ORIGINAL ARTICLE

Correlation of Serum Levels of Estradiol and Her2/neu according to BMI and Menopausal Status in patients with Breast Carcinoma

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ABSTRACT

Background: Breast carcinoma is the second leading cause of deaths in women (after lung cancer) and is the most common carcinoma among women in most countries of the world including Pakistan. The etiology is complex and is discussed in terms of risk factors. Obesity is the disease of diseases and is one of the important risk factor of carcinoma. As estrogen is responsible for cell proliferation so may lead to spontaneous mutations. The discovery of the role of Her2/neu in breast cancer was one of the landmarks in breast cancer research in the last 2 decades. Tumor cells are found to over-express Her2/neuin 20-40% of the cases.

Methodology: So a study was designed to see the serum levels of estradiol and Her2/neu in relation to the BMI and menopausal status of the patients with carcinoma (CA) of the breast.

Results: It was found that although the levels of estradiol were correlated with Her2/neu in postmenopausal CA breast patients but not in relation to BMI.

Conclusion: Estradiol levels affect the Her2/neu levels in postmenopausal CA breast patients.

Keywords: CA breast, estradiol, Her2/neu& BMI.

INTRODUCTION

Carcinoma is caused by mutations in the genes that regulate cell growth and division. Some mutations are inherited, while others are caused by exposure to mutation-inducing agents like chemicals or radiations. Mutations also can occur spontaneously due to mistakes that are made when DNA replicates prior to cell division [1].Mutations in specific genes that control proliferation, such as proto-oncogenes or tumor suppressor genes, are copied with each new generation of cells and can lead to uncontrolled proliferation and the onset of carcinoma[1]. Obese people have large amount of adipose tissue deposited in their body. Adipose tissue contains high levels of aromatase, the enzyme that converts androgens into estrogens. So accumulation of adipose tissue may lead to raised estrogen levels[2].Although estrogen is not directly believed to cause the DNA mutations that elicit the development of human carcinoma, yet it does stimulate cell proliferation. Therefore, if one or more breast cells already possess a DNA mutation that increases the risk of developing carcinoma, these cells will proliferate (along with normal breast cells) in response to estrogen stimulation, thus resulting in an increase in the total number of mutant cells. Any of which might thereafter acquire the additional mutations that lead to uncontrolled proliferation and the onset of carcinoma[3].

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Even in women who do not have any mutant breast cells, estrogen-induced proliferation of normal breast cells may still increase the risk of developing carcinoma. The reason involves DNA. DNA molecules are replicated prior to each cell division, thereby ensuring that each daughter cell receives one complete set of DNA molecules. The process of DNA replicationinfrequently makes mistakes so may result in mutations. If one of these spontaneous mutations occurs in a gene that controls cell growth and division, it could lead to the development of carcinoma[1]. Thus proliferation of normal cells from exposure to estrogen creates a vulnerability to spontaneous mutations, some of which might represent a first step on the pathway to carcinoma.

Mutations, which include over-expression and amplification, transform proto-oncogene- human epidermal growth factor receptor gene (Her2/neu) in the breast epithelium to oncogenes. Her2/neuover expression is found in 20-40% of the cases[4,5]. The proto-oncogene Her2/neuis located on band q21 of chromosome 17 and encodes a trans-membrane tyrosine kinase growth factor receptorthat is expressed on cells of epithelial origin [6, 7]. It has substantial homology with the epidermal growth factor receptor (EGFR) and is one of the family of closely related trans-membrane growth factors designated Her1 to Her 4[8,9]. The human epidermal growth factor receptor (Fig.1) is aglycoprotein and has a molecular mass of 185,000 Daltons. It is composed of an internal tyrosine kinase domain, a shorttrans-membrane portion and an extracellular domain (ECD)[10].

ECD is shed in plasma and serum [11] of healthy individualsas well as of the patients with carcinoma of

the breast[9]. The cleavage of the extracellular domain of Her2/neureceptor leads to increased phosphorylation of the intracellulartyrosine kinase. The ECD concentrations are not only a marker of tumor over-expressionof Her2/neu, but they may also be indicative of the degreeof receptor activation [12]. Studies have shown that the extracellular domain (ECD) of the Her2/neuoncoprotein is released from the cell and can be measured in the circulation of women with carcinoma of the breast. Enzyme-linked immunosorbent assay methods used to measure the circulating Her2/neuECD have shown that the prevalence of elevated ECD levels is approximately 18.1% in women with primary breast cancer and approximately 45.6% in women with metastatic breast carcinoma (MBC). Patients with Her2/ neupositive ca breast have an abnormally high (>15ug/L) serum levels of Her2/ neu(ECD)[13].

Estrogen causes cell proliferation and growth and as we know that it produces it's effects not only through estrogen receptors but also by stimulating Her2/neu receptors. Carcinoma by Her2/neu has been identified as the most promising tumor marker since yet[14]. No study has been conducted so far to determine the correlation between the serum levels of Her2 ECD and estradiol in Pakistani females with carcinoma of the breast. The present study was designed to assess this correlation.

