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Comparative evaluation of selected sex hormones in premenopausal and postmenopausal women with breast cancer

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Abstract

Breast cancer is the second most common cancer among women with respect to gender and manifold evidence support a vital role of hormones in the etiology of this cancer as sex steroids stimulate the development and proliferation of breast cells. The present study was planned to investigate the relationship of selective female sex hormones including estradiol, progesterone and testosterone in premenopausal and postmenopausal women with breast cancer from local areas of Punjab, Pakistan. Total 150 selected subjects divided into three main groups; control group (n=40) (premenopausal and postmenopausal) (n=20 each) not having breast disease or hormone related tumors or any other disease, 59 premenopausal patients and 51 postmenopausal patients having breast cancer. Pre and postmenopausal patients were divided further into three patient groups; a) newly diagnosed b) under treatment and c) treated. After evaluation, the blood hormone levels by Electro Chemi Luminescence immunoassay, one-way analysis of variance (ANOVA) and least significant difference (LSD) tests were used to evaluate results statistically. Results showed that non-significant relationship found between estradiol (p = 0.364), progesterone (p = 0.603), testosterone (p = 0.458) in premenopausal women and breast cancer. Significant relationship was found between estradiol ($p < 0.05$) and testosterone ($p < 0.05$) in postmenopausal females with breast cancer while progesterone showed non-significant association ($p > 0.05$) with breast cancer. The study concluded that serum estradiol and testosterone levels in postmenopausal women could be associated with breast cancer.

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Introduction

Breast cancer is considered as the most common form of cancer, with the estimation of 1.38 million new cases of breast cancer worldwide in 2008. It is the second most frequent malignant cancer with respect to gender. Breast cancer also considered as the 5th highest cause of death from cancer overall (Parkin *et al.*, 2005). Adaptation of western lifestyle, null parity or low parity, no breastfeeding or for short period feeding, using hormone therapy, late age at first full-term pregnancy, early age at menarche, tall height, sedentary lifestyle and alcohol consumption, acculturation are regarded as the major risk factors for high breast cancer ratio (John, 2005; Brenner *et al.*, 2016; Riaz *et al.*, 2016).

In addition, obese postmenopausal women have both higher level of circulating endogenous sex hormone, especially estrogens and risk of breast cancer (van den Brandt *et al.*, 2000). To find out the association of different sex hormones secreted in a female with breast cancer, a pooled analysis was also conducted by The Endogenous Hormones and Breast Cancer Collaborative Group (Key *et al.*, 2015). Female sex steroid hormones have a key role in the cause of breast cancer. Estradiol is the major sex steroid hormone which increases the mitotic rate (cell proliferation) of breast epithelium (Hankinson *et al.*, 2004). Ovarian granulosa cells are responsible for the secretion of estrogen in premenopausal women.

The aromatase enzyme converts testosterone and androstenedione, to estradiol and estrone respectively. This ovarian production of estradiol is regulated by feedback control on the levels of follicle stimulating hormone. In premenopausal women, estradiol hormone secreted cyclically, with large significant variation in the level of circulating estradiol and progesterone throughout the menstrual cycle as oocytes mature and released. Aromatase is also expressed, albeit at lower levels, in peripheral tissues such as adipose tissue and skin, where activity is under control of other factors including c-AMP, prostaglandin E2 and glucocorticoids (Simpson *et al.*, 1993).

After the menopause when estrogen and progesterone output from the ovary declines, production of circulating estradiol continues in the peripheral tissues. Levels of circulating estradiol are not subject to large fluctuations in postmenopausal women, remaining fairly constant within an individual (Hankinson *et al.*, 1995) and comparatively low as those found in younger women. The adrenal production of androgens continues after the menopause but output declines with age (Labrie *et al.*, 2005). Adipose tissues are termed as the major source of estrogens production and in the development of cancer (van den Brandt *et al.*, 2000). Plasma Estradiol concentration decrease in postmenopausal women by 90% (Russo and Russo , 2006). Females with higher mineral density, which is a cumulative measure of endogenous estrogen, have an increased risk of breast cancer (Zhang *et al.*, 1997). Androgens also act as a precursor of estrogens in the body and it was suggested that the conversation of androgens into estrogens might be the possible pathway by which androgens control and stimulate epithelial cell proliferation. The aromatize enzymes are responsible for the conversion of androgens into estrogens (Shufelt *et al.*, 2008). Testosterone has indirectly effect on breast tissues by promoting tumor development (Bernstein L and Ross, 1993).

