SEROLOGICAL CHANGES OF MONOCLONAL ANTIBODY CAMA3C8 AGAINST HUMAN BREAST CANCER CELL LINE

ABSTRACT

Background: Tumor associated antigen are glycoproteins and glycolipids expressed on the surface or in the cytoplasm of tumor cells. The antigenic components are shed from the tumor cells into the tissue culture medium or blood or other human body fluids like human milk. **Objective:** In the present study, investigate the serological assay of the antigen recognized by CAMA3C8 in patients with carcinoma of the breast.**Method:** The study is desgriptive cross section study.The study was divited into four groups based on the expression of tumor associated antigen. **Result:** In breast cancer CAMA3C8 levels were significantly increased in stage 4 when compared with stage 1 breast cancer cell line. **Conclusion:** In the present study, we conclude that significantly recognized CAMA3C8 defined antigens in breast cancer.

KEYWORDS: Monoclonal Antibody; Breast Cancer; Tumor Associated Antigen; Serological Techniques

INTRODUCTION

Immunodiagnosis of human tumors has been based on the detection of TAAs (Tumor Associated Antigen) patient`s sera using antibodies. Since 1981, MAbs have been used in sero diagnostic

studies to detect circulating TAAs (Herlyn Rodeck et al., 1987 Kufe et al., 1984). This procedure might be useful for the early detection of Cancer in distinguishing between patients with malignant and those with benign disease, in prognosticating in monitoring response to therapy, and so detect early any recurrence or metastasis (Kaplon et al., 2019 & Wilkenson et al., 1984).

In women with breast cancer the measurement of serum tumor marker levels a necessary for monitoring. However, in primary breast cancer, the classic tumor markers such as CEA (Carcino Embryonic Antigen) and TPA (Tissue polypeptide antigen) are poorly correlated with disease spread and clinical course. To overcome the drawback due to the low sensitivity and specificity of these non-specific antigens, several specific MAbs have been raised against breast cancer tissues and breast carcinoma cell lines. In recent years, over 20 MAbs have been studied, which are cell surface determinants or secretary products of mammary epithelium. The clinical relevance of most of these MAbs in the early diagnosis and management of breast cancer patients has yet to be proved (Tormey et al., 1978).

Murine MAb CAMA3C8 against has been described previously. The human antigen has been shown to be a secretary glycoprotein with a high molecular weight. The MAb CAMA3C8 was found to give the best signal in immunological assays and was therefore chosen to carryout immunohistological evaluation as well as to develop an ELISA method

MATERIALS AND METHODS

The study was conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu, India. The study was performed after obtaining approval from the institutional ethical committee.

IIa.Cell line

Human breast carcinoma cell line – CAMA was cultured in Dulbecco`s modified Eagle`s medium supplemented with 10% fetal calf serum, 2mM glutamine, 100IU/ml penicillin, 50µg/ml streptomycin.

Collection of clinical Materials

A hundred women with treated and untreated breast cancers in the age group between 20-60 were included for this study. The clinical staging was carried out according to the international union against Cancer (UICC) (17). Based on the available clinical data, the patients were grouped as follows, 11% were stage I, 9 % were stage II, 37% were stage III, and 43% were stage IV. In addition, 25% of these patients were premenopausal, and 75% were postmenopausal. Pathological findings were classified according to the criteria of TNM. The histologic type was 93.8% infiltrating ductal carcinoma, 5.5% lobular, 0.7% medullary.

Sixty-one patients with other types of cancers (non-breast) were also studied. Their age ranged from 5 to 60years. The series included 10 patients with ovarian carcinoma, 12 with colorectal carcinoma, 8 with lung carcinoma, 5 with lymphoma, 7 with leukemia, 14 with endometrial carcinoma, and 15 with the uterine cervix.

30healthy female subjects in the age group of 20-60, 10 smokers, and 20 non-smokers were studied as control.

Serum Collection and Storage

Blood samples were obtained from patients with carcinoma of the breast, other malignant tumor, and from normal female control. Serum was separated by allowing the blood to clot, then spinning at 750Xg for 10 min. The serum was stored at 70°C until use.

Statistical Analuysis

The calculation and statistical analysis were carried out using the Statistical Package for Social Sciences (SPSS) for Windows version 21.0 software, one-way ANOVA method and the group mean were compared by Duncan's Multiple Range Test (DMRT). Statistical probability $P<0.05$ was considered to be significant.

RESULTS

The CAMA3C8 defined antigen was estimated by the immunological procedures, ELISA. Optimal dilution of antigen and CAMA3C8 antibody for the standardization of ELISA was determined using the antisera dilution/titration curve. Antigen concentrations of 20, 40, 60, 80, 100ng and primary antibody dilution of 10,20,30,40 in blocking solution were used to construct the antiserum dilution curve. The optimum primary antibody dilution of 1:30 with an antigen concentration of 60ng showed a high O.D at 405nm. Figure 1.

