RESEARCH ARTICLE

CA 15-3 (Mucin-1) and Physiological Characteristics of Breast Cancer from Lahore, Pakistan

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Abstract

Background: High incidence of breast cancer and its fatal effect has reached an alarming stage across the globe, including the third world countries. Many factors have been reported to be associated with the development of breast cancer but detailed structural and functional information is missing. CA 15-3 is one of the known potential tumor marker of breast cancer; however little is known about structure and functional site of this protein. Present study aims to investigate the functional role of CA 15-3 in breast cancer, especially in development and metastasis. Material and Methods: Hundred female breast cancer patients confirmed by histopathological reports were included in the study. Their physiological characters were recorded in a performa. Enzyme linked immunosorbent assay (ELISA) technique was used to estimate serum CA 15-3 level. Immunohistochemistry was done for estrogen (ER), progesterone (PR) and Her2/neu receptors expression. Results: The study revealed the details of physiological characteristics of female breast cancer. Mean age was 37.72±5.99 and 55.05±7.28 years and serum CA 15-3 (MUC1) level was 60.47±8.59 and 63.17±4.58 U/ml in pre and post-menopause respectively, and both groups of women had sedentary life style. Their receptor status especially of progesterone, estrogen and HER-2/neu were positive in 50% of premenopausal women and 65% of postmenopausal women. Conclusion: There are multiple physiological factors promoting breast cancer. High serum CA 15-3 level and hormonal imbalance of ER, PR and Her2/neu appears to be the main cause of breast cancer. It may be possible that the functional sites of these proteins may be altered which may increase the chances of metastasis in breast cancer.

Keywords: Mucin1 - CA 15-3 - ELISA - estrogen - transmembrane protein - breast cancer - Pakistan

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Introduction

Breast cancer has been reported as the most common causes of death in female (Jemal et al., 2011). Worldwide, over 1.1 million women are diagnosed with breast cancer each year (Cancer Research UK, 2012).The incidence and fatality rate is increasing globally, including third world that had low rate history. Incidence and fatality increase with age, 50% percent of women with age of 65 and above die of the disease (Perkins et al., 2007). Breast cancer causes significant morbidity and disability in surviving woman. Factors related with the woman's risk of breast cancer, include the aggressiveness of the cancer and the woman's life expectancy (Balducci et al., 2005). Risk factors that modulate the development of breast cancer include: age, geographic location (country of origin) and socioeconomic status, reproductive events, exogenous hormones, lifestyle risk factors (alcohol, diet, obesity and physical activity), familial history of breast cancer, mammographic density, history of benign breast disease, ionizing radiation, bone density, height etc (Korde et al., 2004; Dumitrescu RG, 2005; Verkooijen et al., 2006; Gierach et al., 2012; Saxena et al., 2012; Vrieling et al., 2012).

Defining molecular abnormalities in breast cancer is an important strategy for early detection, assessment of prognosis, and treatment selection (Tsuda, 1994; Beenken et al., 2001). Several circulating mucinous markers, including CA 15-3, CA 459 etc are secreted products of the polymorphic MUC1 gene, and are used as diagnostic biomarkers in patients with breast cancer (Kufe, 2012). In clinical practice the measurement of the levels of these

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markers in the blood can give important information on the tumor's response to treatment and its biological behavior during disease monitoring. CA 15-3 has 3 domains. It also consists of an extra cellular subunit and a membrane associated subunit. The extracellular subunit consists of a tandem repeat unit of 20 amino acid residues. It is observed that subunits of CA 15-3 have important functional site and help to study the role of CA 15-3 in breast cancer.

Since the marker levels reflect the activity of the tumor, it is important to know all factors influencing the production/secretion and the blood concentrations of MUC1 mucin. CA 15-3, the carcinoembryonic antigen (CEA) also called MUC1 is often over expressed and is therefore a potential target for immunotherapy (Jiang et al., 2000; Fan et al., 2012). It is a transmembrane glycoprotein belonging to the family of Mucins (Norum et al., 2001), comprising of glycoproteins expressed by many epithelial cells and their malignant counter parts (Rakha et al., 2005). MUC1 is expressed on the apical surfaces of most simple, secretory epithelia including the mammary gland, gastrointestinal, respiratory, urinary and reproductive tracts. Although MUC1 was thought to be an epithelial-specific protein (Tang et al., 2010), it is now known to be expressed on a variety of hematopoietic cells as well (Segal-Eiras et al., 1997). MUC1 functions in protection and lubrication of epithelial surfaces. It provides a protective layer on epithelial surfaces and is involved in cell-cell interactions, cell signaling and metastasis (Gendler, 2001; Truant et al., 2003; Sandari et al., 2012).