Estradiol and progesterone, acting through cognate receptors, have an important role in breast development particularly during puberty, pregnancy and lactation. A recent description by Pal *et al.* described how the mammary epigenome can change in response to changes in hormonal stimuli and in particular the possibility that the high levels of progesterone during pregnancy act to promote histone methylation and hence modify gene activity (Pal *et al.*, 2013). Histone methylation has been implicated in silencing the expression of tumor suppressor genes and this suggests that sustained progesterone exposure could have a role in oncogenesis through disruption of the normal balance of the methylation of chromatin and specific gene expression. Studies have indicated that serum estradiol levels can feedback at a genetic level,

influencing the expression of estrogen-sensitive genes in breast tissue (Falk *et al.*, 2013). Importantly this behavior suggests that there could be a functional link between the clinical expression of some breast cancers and hormone levels and this is likely to be an important factor in the progression of the disease. Dunbier *et al.* (2010) demonstrated in postmenopausal women, a significant association stuck between plasma estrogen and expression of genes also known as estrogen responsive genes (TFF1 (trefoil factor 1), GREB1 (growth regulation by estrogen in breast cancer 1), PDZK1 (PDZ domain containing 1) and PGR (progesterone receptor) in ER-positive breast tumors.

The aim of the study was to investigate the relationship of estradiol, progesterone and testosterone in premenopausal and postmenopausal women with breast cancer.

Material and methods

Place of Work

Blood samples were collected from diagnosed breast cancer patients from a local hospital of Lahore, Punjab-Pakistan. Analytical work was performed at Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan.

Experimental design

Total 150 individuals were included in the present study and grouped into three groups. Group A included only normal healthy females, who were not used any hormone related treatment. Group A further classified into subgroups, on the basis of menopausal status, premenopausal and postmenopausal women n=20 in each subgroup. Group B (n=59) and C (n=51) were contained premenopausal and postmenopausal women with breast cancer respectively. Both groups were also further divided into three subgroups a) newly diagnosed patients, b) under treatment patients and c) treated women. Newly diagnosed patients comprised those breast cancer patients who were not receiving any type of treatment therapy. While Under treatment patients group were using chemotherapy or radiotherapy for 2-3 month and treated women included those who had completed their chemo and radiotherapy. Pregnant women, with other carcinoma, tuberculosis patients, diabetic patients and women taking contraceptive were excluded during selection.

Analytical Work

Blood samples were collected from all volunteers and processed for the evaluation of serum estradiol, serum progesterone and serum testosterone. Elecsys Estradiol II regeant kit (Cat. No. 3000079), Elecsys Progesterone II regeant kit (Cat. No. 2145383) and Elecsys testosterone II reagent kit (Cat. No. 520067) were used for the quantitative estimation of Serum estradiol (E_2), serum progesterone and serum testosterone at the Electro Chemi Luminescence immunoassay (ECLIA) analyzer by Roche Diagnostics Elecsys®, respectively. In ECLIA analyzer electrochemiluminescent labeled ruthenium complex [Ru(bpy)32+] is used which during reaction Ru(bpy)32+ is firstly oxidize to Ru(bpy)33+ at the surface of electrode. At the same time tripropylamine (TPA, component of Pro Cell) which is present in excess, is oxidize to produce TPA+ cation radical and it is able to lose a proton spontaneously.

The powerful oxidant Ru(bpy)33+ react with TPA free radical, a strong reductant and generate the excited state of [Ru(bpy)32+] complex. Which after emit photon and returns to the ground state at 620 nm and is then again available for a new light-generating reaction cycle (Blackburn *et al.*, 1991, Kenten *et al.*, 1991, Kenten *et al.*, 1992, Leland and Powell, 1990, Obeng and Bard, 1991, Xu and Bard, 1994). Finally, obtained data was subjected to analysis of variance using ANOVA and LSD by using SPSS 16.0 version, USA.

