

m-RNA mammaglobin expression in metastatic breast cancer patient at Medan city, Indonesia

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Abstract. Breast cancer is the most common causes of women's death in the world. Metastatic spread presents a major clinical problem in about 30% of the patients. The study aims to investigate the clinical reliability of mammaglobin mRNA as a marker of circulating cancer cells in breast cancer patients. The positivity of blood was analyzed in relation to clinical and pathological characteristics. This study was on 29 breast cancer patients (13 metastatic, 16 non-metastatic patients), where 28 were invasive intraductal carcinoma type and 1 was invasive lobular carcinoma type. Breast cancer patients were according to the histologic grade into grade I (7 patients), grade II (6 patients) and grade III (15 patients). All individuals included in this study were subjected to detection of mammaglobin m-RNA of circulating tumor cells in peripheral blood using RT-PCR technique. Positivity for mammaglobin in blood samples was in 38% of patients with metastatic but not in the non-metastatic patients. The presence of mammaglobin correlated with metastatic tumor ($P = 0.011$). Mammaglobin overexpression in breast tissue was significantly positive in low-grade tumors (I and II).

1. Introduction

Breast cancer is one of the most common causes of women's death in the world.[1] The incidence rate of breast cancer worldwide is 2,920,000 women, with the death rate of 1,778,000 women per year, more than half cases were in the developing countries.[2] In Indonesia, breast cancer is the 2nd most common female malignancy after cervical cancer; the incidence rate was 18%.[3]

At the moment of diagnosis, the most patient with breast cancer does not present metastasis. But from time to time subsequent development of metastasis spread, presents a main clinical problem.[4] Because of this reason, to identify the specific and sensitive marker of breast cancer cells from circulation is very important for predicting of metastasis.[5]

In recent years, PCR technique has been used to detect the circulating tumor cells, which included Cytokeratin (CK 19 and CK 20) and Maspin. However, these markers are not significant for breast tumor cells.[6]

H-Mammaglobin gene is a member of uteroglobin family, localized on chromosome 11q12-13.[7] Normally, the h-Mammaglobin expression is confined to the mammary gland, and overexpression was observed in 25% of primary human breast tumors.[8-10]

The study aims to investigate the clinical reliability of h-Mammaglobin mRNA expression as a marker of circulating cancer cells in metastatic breast cancer patient.



2. Material and Methods

2.1. Subjects

This study is a cross sectional study with analytic description. The study was conducted on 29 breast cancer female patients. Their ages ranged from 34 to 69 years.

Subject's venous blood was obtained from the oncology ward of Pirngadi Hospital, Medan, Indonesia. The diagnosis of breast cancer was based on the clinical finding, histopathological examination. X-ray, ultrasonography and CT Scan is used for the detection of metastasis. The protocol of this study was approved by the Health Research and Ethical Committee of Universitas Sumatera Utara Medan, Indonesia.

28 of the breast cancer cases were an invasive intraductal carcinoma, and another 1 was invasive lobular carcinoma type. The patient group was two groups:

1. Localized breast cancer group: This included 16 patients with localized cancer disease, with no evidence of metastasis
2. Metastatic group: This group included 13 patients with metastases

Histopathologically, all breast cancer patients were according to the system of Bloom and Richardson into:

- Grade 1 (Well differentiated): it included five patients.
- Grade 2 (Moderately differentiated): it included four patients.
- Grade 3 (Poorly differentiated): it included 13 patients.

2.2. Sampling

2.2.1. Blood samples. Under the complete aseptic condition, 5 ml of venous blood was collected by venopuncture in sterile EDTA-treated tubes from breast cancer patients and were placed in the minus 20°C freezer in the Biomedical Science Laboratory of Medical Faculty, Universitas Sumatera Utara until the day of PCR examination.

2.3. Analytical Procedures

RT-PCR for detection of circulating H-Mammaglobin mRNA was as follow:

1. RNA isolation from venous blood sample was immediately performed after sample collection using Instagene Matrix reagent (BIORAD Inc. USA).
2. RNA extraction was subjected to Reverse Transcription synthesized into cDNA using iScript cDNA synthase (BIORAD Inc. USA).
3. Isolation of specific gene from cDNA using IQ Supermix reagent (BIORAD Inc. USA). The primer and reaction parameters for RT-PCR were according to:
Forward primer 5'-CAGCGGCTTCCTTGATCCTTG3'
Reverse primer 5'-ATAAGAAAGAGAAGGTGTGG3'

electrophoresis analyzed the amplified products were on Certified PCR Low Melt Agarose (BIORAD INC. USA) and stained using Biotium Gel Red. The band if present was compared to the DNA marker for the site of target DNA that was 402 bp (figure 1 & 2).

2.4. Statistical analysis

Statistical analysis was done using SPSS software package. The results were as number and percentages. The comparison of the frequency of positivity of h-Mammaglobin between the two groups was by using Chi-Square test (X^2 test) or Fischer exact test where necessary. A p-value of < 0.05 was considered statistically significant.

3. Results

The minimum and maximum ages were 34 and 69 years, respectively. A general characteristic of the subjects involved in this study is shown in (Table 1).

Table 1. A general characteristic of subjects (n=29).

Total Cases	29 Female
Ages range	51.5 years (34-69 years)
Metastasis subjects	13 cases (subject no 1,2,3,4,5,21,22,23,24,25,26,27,28)
Non-metastasis subject	16 cases

Table 2. Descriptive statistics of the frequency of h-Mammaglobin expression in peripheral blood.

