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Estrogen and Progesterone Receptor Status in Tumor Samples of Iranian Breast Cancer Patients

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Abstract: Breast cancer is the most common neoplasm affecting women in the western world where the incidence is very high and the risk of developing breast cancer is 1 out of 10. The incidence of breast cancer in Iran is much lower; although it is the most common cancer among Iranian women (skin cancer is excluded). For optimum management of these patients, assay of certain biochemical markers is necessary. Breast Carcinomas that express Estrogen Receptor (ER) and Progesterone Receptor (PR) are more likely to benefit from adjuvant hormonal therapy and have a better prognosis, stage for stage, than those with receptor-negative tumors. Clinically; the most useful markers in breast cancer are estrogen and progesterone receptors that are used to predict response to hormone therapy. In the present study the levels of Estrogen Receptor (ER) and progesterone receptor (PgR) were determined in 48 specimens of primary breast cancers using gold standard biochemical assay of steroid hormone receptors by the Dextran-coated Charcoal method (DCC). The commonest histological type of breast tumor was infiltrating ductal carcinoma that accounts for the 81.5 percent of breast cancers. The results demonstrated that 39.6% were ER^+, PgR^+; 22.9% ER^+, PgR^-; 31.3% ER^-, PgR^- and 6.3% ER^-,PgR^+. In postmenopausal, the incidence of estrogen receptor positive tumors (ER^+) was significantly higher than premenopausal ($p<0.05$). Although the frequency of progesterone receptor positive (PgR^+) breast tumors is higher in postmenopausal than premenopausal but the difference is not significance ($p>0.05$).

Key words: Breast cancer, estrogen receptor, progesterone receptor

INTRODUCTION

Breast cancer is by far the most frequent cancer in women worldwide and ranks third overall when both sexes are considered. It is the most common cancer in all developed countries (except Japan), as well as North Africa, South America, Southeastern and Western Asia (Parkin *et al*., 1999). Estrogen receptor (ER) belongs to the steroid/thyroid nuclear receptor family and is an estrogen-dependent transcriptional factor that regulates growth, development, differentiation, and homeostasis by binding to estrogen response element in DNA to modulate transcription of target genes, such as progesterone receptor (Klijn *et al*., 1993). Like other members of this family, they consist of an amino terminal transcriptional activation domain, a central DNA-binding domain that contains two Zinc-binding fingers a hinge region, and a hormone binding domain (Evans, 1988). The measurement of estrogen receptor (ER) in breast cancer tissue is an important procedure used to discriminate between the hormone dependent and independent tumors in order to determine whether endocrine treatments should be administered. About 60% of the patients with ER-positive tumors respond to such therapies, but less than 10% of the case without ER also responded (Rubens and Hayward, 1980). ER is now more widely used to select patients with early breast cancer likely to respond to the antiestrogen, tamoxifen therapy. Assay of the progesterone receptor (PgR) may also help in selecting hormone-responsive breast cancer. Early work showed that patients with advanced breast cancer were more likely to respond to hormone therapy if their primary cancer expresses both ER and PgR compared to those tumors containing ER but lacking PgR (Osbone *et al*., 1980). In this study we assessed the estrogen (ER) and progesterone receptors (PgR), in the primary breast cancer.

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MATERIALS AND METHODS

Materials: Radioactive 2,4,6,7 ^{3}H-estradiol (83 ci/mmol) and 2,4,6,7 ^{3}H-progesterone (83 ci/mmol) were obtained from Amersham Radiochemical center. Diethylstilbestrol (DES), progestrone, cortisol, Tris, dextraneT-70, gelatin, POPOP [1,4-bis-(4-methyl-1,5-phenyl oxazolyl)-benzene], PPO [2,5-diphenyl-oxazol], dithiothreithol (DDT), Charcoal, EDTA, bovine serum albumin, glycerol were all analytical grade.

Tissue collection: Immediately after surgery a piece of tumor specimens were collected in liquid nitrogen. The samples were stored at -80°C until the analyses were performed. All patients were diagnosed at the cancer institute and Day general hospital, Tehran, Iran.

