gazzaniga P., Silvestri I., Gandini O., Trasatti L., Lauro S., Frati L.and Aglianò A.M. (2003).Detection of CK19, CK20 and EGFR mRNAs in peripheral blood of carcinoma patients: correlation with clinical stage of disease. Oncol Rep.10:217–222.
- Greene F.L., Page D.L.and Fleming I.D. (2002).American Joint Committee on Cancer (AJCC) cancer staging manual. 6th edition. New York: Springer-Verlag.
- Grunewald K., Haun M., Urbanek M., Fiegl M., Muller-Holzner E., Gunsilius E., Dunser M., Marth C., and Gastl G. (2000).Mammaglobin Gene Expression: A Superior Marker of Breast Cancer Cells in Peripheral Blood in Comparison to Epidermal-Growth-Factor Receptor and Cytokeratin-19. Laboratory Investigation; 80(7):1071.

- Ignatiadis M., Kallergi G., Ntoulia M., Perraki M., Apostolaki S., Kafousi M., Chlouverakis G., Stathopoulos E., Lianidou E., Georgoulias V., and Mavroudis D.(2008). PrognosticValue of theMolecular Detection of Circulating TumorCells Using aMultimarker ReverseTranscription-PCR Assayfor Cytokeratin 19,Mammaglobin A, and HER2 in Early Breast Cancer. Clin Cancer Res.;14:2593-600
- Jaffer, S.G. (1999). Cytogenetic study of breast cancer in Iraq. M.S.c thesis . College of Science,Baghdad university.

- Jasim, S. L.(2004). Genetic polymorphism of breast tumor using polymerase chain reaction based techniques. Ph.D thesis . College of Science, Baghdad university.

- Kadry D., Fawzy A., Abdelgawad I.A., Abulkheir I.L. and Abou El Kasem F.M.A. (2013).Detection of Mammaglobin mRNA in the Blood of Breast Cancer Egyptian Female Patients and Its Relation to Established Prognostic Parameters.. Life Science Journal;10(2).
- Labib S., Elsaied W.A., Abd M.E. Fathy N.A.and Fathy M.Y.(2007). Mammaglobin: A novel tumor marker for breast cancer. Turkish Journal of Cancer .Volume 37, No. 3, 2007.
- Leelawat K., Leelawat S., Ratanachu-Ek T., Trubwongchareon S., Wannaprasert J., Tripongkaruna S., Chantawibul S.and Tepaksorn P. (2006). Circulating hTERT mRNA as a tumor marker in cholangiocarcinoma patients. World J Gastroenterol.;12:4195–4198.
- Madhavan, M. ; Priya, S. ; Elizabeth, A. ; Iqbal, A. ; Vijayalekshmi, N. R. and Prabha, B. (2002). Down regulation of endothelial adhesion molecules in node positive breast cancer: possible failure of host defence mechanism. Patho. Onco. Res. Vol. 8 :125-128.

- Mikhitarian K., Martin R.H., Ruppe M.B., Gillanders W.E., Hoda R., Schutte D.H., Callahan K., Michael Mitas M. and Cole D.J. (2008). Detection of mammaglobin mRNA in peripheral blood is associated with high grade breast cancer: Interim results of a prospective cohort study. BMC Cancer ; 8:55
- Reinholz M.M., Nibbe A., Jonart L.M., et al. (2005).Evaluation of a panel of tumor markers for molecular detection of circulating cancer cells in women with suspected breast cancer. Clin Cancer Res.;11:3722- 32.
- Roncella S., Ferro P., Bacigalupo B., Dessanti P., Giannico A., Gorji N., Moroni M., Tozzini S., Pensa F., Gianquinto D., Fais F., Pronzato P., and Fedeli F.. (2006). Relationship between human Mammaglobin mRNA expression in Breast cancer tissues and clinco-pathological features of the tumors. J. Exp. Clin. Cancer Res., 25, 1.
- Silva A.L.,Tome M.J., Correia A.E.and Passos-Coelho J.L. (2002).Human mammaglobin RT-PCR assay for detection of occult breast cancer cells in hematopoietic products. Ann Oncol.;13:422^9.
- Strati A., Markou A., Parisi C., Politaki E., Mavroudis D., Georgoulias V. and Lianidou E. (2011).Gene expression profile of circulating tumor cells in breast cancer by RT-qPCR. BMC Cancer; 11:422.
- Suchy B., Austrup F., Driesel G., Eder C., Kusiak I., Uciechowski P., Grill H., and Giesing M. (2000). Detection of mammaglobin expressing cells in blood of breast cancer patients. Cancer Lett 158: 171–178.
- Van Dongen J.J.M., Macintyre E.A., Gabert J.A., Delabesse E., Rossi V., Saglio G., Gottardi E., Rambaldi A., Dotti G., Griesinger F., Parreira A., Gameiro P., Gonzalez Diaz M., Malec M., Langerak A.W., San Miguel J.F.and Biondi,T.(1999). Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease: report of the BIOMED-1 concerted action—nvestigation of minimal residual disease in acute leukemia. Leukemia;13:1901–28.
- Watson M.A.and FlemingT.P. (1996).Mammaglobin, a mammary- specific member of the uteroglobin gene family, is overexpressed in human breast cancer. Cancer Res.;56:860-5.
- Winer E.P., Morrow M., Osborne C.K.and Harris J.R. (2001).Cancer of the breast. In: Cancer: Principles and Practice of Oncology. eds. De Vita Jr VT, Hellman S, Rosenberg SA. Philadelphia:Lippincott Williams & Wilkins; 1264-1333.
- Wulfing P., Borchard J., Buerger H., Heidl S., Zänker K.S., Kiesel L.and Brandt B. (2006).HER2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients. Clin Cancer Res.;12:1715–1720.
- Zach O., Kasparu H., Krieger O., Hehenwarter W., Girschikofsky M. and Lutz D.(1999). Detection of circulating mammary carcinoma cells in the peripheral blood of breast cancer patients via a nested reverse transcriptase polymerase chain reaction assay for mammaglobin mRNA. J Clin Oncol 17: 2015-2019.
- Zehentner B.K., Persing D.H., Deme A., Toure P., Hawes S.E., Brooks L., Feng Q., Hayes D.C, Critichlow C.W., Houghton R.L.,Nancy B.and Kiviat N.B.(2004). Mammaglobin as a Novel Breast Cancer Biomarker: Multigene Reverse Transcription-PCR Assay and Sandwich ELISA.. Clinical Chemistry; 50:11 2069–2076.