International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 6(12) December -2015

MAMMAGLOBIN: AS A DIAGNOSTIC MARKER FOR BREAST CANCER

Ritu Yadav, Preeti Chauhan, Rajeev Sen and Minakshi Vashist

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 12, pp. 7703-7706, December, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

MAMMAGLOBIN: AS A DIAGNOSTIC MARKER FOR BREAST CANCER

***Ritu Yadav[1], Preeti Chauhan[2], Rajeev Sen[3] and Minakshi Vashist[4]**

[1,2]Department of Genetics, M.D. University, Rohtak-124001
[3,4]Department of Pathology, Pt. B.D. Sharma University of Health Science, Rohtak

ARTICLE INFO

Article History:

Received 05th September, 2015
Received in revised form 08th October, 2015
Accepted 10th November, 2015
Published online 28st December, 2015

Key words:

Breast cancer, Immunohistochemistry, Mammaglobin gene, clinicopathological parameters.

ABSTRACT

The expression of mammaglobin gene is predicted to be highly specific in breast cancer. The role of mammaglobin expression as a prognostic and therapeutic tool in breast cancer is widely accepted. The aim of this study was to investigate immunohistochemical expression of mammaglobin, and to determine the correlations with clinicopathological parameters. In the present study, immunohistochemical analysis of two hundred breast carcinoma patients was performed. The immunohistochemical reaction was scored based on the percentage of positive tumor cells. The positive expression rate of mammaglobin biomarker was observed in 69% of breast cancer patients. Tumor grade was found to be significantly associated with the Expression of mammaglobin (p=0.0017). Maximum patients (45%) were observed with tumor size 2-4.9 cm. No statistically significant association was observed among mammaglobin expression, tumor size, lymph node status and histological types. In conclusion, we suggested mammaglobin gene as excellent candidate for a novel and clinically useful breast tumor marker and help in management of breast cancer patients.

INTRODUCTION

Breast cancer is a major problem among females all over the world. It is a heterogeneous disease with a varying propensity for spread (Leygue and Dotzlaw, 1999). 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 (McPherson and Dixon, 2000). Breast cancer is known for its morphologic diversity and unpredictable clinical behavior. Immunohistochemistry has an expanding role in mammary pathology that has been facilitated by a growing list of available antibodies and a better understanding of biology.

The currently available immunohistochemical markers used for the diagnosis of metastatic breast carcinoma include estrogen receptor (ER), progesterone receptor (PR) and gross cystic disease fluid protein (GCDFP-15) (Bernstein *et al*, 2005). They are valuable diagnostic tools, but there is a need to further improve the sensitivity and specificity of the existing panel of breast markers. Additionally, the lack of organ specificity of these breast carcinoma markers further demonstrates the need for new markers in the diagnosis of metastatic breast cancer.

Mammaglobin is a 93 amino acid glycoprotein with homology to other secretoglobin-uteroglobin family members. The gene has been mapped to chromosome 11q12.3-q13.1 (Bernstein *et al*, 2005). Mammaglobin was first identified as a breast cancer restricted biomarker by differential screening approach directed at the isolation of novel, human breast cancer-associated genes (Watson and Fleming, 1996; Watson *et al*, 1999). Further studies have focused on evaluating its function and expression profile (Leygue *et al,* 1999; Min *et al*, 1998; Colpitts *et al*, 2001; Carter *et al*, 2002; Ouellette *et al*, 2004). Many studies have indicated that mammaglobin expression is specific for breast tissues compared with other tissues, leading to its application in the detection of metastasis of breast tumors (Leygue *et al*, 1999; Min *et al,* 1998). Although data has been accumulating regarding the clinical utility of mammaglobin as a biomarker for diagnostic purposes. Few reports have been focusing on its utility in identifying metastatic breast cancer (Bernstein *et al*, 2005; Han *et al*, 2003).

In spite of all recent effort in breast cancer marker research, relatively little is published on markers that discriminate healthy from cancerous breast tissues. Normal breast tissues from healthy individuals allowed us to identify novel tissue markers that can work as protein blood markers. Some of the markers are associated with aggressive disease or with predisposition to breast cancer (Sjödin *et al*, 2003). Present study based on investigation of immunohistochemical expression of mammaglobin, and to determine correlations with conventional prognostic parameters.