Preparation of cytosol: The specimens were powdered in liquid nitrogen by microdismemberator (Braun Germany). The pulverized tissue is then homogenized using TEDG buffer (10 mM tris, 1.5 mM EDTA, 0.5 mM dithiothreithol, 10% glycerol, pH 7.4). The homogenate is centrifuged at 100,000 g for 60 min at 4°C.

Estrogen and progestrone assay: The content of ER and PgR were measured using single-point dextrane-coated charcoal (DCC) assay (McGuire *et al*., 1977; Pichon and Milgrom, 1977). Briefly, aliquots of cytosol obtained from breast tissues were incubated with saturating concentration of [^{3}H]-estradiol in the presence or absence of a 200-fold excess of diethylstilbestrol for ER determination and with saturating concentration [^{3}H]-progesterone and cortisol in the presence or absence of a 200-fold excess of unlabelled progesterone for PgR determination. The unbound fraction of ligands was separated by dextrane-coated charcoal technique. Protein content of the cytosol was evaluated by the method of Bradford (Bradford, 1976). Results were expressed as fmol/mg protein. ER and PgR positivity are defined as tumors having 10 fmol/mg protein or more and 5 fmol/mg protein or more ER and PgR, respectively.

RESULTS

Fourthly eight patients with primary breast cancer were analyzed. All patients were females between 28 and 65 years of age. The maximum incidence of breast cancer was between 45-49 years old (Table 1). As shown in Table 2, 81.5% of tumors were invasive ducal carcinomas and remainders were other histological type and 68.2% of patients were identified as axillaries lymph node metastasis. As regards histological grade there were 15.9% grade I, 45.5% grade II and 38.6% grade III tumors.

Table 1: Age incidence rates of breast cancer

Age groups (year)	No. of total	Total (%)
<30	3	6.3
30-34	4	8.3
35-39	6	12.5
40-44	4	8.3
45-49	11	22.9
50-54	10	20.8
55-59	4	8.3
60-64	4	8.3
>65	2	4.2

Table 2: Histological types of breast cancer tumor

Histological type	Total (%)
Infiltrating ductal carcinoma	81.5 (39/48)
Infiltrating lobular carcinoma	4.2 (2/48)
Invasive breast carcinoma	2.1 (1/48)
Ductal carcinoma	4.2 (2/48)
Breast carcinoma with sarcomatous neoplasia	2.1 (1/48)
Intraductal carcinoma with pagets disease	2.1 (1/48)
Infiltrating carcinoma medullary type	2.1 (1/48)
Infiltrating breast carcinoma cribriform	2.1 (1/48)

Estrogen and progesterone receptor content of tumors varied from 0-428 fmol mg^{-1} cytosol protein and 0-270 fmol mg^{-1} cytosol protein, respectively.

There were 62.5% estrogen receptor positive (ER^{+}) and 37.5% estrogen receptor negative tumors (ER^{-}). There were 45.8% progesterone receptor positive (PgR^{+}) and 54.2% progesterone receptor negative tumors (PgR^{-}).

Twenty eight out of the 48 patients (58.3%) were at the premenopausal, while the remaining 20 (41.7%) were at the postmenopausal state. Table 3 summarizes the comparison of estrogen and progesterone receptor status in pre- and postmenopausal breast cancer tumors. In postmenopausal, the incidence of estrogen receptor positive tumors (ER^{+}) was significantly higher (χ^{2}-test) than premenopausal ($p<0.05$).

Although the frequency of progesterone receptor positive (PgR^{+}) breast tumors is higher in postmenopausal than premenopausal but the difference (χ^{2}-test) is not significance ($p>0.05$).