Statistical analysis

Mean ± SD of studied hormones levels were calculated using Microsoft Excel 2007. The obtained data was subjected to analysis of variance (ANOVA) test followed by multiple comparison analysis through Least Significant Difference (LSD) test using SPSS 17.0 version.

Results

Premenopausal women with breast cancer

Results of blood samples collected from Premenopausal women (Fig. 1) were found (mean ± SD) as the mean value of estradiol (pg/ml) 50.844 ± 18.80 in control individuals, 206.22 ± 20.599 for newly diagnosed patients, 18.629 ± 10.786 for under treatment patients and 256.687 ± 93.187 for treated women with breast cancer, Progesterone (ng/ml) as (mean ± SD) 1.243 ± 0.529 for controls, 12.51 ± 1.495 for newly diagnosed patients, 0.508 ± 0.380 for under treatment patients and 3.261 ± 1.2902 for treated women and serum Testosterone (ng/dl) was 14.566 ± 2.647, 15.291 ± 9.653 8.245 ± 6.755 and 32.66 ± 9.3572 in controls, newly diagnosed, under treatment patients and treated women, respectively. Statistical analysis revealed non-significant difference *(p>0.05)* in estradiol, progesterone and testosterone levels among all the groups. Further, results of multiple comparisons of all variables among the different groups also found non-significant *(p>0.05)* in premenopausal women breast cancer as compared to control group (Table 1).

Table 1. Multiple comparisons (LSD) of female sex hormones in premenopausal women with breast cancer and controls.

	Estradiol (pg/ml)			Progesterone (ng/ml)			Testosterone (ng/dl)		
Premenopausal	Newly diagnosed (a)	Under treatment (b)	Treated women (c)	Newly diagnosed (a)	Under treatment (b)	Treated women (c)	Newly diagnosed (a)	Under treatment (b)	Treated women (c)
Patients (n=59)	206.4±98.9	18.7±10.7	256.7±93.2	1.3±0.5	0.5±0.3	3.3±1.3	15.3±9.7	8.3±6.8	32.1±9.3
Controls (d) (n=20)	50.8±18.8	50.8±18.8	50.8±18.8	1.24±0.6	1.24± 0.6	1.24±0.6	14.5±2.7	14.5±2.7	14.5±2.7
Standard Error	163.38$^{(a*d)}$	161.10$^{(a*d)}$	155.36$^{(c*d)}$	2.23$^{(a*d)}$	2.20$^{(b*d)}$	2.12$^{(c*d)}$	16.19$^{(a*d)}$	15.97$^{(b*d)}$	15.40$^{(c*d)}$
	165.40$^{(a*b)}$	165.40$^{(b*a)}$	159.82$^{(c*a)}$	2.26$^{(a*b)}$	2.26$^{(b*a)}$	2.18$^{(c*a)}$	16.39$^{(a*b)}$	16.39$^{(b*a)}$	15.84$^{(c*a)}$
	159.82$^{(a*c)}$	157.49$^{(b*c)}$	157.49$^{(c*b)}$	2.18$^{(a*c)}$	2.15$^{(b*c)}$	2.15$^{(c*b)}$	15.84$^{(a*c)}$	15.61$^{(b*c)}$	15.61$^{(c*b)}$
p value	0.34$^{(a*d)}$	0.84$^{(a*d)}$	0.19$^{(c*d)}$	0.99$^{(a*d)}$	0.74$^{(b*d)}$	0.34$^{(c*d)}$	0.96$^{(a*d)}$	0.69$^{(b*d)}$	0.26$^{(c*d)}$
	0.26$^{(a*b)}$	0.26$^{(b*a)}$	0.75$^{(c*a)}$	0.74$^{(a*b)}$	0.74$^{(b*a)}$	0.36$^{(c*a)}$	0.66$^{(a*b)}$	0.67$^{(b*a)}$	0.29$^{(c*a)}$
	0.75$^{(a*c)}$	0.13$^{(b*c)}$	0.13$^{(c*b)}$	0.36$^{(a*c)}$	0.20$^{(b*c)}$	0.20$^{(c*b)}$	0.29$^{(a*c)}$	0.13$^{(b*c)}$	0.13$^{(c*b)}$

The values are Mean ± SD, Alphabets in parenthesis represent significant differences between study groups with control group. Significant (p< 0.05), Non-Significant (p>0.05).