Corresponding author:* **Ritu Yadav
Department of Genetics, M.D. University, Rohtak-124001

MATERIALS AND METHODS

Sample Collection

Tumor block of two hundred patients ranging in age from <40 to ≥60 years were collected from Pt. B. D. Sharma University of Heath Sciences Rohtak, Haryana for this study. Out of 200 patients only 150 were evaluated for the histological examination, 50 patients being omitted due to the incomplete information.

Histological Examination

Diagnosis age was categorized as <40, 40–49, 50–59, and ≥60 years. Histological assessment of tumor grade (low, intermediate, and high) was based on cellular and nuclear pleomorphism, ductular differentiation, necrosis and infiltration of surrounding adipose tissue. Tumor size detection ranges as <2 cm, 2–4.9 cm, ≥5 cm. Lymph node status (positive or negative) was counted on basis of two parameters less than 4 and more than 4 lymph nodes. Two Histological types i.e. infiltrate ductal carcinoma (IDC) and lobular carcinoma (LC) was studied. The histological parameters of all cases were reviewed by pathologist and the histological grades were determined for each case according to Nottingham modification of the Bloom and Richardson Grading System.

Immunohistochemical Scoring

The mammaglobin results were screened manually and interpreted as positive or negative on the basis of scores for proportion. The expression of mammaglobin was scored from 0 to 4 as follows: 0, no staining; 1, weak and sporadic staining in <50% of tumor cells; 2, weak staining in >50% of tumor cells; 3, strong diffuse cytoplasmic staining in <50% of tumor cells; and 4, strong, diffuse cytoplasmic staining in >50% of tumor cells. Only sections scoring 3 or 4 were considered to be mammaglobin positive (Jonine *et al*, 2000).

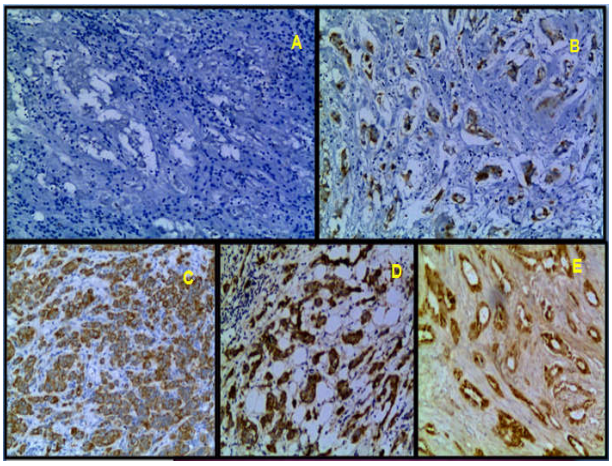

Figure 1 Patterns of mammaglobin immunoreactivity in breast carcinoma. (A) No staining 0; (B) Cytoplasm staining in <50% of tumor cells 1+ve; (C) Weak staining in >50% of tumor cells 2+ve; (D) Strong diffuse cytoplasm staining in <50% of tumor cells 3+ve; (E) Strong, diffuse cytoplasm staining in >50% of tumor cells 4+ve.

Table 1 Distribution of breast cancer patients based on the mammaglobin-quick scoring method.

Sr. No.	Scores	Percentage frequency of Mammaglobin positive breast cancer patients
1.	0	22%
2.	1	42%
3.	2	17%
4.	3	11%
5.	4	8%

Table 2 Relationship of mammaglobin expression in breast cancer patients with clinicopathological factors

Clinicopathological Factors	Percentage frequency of mammaglobin expression in breast cancer patients (%)	p
TUMOR GRADE		
Grade I (Low)	55	
Grade II (Intermediate)	34	
Grade III (High)	11	0.0017
TUMOR SIZE		
<2 cm	37	
2-4.9 cm	45	ns
>5 cm	18	
LYMPH NODE STATUS		ns
<4	57	
>4	43	ns
HISTOLOGICAL TYPE		
Infiltrate ductal carcinoma (IDC)	91	
Lobular carcinoma (LOB)	9	

Significance level, $p<0.05$ (χ^2 test); ns, Non significant

Statistical Analysis

Chi square test, Pearson correlation were performed using software SPSS to find out relation of mammaglobin expression with different clinic pathological factors age, tumor size, grade, lymph node status.