METHODOLOGY

It was a cross sectional study and was conducted at University of Health Sciences in collaboration with INMOL. Her2/neu ECD concentration was measured in serum of 60 patients diagnosed as a case of primary breast carcinoma, 30-60 years of age. Data collection forms were filled to collect the data regarding their name, age, residence, relevant history, physical examination, menopausal status, phase of menstrual cycle and concentration of Her2/ neuECD and estradiol in serum. Serum samples were collected and were stored at -20°C. Grossly hemolyzed or grossly lipemic specimens were excluded. Levels of estradiol and Her2/neu were estimated by ELISA. Correlation between Her2/neu and estrogen was estimated by regression analysis and by calculating correlation coefficient.

RESULTS

Out of 60 cases 38(63.3%) were <50 or equal to 50 years old and 22(53.3%) were >50 years old. 28(46.7%) were of pre menopausal age group and 32(53.3%) were of post menopausal age group. Only 10% of the cases were nulliparous and the remaining multigravida. 10(16.7%) were of normal BMI, 22(36.7%) were overweight and 28(46.7%) were obese (Table 1). Among the premenopausal cases 82.1% had increased BMI. 35.7% of them were over-weight 46.4% obese (Table 2). 87.5% post-menopausal cases were found to have increased BMI. Out of these 87.5% cases 28.1% were over-weight and 59.4% were obese (Table 2). 18(42.85%) cases had raised serum Her2 ECD levels and 42(57.15%) cases had their levels with in normal recommended range (Table 2). No correlation was found between Estradiol and Her2/neu levels according to the BMI (Table 3). Serum estradiol levels of all cases were also determined. Pre-menopausal cases had normal serum estradiol levels. In the follicular phase of menstrual cycle cases had levels 2.87-119pg/ml with a mean of 53.73pg/ml and those in the leuteal phase had levels ranging from 7.91-134.3pg/ml with a mean level of 52.2pg/ml. On the other hand the post-menopausal cases had serum estradiol levels 2.83-58.43pg/ml with a mean level of 19pg/ml.So in these females the serum levels were found to be raised. When the raised levels of estradiol were correlated with raised levels of Her2 ECD in post-menopausal cases they showed statistically significant correlation with a p value=0.034 (Table 4).

Table 1: Characteristics of the Patients with CA Breast

Factors	Cases n = 60	n (%)
Age at interview		
≤ 50Years	38	63.3
> 50 Years	22	36.7
Menopausal Status		
Pre menopausal	28	46.7
Post menopausal	32	53.3
First degree family history of carcinoma		
Yes	8	13.3
No	52	86.7
Parity		
Nulliparous	6	10.0
1-2	12	20.0
3 plus	42	70.0
Abortion or miscarriage		
Yes	14	23.3
No	46	76.7
Body mass index		
Normal (18.5 BMI <25)	10	16.7
Overweight (25 BMI <29)	22	36.7
Obese (BMI > 29)	28	46.7

Table 2: Distribution of cases according to body mass index and menopausal status

Charac-teristics	n	Body Mass Index		
		Normal	Overweight	Obese
Pre M	28	5(17.9%)	10(35.7%)	13(46.4%)
Post M	32	4(12.5%)	9(28.1%)	19(59.4%)

Table 3: Correlation of Her2/neu ECD and Estradiol levels in CA breast patients according to BMI

BMI	Her-2/neu (µg/L)	Estradiol (pg/ml)
Normal		
Mean	30.36 (1.8-200)	55.6 (6.3-134)
±SD	59.83	40.43
SEM	18.92	12.78
P-value	*0.224*	*0.093*
Overweight		
Mean	22.22 (1.4-200)	32.85 (2.87-85.14)
±SD	45.52	28.75
SEM	10.3	6.77
P-value	*0.171*	*0.381*
Obese		
Mean	38.3 (2-200)	30.15 (2.83-90.15)
±SD	58.6	23.93
SEM	10.37	4.22
P-value	*0.172*	*0.363*

Table 4: Correlation of Her2/neu ECD and Estradiol Level according to pre and post menopause

	Her2/neu	Estradiol	p value
Pre M	30.95 (1.4-200)	52.82 (2.87-134.23)	0.148
Post M	33.2 (1.8-200)	19.78 (2.83-58.43)	0.034*

Correlation is significant at the 0.05 level (2-tailed)

DISCUSSION

Advances in the molecular biology have pointed out various tumor markers and the most promising is Her2/neu, a proto oncogene [14]. The extracellular domain of the Her2/neuoncoprotien is shed into the blood and can be detected in the serum [15]. The Percentage of Her2/neu positive carcinomas of the breast found in our study was 42.85% which is more than the limits reported earlier in the studies conducted in other populations [4]. According to some investigators circulating estrogens can stimulate breast carcinoma cell proliferation not only through estrogen receptors but also through Her2/neu receptor [16]. Statistically significant correlation was found between serum levels of Her2/neu ECD and estradiol only in postmenopausal cases (p value=0.034). It means raised serum levels of estradiol can increase the serum levels of Her2/neu ECD in postmenopausal cases of the carcinoma of the breast but not in premenopausal CA breast patients. Our findings were in accordance with the previous findings[16].

CONCLUSION

It is concluded that in the case of postmenopausal CA breast patients the Her2/neu levels can be affected by the estradiol levels. BMI has no affect.

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