A standard curve for CAMA3C8 defined antigen is shown in figure 1. The curve is linear in a range of 20-100ng/ml. More than 10 replicate assays have been carried out, and a standard deviation of less than 17% was observed in the CAMA3C8 defined antigen. The sensitivity of this assay system was observed

CAMA3C8 defined antigen level in breast cancer

In contrast to normal subjects, serum CAMA3C8 defined antigen levels were elevated in the majority of patients with breast cancer (Table1). In stage I diseases 63.6% of the patients (7) had values >60ng/ml, and in stage II, 77.7(7) had values >60ng/ml with median levels of 102ng/ml and 112ng/ml, respectively.

More than 80% of the sera from stage III patients contained over 60 ng/ml of CAMA3C8 defined antigen levels have been found in 93% of the patients having distant metastasis of stage IV with a median value of 295ng/ml. Elevated CAMA3C8 defined antigen levels with histological types of breast cancer. However, the test was not carried out to see whether there was any quantitative amount of antigen present in sera of patients with all histological types of breast cancers.

CAMA3C8 defined levels in patients with other cancers

CAMA3C8 defined levels were determined in patients with non-breast epithelial and non-epithelial tumors (Table 2). Among the 10 patients with clinically and surgically demonstrable Ovarian Cancer, 80% had a serum value over 60ng/ml. The maximum value obtained was over 170ng/ml serum. CAMA3C8 defined antigen was also elevated in tumor of the colorectal carcinoma 66.6%, lung carcinoma 62.5%, endometrial carcinoma 78%, and uterine cervix carcinoma 66.6%, but in general, for all non breast epithelial cell cancer, the median value was higher. None of the patients with lymphoma and leukemia had elevated values of CAMA3C8 defined antigen.

Correlation of Antigen levels with the clinical course of breast cancer

Serum samples were obtained from a group of 10 patients with breast cancer over a two month duration, and changes in CAMA3C8 defined antigen levels compared with the clinical response to therapy. The alterations in CAMA3C8 defined antigen levels from which the correlation

between the progress of the disease and antigen levels is apparent. Out of these patients, 3 had progressive disease, and 2 of them had a significant increase in CAMA3C8 defined antigen levels remained the same. In 5/10, there was a complete or partial remission by surgery, chemotherapy or radiotherapy, or both, and anti-estrogens and the CAMA3C8 defined antigen levels fell by more than 50% in all these patients. Patients with progressive breast cancer disease were significantly different from those with the stable or regressing disease. Overall, there is an 80% of correlation between CAMA3C8 defined antigen variations with the clinical course of the disease.

DISCUSSION

An ELISA has been performed with the sensitivity of 10ng/ml using the CAMA antigen and the MAb CAMA3C8. A study of this circulating levels of CAMA3C8 defined antigen by ELISA in normal women, patients with different stages of breast tumor, and other cancers of epithelial and non-epithelial have revealed that this antigen level has been found to be elevated in patients with localized disease and advanced breast cancer. A level of 60ng/ml has been arrived at based on the values obtained from normal and patients. Of the 30 sera samples of normal women, including 10 smokers analyzed, 2 smokers had significantly elevated levels (>60 ng/ml). Perhaps this might indicate that CAMA3C8 defined antigen level might reveal preneoplastic change, leading to malignancy. The rest of the normal subjects studied had values <60ng/ml (Tormey et al., 1978).

The serum assay of CAMA3C8 defined antigen has been carried out for about 100 cancer patients with different stages of the disease. With an increase in the degree of malignancy, the

serum levels of this tumor antigen have proportionately increased, with significantly high levels median value 295ng/ml in the case of patients with stage IV disease. These results might suggest that the level of this tumor antigen will prove very useful to evaluate the extent of disease and also to detect early any recurrence or metastasis (Kohler et al.,1975).

It can also be seen that difference in the expression of CAMA3C8 antigen between normal patients with low levels of malignancy and those with advanced stage of the disease is in agreement with the staining patterns of CAMA3C8 antibody with the antigen present in the normal, benign, and malignant breast tumor tissues indicating that the expression of this antigen at the cellular level also varies with the advancement of the disease. The determination of the serum levels of these antigens has been found to be very useful clinically as tumor markers, especially for carcinoma of the breast and ovaries. The clinical results of some of these mucin marker assays are described in table-3 **(**Price et al.,1985).

The CAMA3C8 defines antigen as a highly glycosylated mucoprotein, and similarity between this antigen and various other breast cancer associated glycoprotein antigens defined by mucin. MAbs have been observed. A well known immunodominant antigenic family has been found to be mucin present in human milk, breast cancer, and other tumoral and normal epithelia. The present assay has demonstrated that the CAMA3C8 defined antigen correlating linearly with tumor burden represents a useful tumor marker for monitoring during therapy, very similar to MAM-6 antigen assay even though with the less diagnostic and prognostic value found for other similar antigens. This antigen might be a satisfactory alternative to HMFG. In the present study shown that CAMA3C8 defined antigen value in the diagnosis of breast cancer since 66% of stage I and 77% of stage II patients were elevated of this antigen (Hilkens et al.,1986).