Mucin1 is large protein of 1255 amino acid length and 122 kDa molecular weight. MUC1 have 3 domains: a) externally located domain comprising 1-1161 amino acid residues that bears carbohydrate chains, b) middle membrane spanning region comprising 1162-1179 residues consisting mainly of hydrophobic residues and c) a cytoplasmic domain comprising 1180-1255 amino acid residues that has a high proportion of charged and polar residues. MUC1 also consists of an extra cellular subunit and a membrane associated subunit (Parry et al., 2001). The extracellular sub unit also consists of a tandem repeat unit of 20 amino acid residues in the central region (120 to 959) of the protein (Voet et al., 1999). It has been demonstrated that the cytoplasmic domain of MUC1 interacts with CH2 domain containing GRB2 protein, which transude signal to RAS protein (Baruch et al., 1999). Other studies reported that MUC1 expression in human breast cancer cells is altered by factors affecting cell proliferation (Bairoch et al., 1997). Present study was conducted to study the factors related with the woman's risk of breast cancer. Study also tried to find out the functional aspects of CA 15-3 (MUC1) with the help of its structure and physiological characteristics.

Materials and Methods

Subjects

Hundred female breast cancer patients were included in the study based on confirmation by histopathological reports. Forty normal subjects of the same age with no history of breast cancer or any other disease were taken as control. Blood samples were collected before starting the treatment regimen. Study was conducted during August 2010-September 2011. Informed consent was obtained from all the patients and the study was approved by the ethical committees of the participating centers.

Data Collection

Data were collected by trained medical physician, nurses and laboratory persons. General information from each subject was collected through a standard questionnaire including participant's name, age, education, monthly income and living style, ethnicity, gravidity, para, abortions, clinical sign and symptoms, material status, family history, etc. We explained the objectives and important features of the study to all patients prior to the start of study and their consent was taken. The socio economic status subjects were distributed according to the monthly income divided into three groups. Upper class with monthly income Rs. 30,000 and more, Middle class with monthly income Rs. 15,000 to 29,000 and poor class with monthly income of Rs.10,000 or below. And blood pressure was measured by mercury based blood pressure machine.

Blood Collection

Before interviewing and blood collection a verbal consent was taken from each respondent. Venous blood was withdrawn for the investigation. First the skin was cleaned thoroughly with sterilized with 70% Isopropyl Alcohol swab (Kandall Health Care Company, USA) and dried before puncturing. Then 2 ml of blood taken from the antecubital vein with a 5cc disposable syringe (Becton Dickinson, Pak pvt Ltd). The blood sample was transferred to labeled test tube. And then tubes were marked with codes and immediately taken to the lab.

Estimation of protein expression

Enzyme linked immunosorbent assay (ELISA) technique was used for estimation of the level of CA 15-3 (CODA Automated EIA Analyzer, by using MP Biomedicals (USA) Kit. Immunohistochemistry were done for ER, PR and Her2 expression studies.

Results

Physiological characteristics of pre and post menopausal women with breast cancer were tabulated as Table 1. 96% breast cancers were intra-ductal carcinomas, while 4% were of lobular type. Mean age was 37.72±5.99 and 55.05±7.28 years in pre and post-menopause respectively The mean age for menarche and menopause among breast cancer women was 10 and 50 years respectively. Majority of breast cancer women (60% premenopausal and 70% postmenopausal) belong to poor socio-economic class followed by middle class (25% pre and post-menopausal) and upper class (15% pre and 5% post-menopausal). About 76% of post-menopausal women in this study had sedentary lifestyle. Most were married (95% pre and 97% post menopausal) with average 2-4 children. 2% had a close relative with breast cancer.

Serum CA 15-3 (MUC1) level was 60.47±8.59 and

Table 1. Physiological Characteristics of Female Breast Cancer

Variables		Menopausal women	
		Pre	Post
Number of patients		N=40	N=60
Mean age (years)		37.72±5.99	55.05±7.28
Age of menarche (years)		10.0±1.04	11.1±1.06
Age of menopause (years)		--	50.89±2.76
Socio-economic status	Poor	(60%)	(70%)
	Middle	(25 %)	(25%)
	Upper	(15 %)	(05%)
Family History		2-3%	1-2%
Marital status	Married	95%	97%
Parity		3.25±1.67	2.55±1.01
Blood pressure (mmHg)		110/70±100/65	100/60±95/60
Life style	Active	(20%)	(26%)
	Sedentary	(80%)	(74%)
Multivitamin use		20%	10%
Use of oral contraceptive		40%	-
Site of cancer	Right	16	24
	Left	24	36
Type of cancer	Intraductal carcinoma	(95%)	(96%)
	Lobular carcinoma	(5%)	(4%)
Lymph node involvement		65%	80%
Receptor status	ER, PR and Her2 +ve	(50%)	(65%)
	ER, PR and Her2 -ve	(50%)	(35%)
Serum CA 15-3 (U/ml)		60.47±8.59	63.17±4.58

63.17±4.58 U/ml in pre and post-menopause respectively as compared to <31 U/ml in normal healthy indivisuals. We found receptors of progesterone, estrogen and HER-2/neu were positive in 50% patients amongst premenopausal women, while 65% of post menopausal women were associated with ER, PR positive and Her-2/neu negative and remaining 35% was triple negative.