RESULTS

In the present study, Immunohistochemical analysis of breast carcinoma patients was performed and their relation with clinicopathological factors was studied. The positive scores were given on the basis of quick score (cytoplasm stain intensity) from 0 to 4. Negative score was given when cytoplasm didn't attain brown color of DAB (Figure 1). The positive expression rate of mammaglobin biomarker was observed in 69% of breast cancer patients (Figure 1). Percent frequency of patients with scores from 0 to 4+ve is given in Table 1. Maximum quick score showed by patients was 1+ve (42%) and minimum score was counted as 4+ve (8%) (Table 1). Breast cancer patients were categorized in different age groups from less than 40 years to greater than 60 years. The mean age was 45 ±4 years (Figure 2). In grading pattern, majority of the patients were found in grade I as compared to grade II and III. The Positive expression of mammaglobin was observed 55%, 34% and 11% in tumor grade I, II and II respectively. The Expression of mammaglobin was found to be significantly associated with tumor grade (p=0.0017) (Table 2).

Majority of the patients (45%) were found with tumor size 2-4.9 cm as compared to <2 cm (37%) and >5 cm (18%). No

statistically significant association was observed between mammaglobin expression and tumor size. In lymph node status <4 positive lymph nodes showed more reactivity (57%) of the mammaglobin receptor in comparison within lymph node status, >4 positive lymph nodes (43%) in breast cancer patients. Majority of the patients of infiltrate ductal carcinoma type (91%) and only a few were lobular carcinoma type (9%). No significant association of histological types was found with mammaglobin expression (Table 2).

DISCUSSION

In this study potential of mammaglobin gene expression was investigated to serve as a biomarker for breast cancer. Previous studies have shown that mammaglobin mRNA levels were detectable at higher levels in breast tumors as compared with normal breast tissue (Watson and Darrow, 1999). Mammaglobin protein expression in breast tumors was observed independent of stage, or histological type (Watson *et al*, 1999; Grunewald *et al*, 2000; Span *et al,* 2004). This evidence of breast tissue specificity led to the application of mammaglobin as a diagnostic marker for breast cancer (Min *et al,* 1998). In the present study, positive immunohistochemical expression showed strong reaction with diffuse cytoplasmic pattern, even in cases with few positive cells; therefore, the intensity is less useful for the scoring.

Many studies have reported that mammaglobin expression in breast cancers tissue varies from 20% to 75% as compared 69% in our study (Watson *et al*, 1999; Fleming and Watson 2000; Sonia *et al*, 2007). In addition some studies have showed 90% and 93% of breast cancers patients expressed mammaglobin by PCR technique (Gargano *et al*, 2006; Roncella *et al*, 2006). Such broad range might be due to several factors, such as tumors storage methods (fresh/frozen tissue and paraffin-embedded blocks) and/or the different techniques (RT-PCR, immunohistochemical staining or in situ hybridization) used for assessing the different expression levels (Han *et al*,2003). Mammaglobin expression in lymph node is an important marker of metastatic breast carcinoma. (Han *et al*, 2003; Fleming and Watson, 2000; Shira *et al*, 2001). In this study, we used immunohisochemical technique because this method is easier, practical and give enough sensitivity. In quick scoring detection method 1+ve strong staining of mammaglobin score in most common (42%) similar to other results (Raica *et al*, 2009). In contrast Abbas, (2012) reported majority of results have 3+ve quick score. The age of patients was found to be non significant with the expression of mammaglobin marker consistent with other studies (Raica *et al*, 2009; Zhiqiang *et al*, 2009).