CAMA3C8 defined antigen has also been identified the sera of proteins with other epithelial cancers. The expression of CAMA3C8 defined antigen in a substantial number of proteins with non breast tumors has also been demonstrated by tissue distribution study of this antigen on the secretory epithelium. The antigenic levels have been found to be most frequently increased in ovarian, lung, and endometrium (Bombardieri et al., 1989). This antigen has been recognized at high levels by immunocytochemical staining of ovarian carcinoma cells, and in a very small preliminary study carried out, elevated levels of this antigen have been found in 10/10 sera of ovarian cancer patients. An early diagnosis of ovarian cancers might be possible by serum assay of this tumor antigen and is of great clinical significance since the majority of these patients have disseminated diseases at the time of initial presentation. This warrants further investigations. It is pertinent to mention in this context that CAMA3C8 has been found very useful in the identification of tumor cells in the effusion fluids of ovarian cancer patients with an initial diagnosis of malignancy with no confirmatory evidence for the presence of malignant cells by histopathology.

The very low antigen levels present in these patients indicate that this antigen is of epithelial origin. However, in the case of non-epithelial Cancer, this antigen could not be identified.

Serum levels of CAMA3C8 defined have been compared with various parameters like age, menopausal status, ER status, and CEA expression for a few patients with carcinoma of the breast. Unfortunately, no correction has been observed.

Detection of increased levels of CAMA3C8 defined antigen in other epithelial tumors of non-breast origin might reflect the expression of heterogeneity in antigenic epitopes with different degrees of glycosylation known to be recognized by these monoclonals (Hilkens et al., 1986).

This study has also confirmed that CAMA3C8 recognized antigen levels should be useful for monitoring the clinical course of breast cancer. CAMA3C8 defined antigen values significantly increased or decreased in consistency with progression or regression of disease. This correlation was observed in 50% of cases studied. Since it shows that the patients with significant increasing or decreasing CAMA3C8 defined antigen levels have the progressive or regressive disease with more than 80% reliability. Ten patients with metastatic breast cancer 8 (80%) had altered levels of CAMA3C8 defined antigen, which correlated with either progression, regression of disease.

CAMA3C8 defined antigen has been identified in the sera of a significantly greater number of advanced breast cancer patients compared to healthy controls. However, repeating the ELISA assay of positive serum samples alters freezing and thawing at least twice the test results obtained, even consistently positive. Hilken`s et al. using a monoclonal 115D8antibody raised against HMFG have found that this MAb recognizes mucin, and assays carried out on serum samples that had been frozen and thawed or stored at 4°C or room temperature several times gave identical results (Burchell et al., 1984).

CONCLUSION

We conclude that the determination of the serum levels of CAMA3C8 recognizes an antigen has been found to be very useful clinically as tumor markers, especially for carcinoma of the breast and ovaries.

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Table.1. Levels of CAMA3C8 defined antigen (ng/ml) in Normal and Breast Cancer Patients determined by ELISA

Group	No. of studied	CAMA3C8 defined antigen level		
		Median (ng)	No of Patients >60ng/ml	% of Patients with levels >60ng/ml
Normal Individuals				
Smokers	10	47	2	20
Non- Smokers	20	39	-	0
Total	30	43	2	6.6
Breast Cancer				
Stage-I	11	102	7	63.6
Stage-II	9	112	7	77.77
Stage –III	37	175	30	81
Stage-IV	43	295	40	93

Table.2.

Levels of CAMA3C8 defined Antigen in Sera of other types of cancers

Group	No. of Patients	CAMA3C8 defined antigen level		
		Median (ng)	No of Patients >60ng/ml	% of Patients with levels >60ng/ml
Ovarian carcinoma	10	167	8	80
Colorectal carcinoma	12	102	8	66.6
Lung Carcinoma	8	134	5	62.5
Endometrial Carcinoma	14	154	11	78
Carcinoma of Uterine Cervix	15	98	10	66.6
Lymphoma	5	40	0	0
Leukemia	7	30	0	0

Table.3. Clinically Useful Mucin tumor Markers in breast Cancer

Immunoassay	**Monoclonal Antibody**	**Immunogen**	**% of Sero Positive on Assay**	
			Control % Range	**Advanced Cancer % Range**
CA15-3	115D8DF3	HMFG Cancer Cell Membranes	1-1.3	63-94
HMFG-2	HMFG-2	HMFG	3-16	53-72
M29/M28	M29/M28 M26/M38	Purified mucin from milk ascites cell lines	0-1	67-76
MSA	3E1.2	Breast cancer tissue	0-3	82-93
CTA	M85/34 F36/22	Ascites from ovarian cancer	0	72
CA549	CA549	Breast cancer cell line	0	91
Ca of uterine cervix	15	10	67	66.6

Figure.1. Standard Curve for CAMA3C8 defined antigen (ng/ml) levels by ELISA