	N	h-Mammaglobin +	% in PB
Staging			
All patients	N=29	5/29	17.2%
Localized	N=16	0/16	0%
Distant metastasis	N=13	5/13	38.4%
Histologic grade			
Grade I	N=7	3/7	42.8%
Grade II	N=6	2/6	33.3%
Grade III	N=15	0/15	0%

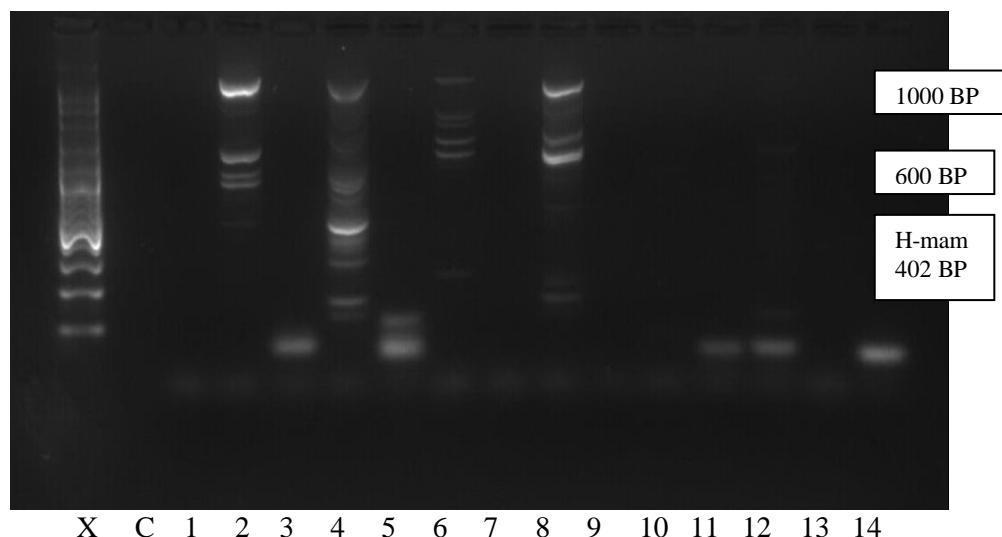
PB: Peripheral Blood

Table 3. Imaging show of metastases.

Lung Nodules	3 cases
Lung nodules+Pleural effusion	3 cases
Lung nodules+Pleural Effusion+Liver nodules	1 case
Osteolytic process	2 cases
Liver nodules	4 cases

3.1. Expression of mRNA h-Mammaglobin

Circulating mRNA h-Mammaglobin positive cells expression were not in 16 non-metastatic patients but were detected in 38% metastases breast cancer patients (5 out of 13 cases).

**Figure 1.** Electrophoresis mammaglobin (sample no 1-14).

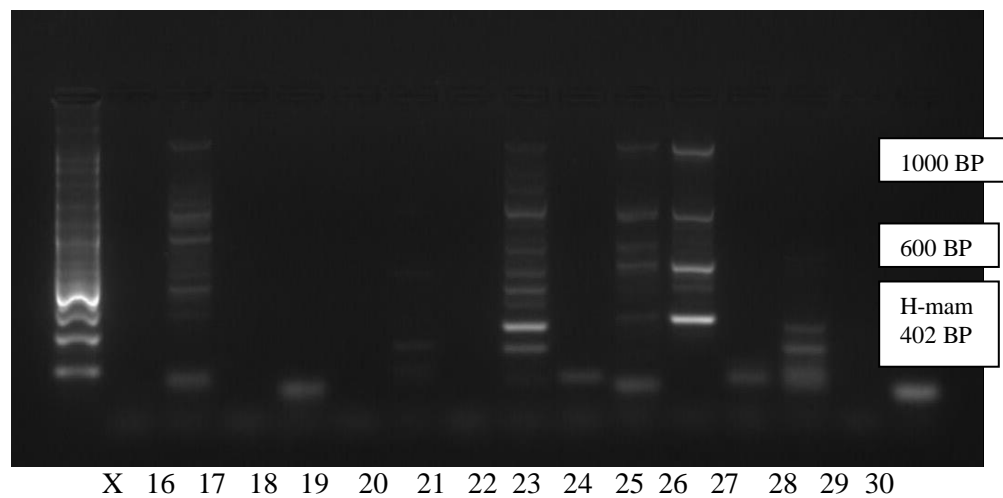


Figure 2. Electrophoresis mammaglobin (sample no 15-29).

Figure 1& 2 showed sample no **1** to **5** and **21** to **28** were subjects with metastases. The sample no 4,23,25,26 and 28 showed positivity of Mammaglobin (402BP).

4. Discussion

Our results were detecting mammaglobin in 38 % of patients with metastatic but not detected in any of non-metastatic patients. The existence of circulating tumor cells and the development of metastatic diseases is not fully understood. In the recent years, many biomolecular markers have been identified. One of this marker is mammaglobin which is the matter of researchers nowadays.[4,5,11,12]

Mammaglobin gene was the first by Watson and Fleming in 1996 and is exclusively expressed in breast tissue and its mRNA only detected in breast cancer.[8,9] Mammaglobin expression is a sensitive and specific marker of epithelial cells of breast cancer and also an independent prognostic factor of recurrence.[8,14-16]

Our study has shown the expression of mRNA h-Mammaglobin were 5 out of 13 metastatic breast cancer patients, and in the statistical analysis done by Fischer exact test, the p-value was 0.011 (less than 0.05) and considered statistically significant.

In conclusion, h-mammaglobin is a promising marker of metastatic breast cancer as its expression was detected a substantial number of examined breast cancer peripheral blood. The finding suggests that h-Mammaglobin has the potential to be used as a marker of tumor progression. This work may require further expansion through acquiring a bigger sample and the use of an inner primer for a better result.

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