The histological grade I tumors were found more common in our study followed by grade II and grade III similar to other studies (Sonia *et al*, 2007; Abbas, 2012) High grade was found in more common in other study (Zhiqiang *et al*, 2009). We found a correlation between mammaglobin expression and grade of the tumor (p=0.0017) similar to many other studies (Bernstein *et al*, 2005; Span *et al,* 2004; Gargano *et al*, 2006; Raica *et al*, 2009; Abbas,2012; Gilbey *et al*, 2004). On the other hand, some authors found no correlation between mammaglobin expression and cancer stage, grade, or histology of the tumor (Watson *et al*, 1999; Span *et al*, 2004). No significant association was observed between tumor size and the expression of mammaglobin marker similar to the other studies (Raica *et al*, 2009; Gilbey *et al*, 2004).

Breast cancer prognosis is well known to be correlated with axillary lymph node status. No significant association was observed between mammaglobin expression and lymph node metastasis contrast to other studies who have found significant correlation between lymph node status and mammaglobin expression (Raica *et al*, 2009; Gilbey *et al*, 2004). No significant association of histological types was found with mammaglobin expression similar to other studies (Watson and Fleming, 1996; Nunez-Villar *et al*, 2003). This is in contrast to the study in which infiltrating lobular carcinomas showed strong; diffuse immunostaining for mammaglobin as compared to infiltrating ductal carcinomas (Bernstein *et al*, 2005; Aaronson and Fleming, 2005; Goedegebuure *et al*, 2004).

CONCLUSION

In conclusion, mammaglobin is a highly specific marker of breast cancers, and its expression was detected in about more than half the breast cancers patients. In prognostic factor tumor grade was significantly correlated with expression of mammaglobin. Based on its breast cancer specific pattern of expression, we believe that mammaglobin would be an excellent candidate for a novel and clinically useful breast tumor marker and help in management of breast cancer patients.

Acknowledgment

Authors are grateful to the Department of pathology Pt. B.D. Sharma University of Health Science, Rohtak, for providing tumor block and guidance in research work. The authors acknowledge the Department of Genetics M.D. University Rohtak and Health University, Rohtak for providing the infrastructure and support.

Conflict of Interest - Nil

Ethical Clearance

Permission was obtained from the institutional ethical committee of M.D. University, Rohtak .

References

1. Leygue, E., Snell, L., and Dotzlaw, H. 1999. Mammaglobin, a potential marker of breast cancer nodal metastasis. *JP athol*. 189: 28-33.
2. McPherson, K., Steel, C., and Dixon, J. 2000. ABC of breast diseases: Breast cancer epidemiology, risk factors and genetics. *Br Med J*. 321: 624-28.
3. Bernstein, J., Godbold, J., and Raptis, G. 2005. Identification of mammaglobin as a novel serum marker for breast cancer. *Clin Cancer Res*. 11: 6528-35.
4. Watson, M., and Fleming, T. 1996. Mammaglobin, a mammary-specific member of the utero globin gene