Discussion

One of the objectives of the present study was to find out the possible relationship between the age of the patients with the disease. The study revealed the mean age of premenopausal patients to be 37 years and of post menopausal women to be 55 years. Study also observed that the number of post menopausal women with breast cancer was higher than that to premenopausal women who visited the Oncology department of hospitals in Lahore, Pakistan. The age distribution of Pakistani women suffering from breast cancer ranged from 32-75 years with a mean of 48.3 years. Ahmed et al. (2006) had found 62.5% (>40 years) and 37.5% (<40 years) on the basis of 24 cases.

We found menarche and menopause age of breast cancer women was 10-11years and 50 year which is similar to the finding of Missmer et al. (2004) where mean age of menarche and menopause was 12 and 49 years. It was reported that breast cancer in postmenopausal women is due to naturally altered hormonal homeostasis whereas in pre menopausal women it was related to an endocrine imbalance due to some defect involving the ovaries (Hindle, 1990). 96% of breast cancer women were married and had 2-4 children, similar to previous finding of an average of 3-4 children in women with breast cancer (Missmer et al., 2004) and higher incidence of breast cancer amongst married women (Miller et al., 1994). However, another study assert that there is a link between infertility and increased breast cancer incidence (Baum et al., 1981). It has been widely reported that family history of breast cancer is risk factor for breast cancer (Barnes et al., 2009; Zhang et al., 2009). In our study ~2% women had a close relative with breast cancer.

We found majority of women (60-70%) with breast cancer belong to poor class that is contradicting with the previous report saying breast cancer is more common among women of higher socio-economic status in developed countries (Muttlin, 1984; Babu et al., 2011; Saxena et al., 2012). In present study, 76% of post-menopausal women had sedentary lifestyle, similar to previously reported finding: women with a sedentary lifestyle are at a higher risk of developing breast cancer (Sattin et al., 1985).

Invasive ductal carcinoma is the most common type of breast cancer (Howe et al., 1991) and we also found 96% breast cancers were intra-ductal carcinomas, while 4% were lobular. The incidence of ductal carcinoma *in situ* has increased in the last 25 years, mainly due to better detection rates due to mammography advances (Khan et al., 2004).

Breast cancer may be related to different hormonal environments, depending on different hormone receptor status (Kakugawa et al., 2007). The progression of breast cancer is often linked to changes in the expressions of PR, ER, and HER-2/neu receptor status (Liu et al., 2010). The present study found that these receptors (PR, ER, and HER-2/neu) were positive in 50% patients amongst premenopausal women, while 65% of menopausal women's cancer was associated ER, PR positive and Her-2/neu negative and remaining 35% were triple negative (ER, PR and HER2/neu -ve). Our results were in accordance with another study that found a significant association between HER-2/neu receptor positivity and tumour size and negative ER/PR status (Missmer et al., 2004).

Present study observed a significant increased level of CA 15-3 in both group of pre and post menopausal women as compared to normal subjects. A number of studies are in accord to our study. A study revealed that most commonly used serum marker that is helpful in diagnosing and monitoring the breast cancer disease activity is CA 15-3 (Molina et al., 2010; Kufe, 2012). This protein is over expressed and less glycosylated and their high levels may be responsible for metastasis (VanLith et al., 2002). Studies reported that decreased immunity (cellular and humoral) to MUC 1 is unable to suppress the growth of tumor. Therefore, MUC 1 has a high potential for immune intervention (Mukherjee et al., 2001; Rahn et al., 2005). Present study observed that the level of CA 15-3 is significantly increased in patients with positive hormonal receptor status. A study reported the relationship of CA 15-3 with estrogen receptor, binding takes place between CA 15-3 and domain of ER alpha. CA 15-3 stimulates ER alpha-mediated transcription and contributes to E-2 mediated growth and survival of breast cancer cells (Wei et al., 2006).

In conclusion, there are multiple physiological factors promoting breast cancer. High serum CA 15-3 level and hormonal imbalance of ER, PR and Her2/neu appears

to be the main cause of breast cancer. The hormonal imbalance may cause a multifold cell division. It may be possible that the functional sites of these proteins may be altered and the extra cellular subunit of CA 15-3 might be interacting with α domain ER which may increase the chance of metastasis in breast cancer by stimulating RAS protein Pathway.

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