DISCUSSION

The incidence of breast cancer is increasing in all industrialized countries. Changes in diet and reproductive patterns and altered exposure to endogenous and exogenous substances with hormonal activity have been suggested as contributing to this increase (Colditz and Frazier, 1995; Hansen, 1999; Millikan *et al*., 1995; O'Brien and Caballero, 1997; Weiss *et al*., 1997; Wolff *et al*., 1996). Today the prognosis and type of treatment for breast cancer is still determined by the clinical stage of disease, which is estimated by pathological parameters such as

Table 3: Estrogen and progesterone receptor status of breast cancer tumor tissues in pre- and postmenopausal patients. Estrogen and progesterone receptor content (fmol/mg cytosol protein) in tissues of breast cancer are expressed as Mean±SD

	Premenopausal		Postmenopausal	
Receptor status	(%)	Mean±SD	(%)	Mean±SD
ER+	50.0 (14/28)	57.29±5.59	80 (16/20)	83.40±28.8
PgR+	39.3 (11/28)	49.48±28.57	55 (11/20)	27.93±5.39

Table 4: Comparison of estrogen and progesterone receptor Breast cancer tumor can be divided to 4 groups based on estrogen (ER) and progesterone receptor (PgR) Status. Present results are compared with other investigation

	Receptor status			
Studies	ER^+PgR^+(%)	ER^+,PgR^-(%)	ER^-,PgR^-(%)	ER^-,PgR^+ (%)
Degenshein *et al.* (1980)	41	17.1	38	3.9
Young *et al.* (1980)	51.4	22.2	21.2	5.2
Mehta *et al.* (1992)	30.3	20.9	41.2	7.7
Chua *et al.* (1985)	55	10	17.5	17.5
Current study	44.4	18.4	29	8.1

Table 5: Comparison of estrogen receptor in pre- and post menopausal patients with other investigation

	ER Status	
Studies	Premenopause ER^+ (%)	Postmenopause ER^+ (%)
Allegra *et al.* (1980)	37	65
Elwood and Godolphin (1980)	39	51
Leake *et al.* (1981)	50	49
Ziaee *et al.* (1981)	89	92.5
Current study	50	80

Table 6: Comparison of progestrone receptor in pre- and post menopausal patients with other investigation

	PgR status	
Studies	Pre menopause PgR^+ (%)	Post menopause PgR^+ (%)
Raemaekers *et al.* (1987)	37	62
Saez *et al.* (1984)	42	37
Bhatavdekar *et al.* (1992)	66.6	71.4
Current study	39.2	55

tumor size, grade and lymph node involvement. These three parameters are considered the gold standard in clinical practice since they can provide very important prognostic information (Hlupic *et al.*, 2004).

Estrogen and progesterone receptor content of the breast tumors is used to predict response to hormone therapy (Duffy, 2001; McGuire *et al.*, 1990; Pertschuk *et al.*, 1996). Patients whose breast tumors contain ER and PgR are likely to respond to hormone therapy and also have better prognosis (Degenshein *et al.*, 1980; Furmanski *et al.*, 1980). Survival and response to hormonal therapy are most favorable among women diagnosed with tumors positive for both the ER and PR, intermediate for tumors discordant on receptor status (ER^+PR^-, ER^-PR^+) and least favorable for tumors negative for both receptors (Allegra *et al.*, 1980; Campbell *et al.*, 1981; Hansen, 1999).

The following variables have been found to correlate significantly with shortened recurrence-free survival in premenopausal women: Young age, large tumor size, high number of metastasis lymph nodes in the axilla, high histological grade, and negative ER and PR status of the tumor (Davis, 1996). The action of estrogen and progesterone on breast cell proliferation appears to be mediated by the estrogen receptor (ER) and progesterone receptor (PgR) (Clemons and Goss, 2001).

In the present study, we aimed to determine estrogen and progesterone receptor status in breast cancer tumor. Breast cancer tumors can be divided in 4 groups based on estrogen and progesterone receptor status. As showed Table 4 present results are compared with other investigation and our results are in agreement with others investigation.

In postmenopausal women the incidence of ER^+ tumors were significantly higher than in postmenopausal ($p<0.05$). Although the incidence of PgR^+ is higher in postmenopausal women but the difference is not significance ($p<0.05$). As showed in Table 5 present results are in close agreement with those obtained by other investigators.

There is only one report about the estrogen receptor in Iranian breast cancer women (Ziaee, *et al.*, 1981), They reported that the incidence of ER^+ tumors did not differ between pre-menopausal (89%) and postmenopausal (92.8%) and present results are not agreement with that. In Table 6 present results are compared with other results. As shown in Table 6 present results showed that the incidence of progesterone receptor positive (PgR^+) tumors does not significantly different between pre- and post-menopausal.

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