family, is over expressed in breast cancer. *Cancer Res*.56: 60-65.
5. Watson, M., Dintzis, S., and Darrow, C. 1999. Mammaglobin expression in primary, metastatic, and occult breast cancer. *Cancer Res*. 59: 3028-31.
6. Min, C., Tafra, L., and Verbanac, K. 1998. Identification of super biormarkers for polymerase chain reaction detection of breast cancer metastases in sentinel lymph nodes. *Cancer Res*. 58: 4581-84.
7. Colpitts, T., Billing-Medel, P., and Friedman, P. 2001. Mammaglobin is found in breast tissue as a complex with BU101. *Biochemistry*. 40: 11048-59.
8. Carter, D., Douglass, J., and Cornellison, C. 2002. Purification and characterization of the mammaglobin/ lipophilin B complex, a promising diagnostic marker for breast cancer. *Biochemistry*. 41: 6714-22.
9. Ouellette, R., Richard, D., and Maicas, E. 2004. RT-PCR for mammaglobin genes, MGB1and MGB2, identifies breast cancer micro metastases in sentinel lymph nodes. *Am J Clin Pathol*. 121: 637-43.
10. Han, J., Kang, Y., and Shin, H. 2003. Mammaglobin expression in lymph node is an important marker of metastatic breast carcinoma. *Arch Pathol Lab Med*. 127: 1330-34.
11. Sjödin, A., Guo, D., Hofer, P., Henriksson, R., and Hedman, H. 2003. Mammaglobin in normal human sweat glands and human sweat gland tumors, *J Invest Dermatol*. 121: 428–29.
12. Jonine, L., James, H., Godbold, G., Mark, A., and Watson, B. 2000. Utero globin/Clara cell 10-kDa family of proteins: nomenclature committee report. *Ann N Y Acad Sci*. 923: 348-54.
13. Grunewald, K., Haun, M., and Urbanek, M. 2000. Mammaglobin gene expression: a superior marker of breast cancer cells in peripheral blood in comparison to epidermal- growth-factor receptor and cytokeratin-19. Lab Invest. 80: 1071-77.
14. Span, P., Waanders, E., and Manders, P. 2004. Mammaglobin is associated with low-grade, steroid receptor- positive breast tumors from postmenopausal patients, and has independent prognostic value for relapse-free survival time. *J Clin Oncol*. 22: 691-8.
15. Fleming, T., and Watson, M. 2000. Mammaglobin, a breast specific gene and its utility as a marker for breast cancer. *Ann N Y Acad Sci*. 923: 78-89.
16. Sonia, L., Wafaa, E., Marwa, A., Naglaa, F., and Monaa, F. 2007. Mammaglobin, A novel tumor marker for breast cancer. *Turkish journal of cancer*. 37: 89-97.
17. Gargano, G., Agnese, V., and Calo, V. 2006. Detection and quantification of mammaglobin in the blood of breast cancer patients: can it be useful as a potential clinical marker? Preliminary results of a GOIM (Gruppo Oncologicodell' Italia Meridionale) prospective study. *Ann Oncol*. 17: 41-5.
18. Roncella, S., Ferro, P., and Bacigalupo, B. 2006. Relationship between human mammaglobin mRNA expression in breast cancer tissue and clinico pathological features of the tumors. *J Exp Clin Cancer Res*. 25: 65–72.
19. Shira, G., Carrie, F., and Dennis, L. 2001. Detection of mammaglobin- RNA in the plasma of breast cancer patients. *Ann N Y Acad. Sci*. 945: 192-194.
20. Raica, M., Cîmpean, A., Meche, A., and Alexa, A. 2009. Analysis of the immunohistochemical expression of mammaglobin A in primary breast carcinoma and lymph node metastasis Romanian *Journal of Morphology and Embryology*. 50: 341–47.
21. Abbas, M. 2012. Over expression of mammaglobin in tissue breast carcinoma by immunohistochemistry. *QMJ*. 8: 59-68.
22. Zhiqiang, W., Betsy, S., Anna, S., Yiaoming, L., and Hongbao, L. 2009. Mammaglobin, a Valuable Diagnostic Marker for Metastatic Breast Carcinoma. Int. *J Clin. Exp Pathol*. 2: 384-89.
23. Gilbey, A., Burnell, D., and Coleman, R. 2004. The detection of circulating breast cancer cells in blood. *J Clin. Pathol*. 57: 903- 11.
24. Nunez-Villar, M., Martinez-Arribas, F., Pollan, M., Lucas, A., Sanchez, J., Tejerina, A. 2003. Elevated mammaglobin (h-MAM) expression in breast cancer is associated with clinical and biological features defining a less aggressive tumor phenotype. *Breast Cancer Res*. 5: 65-70.
25. Aaronson, S., and Fleming, T. 2005. Identification of Mammaglobin as a Novel SerumMarker for Breast Cancer. *Clin Cancer Res*.11: 6528-6535.
26. Goedegebuure, P., Watson, M., Viehl, C., and Fleming, T. 2004. Mammaglobin-based strategies for treatment of breast cancer, Curr. *Cancer Drug Targets*; 4: 531–42.

How to cite this article:
Ritu Yadav *et al*., Mammaglobin: As A Diagnostic Marker For Breast Cancer. *International Journal of Recent Scientific Research Vol. 6, Issue, 12, pp. 7703-7706, December, 2015*

****

ISSN 0976-3031