Postmenopausal women with breast cancer

Results (Fig. 2) revealed that Serum estradiol (pg/ml) (mean ± SD) was 14.027 ± 4.1 in control individuals, 22.287 ± 12.31 in newly diagnosed patients, 9.583 ± 6.45 in under treatment patients and 12.538 ± 7.71 in treated breast cancer women at Postmenopausal phase.

The serum progesterone (ng/ml) in control was 0.422 ± 0.156, 0.301 ± 0.272 in newly diagnosed, 0.301 ± 0.272 in under treatment and 0.269 ± 0.234 in treated women. The results of serum testosterone (ng/dl) (mean ± SD) were as 6.5885 ± 0.889, 13.886 ± 7.48, 15.797 ± 5.65, 16.944 ± 11.55 in control, newly diagnosed, under treatment and treated women, respectively, with breast cancer. Statistical analysis revealed overall significance difference *(p<0.05)* in estradiol and testosterone levels while insignificant difference *(p>0.05)* in progesterone in all the groups of postmenopausal women with breast cancer relative to control group. Multiple comparisons showed significant results *(p<0.05)* of estradiol levels in newly diagnosed patients as compared to control group, under treatment and treated postmenopausal women. It was also found on multiple comparison analysis that there is no significant *(p>0.05)* association between and within all other groups.

Insignificant *(p>0.05)* difference in progesterone levels was observed in all the groups of postmenopausal women with breast cancer except treated groups *(p<0.05)* on multiple analyses of data.

Further, it was also reported that insignificant *(p>0.05)* association between and within groups is found on comparing the serum testosterone levels of postmenopausal women with breast cancer and only treated group showed a significant difference in testosterone level as compared to control group *(p<0.05)* (Table 2).

Table 2. Multiple comparisons (LSD) of female sex hormones in postmenopausal women with breast cancer and controls.

	Estradiol (pg/ml)			Progesteron (ng/ml)			Testosterone (ng/dl)		
Post-manopausal	Newly diagnosed (a)	Under treatment (b)	Treated women (c)	Newly diagnosed (a)	Under treatment (b)	Treated women (c)	Newly diagnosed (a)	Under treatment (b)	Treated women (c)
Patients (n=51)	22.3±12.3	9.5±6.5	12.5±7.7	0.33 ±0.22	0.30±0.27	0.27±0.23	13.8±7.4	15.8±5.7	16.9±11.6
Controls (d) (n=20)	14.03±4.1	14.0±4.1	14.03±4.1	0.4±0.15	0.42±0.15	0.42 ±0.15	6.9±0.89	6.9±0.89	6.9±0.89
Standard Error	4.11$^{(a*d)}$	4.19	3.93$^{(c*d)}$	0.07$^{(a*d)}$	0.07$^{(b*d)}$	0.07$^{(c*d)}$	3.73$^{(a*d)}$	3.80$^{(b*d)}$	3.56(c*d)
	4.40$^{(a*b)}$	4.41$^{(a*b)}$	4.16$^{(a*c)}$	0.08$^{(a*b)}$	0.08$^{(a*b)}$	0.07$^{(a*c)}$	4.00$^{(a*b)}$	4.00$^{(a*b)}$	3.77(a*c)
	4.16$^{(a*c)}$	4.23$^{(b*c)}$	4.23$^{(b*c)}$	0.07$^{(a*c)}$	0.07$^{(b*c)}$	0.07$^{(b*c)}$	3.77$^{(a*c)}$	3.84$^{(b*c)}$	3.84(b*c)
p value	0.04$^{(a*d)}$	0.29$^{(b*d)}$	0.70$^{(c*d)}$	0.22$^{(a*d)}$	0.11$^{(b*d)}$	0.03$^{(c*d)}$	0.06$^{(a*d)}$	0.02$^{(b*d)}$	0.01 (c*d)
	0.01$^{(a*b)}$	0.48$^{(b*c)}$	0.48$^{(b*c)}$	0.70$^{(a*b)}$	0.66$^{(b*c)}$	0.66$^{(b*c)}$	0.62$^{(a*b)}$	0.76$^{(b*c)}$	0.76(b*c)
	0.02$^{(a*c)}$	0.01$^{(a*b)}$	0.02$^{(a*c)}$	0.40$^{(a*c)}$	0.71$^{(a*b)}$	0.40$^{(a*c)}$	0.41$^{(a*c)}$	0.62$^{(a*b)}$	0.41(a*c)

The values are Mean ± SD, Alphabets in parenthesis represent significant differences between study groups with control group. Significant (p< 0.05), Non-Significant (p>0.05).

Discussion

Multiple epidemiological studies to investigate the relationship between sex hormones and premenopausal breast cancer risk have been conducted, but notwithstanding the strength of sex hormone paradigm in breast cancer etiology, the investigations have been equivocal (Key and Pike, 1988). The present research was based on the evidence that the serum estradiol (Eliassen *et al.*, 2006), progesterone and testosterone (Dorgan *et al.*, 2010) gradients altered significantly in postmenopausal women with breast cancer.

The outcomes of present research work showed non-significant alteration in serum estradiol (p=0.346) serum progesterone (p=0.603) and serum testosterone (p=0.458) concentrations in premenopausal female breast cancer patients on comparing to control individuals which are comparative to the work of Ho *et al.* (Ho *et al.*, 2009). The outcomes of present research work are in agreement with the largest prospective study conducted on premenopausal females in the European Prospective Investigations into Cancer and Nutrition (EPIC) cohort. In this 258 invasive breast cancer patients and 555 controls were studied which revealed non-significant association for estradiol with breast cancer risk in premenopausal women (Kaaks *et al.*, 2005). A second large prospective study, exposed that follicular, but not luteal, total and free estradiol was significantly related to breast cancer risk in premenopausal female conducted within the nurses' Health study II (NHSII). Further, in the NHSII, study modest but not statistically significant relations were observed for testosterone and progesterone concentration and breast cancer risk (Eliassen *et al.*, 2006). In the study, conducted by Ho *et al.,* (Ho CC *et al.*, 2009) also showed non-significant association with serum testosterone and premenopausal breast cancer.

The current study showed the significantly elevated levels of estradiol (p=0.031) as compared to control in postmenopausal women with breast cancer. Progesterone was observed non-significant in all groups except control and treated women (p=0.033)

in postmenopausal women. It was well established by various experimental studies that estradiol have a preoperative consequence on breast tissues (Thomas DB, 1984) linked most likely by increasing the proliferation and mitotic activity of breast tissue cells. It was also believed that this is related to be promoting influence relatively than initiating effect (Telang *et al.*, 1997; Key *et al.*, 2015).

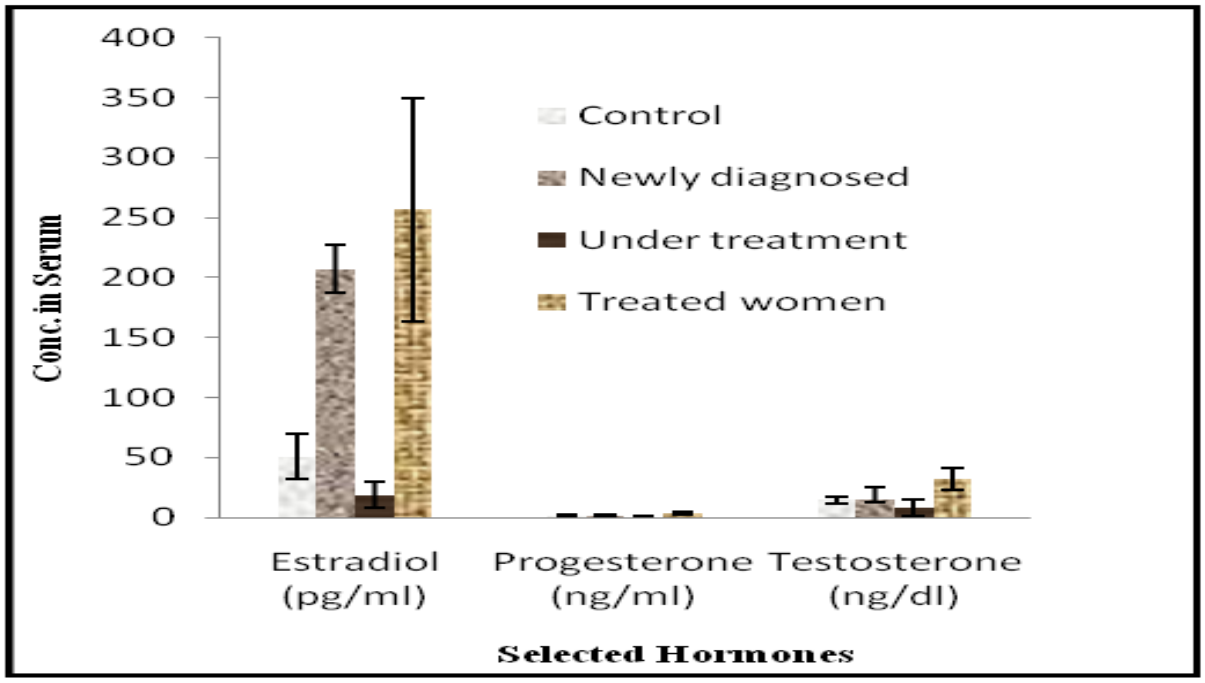

Fig. 1. Levels of selected (Mean ± SD) female sex hormones in premenopausal patients with breast cancer and controls.

The consistent increase in the chemical signals in the form of different sex hormones probably increased the proliferation of the cells many times might be lead to carcinogenesis, due to increased risk of DNA mutations occurred during persisted replication of cells genetic material, which if not corrected, may lead to cancer (Osborne *et al.*;1993, Ursin *et al.*, 2001). For that reason, it is believed that estrogen increased the risk of breast cancer. Zhang *et al.* have astonishingly reported that one measurement of endogenous hormone levels in a postmenopausal woman can envisage risk of hormone-responsive breast cancer for up to 16 to 20 years (Zhang *et al.*, 2013). Findings of the current study are compatible with Key *et al.*, findings who reported, in a meta-analysis of nine prospective studies, statistically significant relationship of sex hormones including estradiol with breast cancer risk in postmenopausal women (Key *et al.*, 2002). It was also found in sixteen case-control studies that estrogen concentration in seven of the studied groups was significantly high in breast cancer cases than control (Thomas *et al.*, 1997) at the same time as the present study also showed the same result of estradiol in newly breast cancer diagnosed patients (2287 ± 23.1) than a control (14.27± 4.1). The correlation between the breast cancer risk and blood estradiol concentration in postmenopausal is well established, with an about two-fold higher risk among women in the top 20-25% (versus bottom 20-25%) of levels (Falk *et al.*, 2013; Brown and Hankinson, 2015).

Progesterone, an ovarian steroid hormone, essentially involve in the development of breast during puberty and in preparation for lactation and breastfeeding. Two types of progesterone receptors including progesterone receptor (PR)-A and PR-B, located in various tissues including the brain where progesterone controls reproductive behavior, and the breast and reproductive organs, progesterone have high affinity to bind these classical receptors to mediate its actions (Lange and Yee, 2008). The

results of present study are in line with the work of Missmer *et al.* (2004) they conducted a large prospective study, with 270 cases, to evaluate the association of postmenopausal circulating progesterone and breast cancer risk to observe the serum progesterone levels in postmenopausal women, where no statistically significant association was observed. No association was observed either overall (top versus bottom quartile of levels: RR=0.9; 95% CI=0.6–1.5; p-trend=0.90) when evaluated by tumor hormone receptor status or stratified by circulating estradiol levels. It was also reported that progesterone have both positive and null associations with estrogens for breast cancer risk and clearly more evaluation is needed to find out the impact of this steroid hormone (Hankinson and Eliassen, 2007).

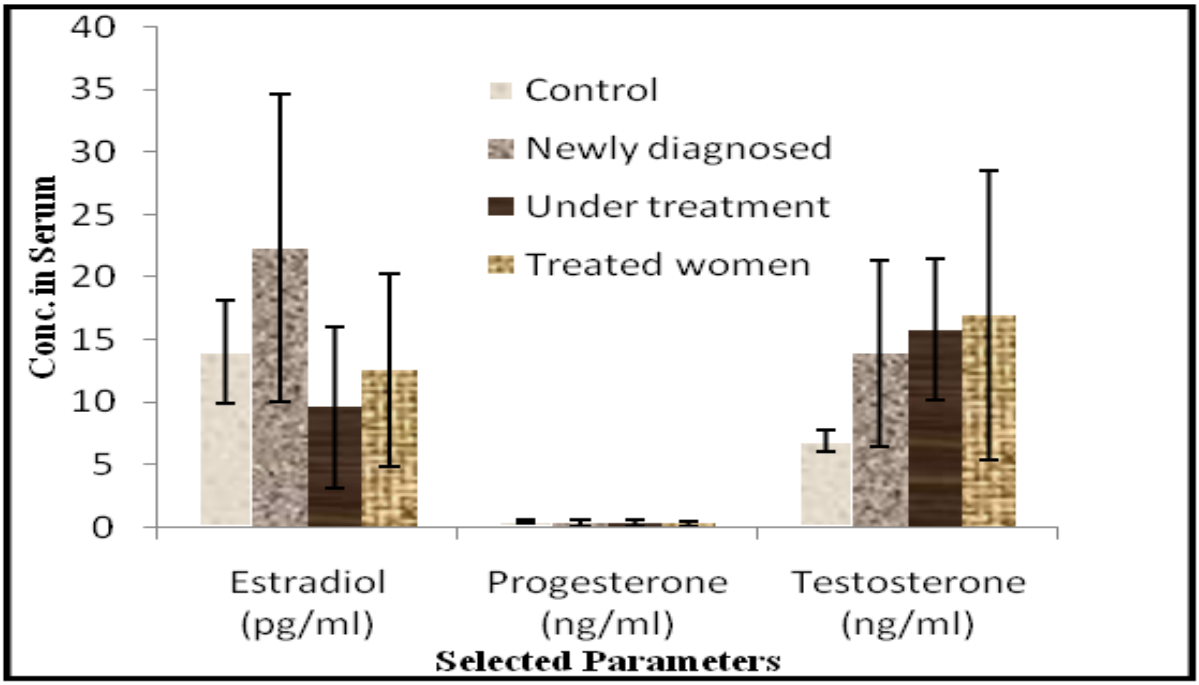

Fig. 2. Levels of selected (Mean ± SD) female sex hormones in postmenopausal patients with breast cancer and controls.

Testosterone believed to indirectly affect breast tissue by promoting tumor development (Bernstein and Ross , 1993).

Testosterone also influences the bioavailability of estradiol because it has the stronger competitive binding ability to Sex Hormone Binding Globulin (SHBG) due to its higher affinity to this protein. So high concentration of circulating testosterone increases the quantity of unbound estradiol and therefore circuitously increases the breast cancer risk.

The results of the present study were consistent with the argument that the contribution of testosterone to the development breast cancer might be largely due to its role as estrogens precursor, and that older ages aromatase activity increase to keep the high concentration of estrogens in the breast tissues (Somboonporn and Davis, 2004). Key *et al.*, 2015 also described through their finding that testosterone significantly associated with breast cancer risk along with other sex steroid hormones.

Conclusion

It was inferred from the study that no significant association was present between female sex hormones and the breast cancer in premenopausal women while in postmenopausal women serum estradiol and testosterone could be associated with development of breast cancer as significantly elevated levels of these hormones were found in newly diagnosed patients as compared to control and treated group patients.

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Conflict of Interests

The authors declared no conflict of interest with respect to the research, authorship, and/or publication of this article.

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