

## Study of endogenous hormones in breast cancer patients of premenopausal Women

GSR Kedari<sup>1</sup>, PM Abraham Sam Rajan<sup>2,\*</sup>, Ratnashree Biswas<sup>3</sup>

<sup>1</sup>Professor, <sup>2</sup>Associate Professor, <sup>3</sup>PG Student, Dept. of Biochemistry, Saveetha Medical College, Chennai

**\*Corresponding Author:**

Email: salomi.jayasam@gmail.com

### Abstract

**Introduction:** Several studies were conducted worldwide to estimate various endogenous hormone levels in breast cancer patients of post-menopausal women and very few studies were done in premenopausal women. In India, our study is the first study conducted to estimate the endogenous hormones in breast cancer patients of premenopausal age group.

**Materials and Method:** 50 breast cancer patients of premenopausal age group were taken as cases and 75 age matched controls were selected for this study. Total testosterone, follicular estrogen, mid luteal estrogen, luteal estrogen, follicular progesterone, mid luteal progesterone, luteal progesterone were estimated for all the subjects.

**Results and Conclusion:** Statistical significant increase was observed in the levels of testosterone in cases when compared to controls. Statistical significant difference was observed in the levels of follicular progesterone between cases and controls. No statistical significant difference was observed in other parameters between cases and controls.

**Keywords:** Premenopausal women, Testosterone, Estrogen, Progesterone.

**Received:** 9<sup>th</sup> June, 2017

**Accepted:** 25<sup>th</sup> July, 2017

### Introduction

Breast cancer is one of the most common neoplasms in women, and is the leading cause of cancer related deaths worldwide.<sup>(1)</sup> Epidemiological studies have identified many risk factors that increase the chance of women developing breast cancer include early age at menarche, late age of menopause, null parity, obesity, oral contraception, hormone replacement therapy, diet, family history, prior history of benign breast disease and lactation.<sup>(2)</sup> The common denominator of these risk factors is their effect on the level and duration of exposure to endogenous or exogenous estrogens.<sup>(3)</sup> According to global health estimates WHO 2013, it is estimated that worldwide, over 508,000 women died in 2011 due to breast cancer.<sup>(4)</sup> Although breast cancer is thought to be a disease of the developed world, almost 50% of the breast cancer cases, and 58% of deaths occur in less developed countries.<sup>(5)</sup> A recent study of breast cancer risk in India revealed that 1 in 28 women develop breast cancer during their lifetime.<sup>(6)</sup> In south India, the prevalence rate of breast cancer is 12.6%.<sup>(7)</sup>

Several studies have shown the relationships between circulating estrogen and androgen levels and breast cancer risk are well established among postmenopausal women,<sup>(8-11)</sup> most previous prospective studies among premenopausal women have been small and, for estrogens, have produced inconsistent results.<sup>(12-15)</sup> Epidemiologic studies have produced results which were not only unclear but also very inconsistent among breast cancer risk and premenopausal plasma levels of sex steroid hormones and no study is done in Indian population which has estimated the levels of endogenous hormones in breast cancer patients. This kind of study is challenging as the

circulating levels of estrogen and progesterone vary greatly during the menstrual cycle and the length of menstrual cycle varies individually which will also alter intra individually. According to literature, only five prospective studies were conducted to study the relationship between breast cancer risk and premenopausal blood levels of estradiol.<sup>(16-20)</sup>

This study is the first of its kind in Indian population as far as our knowledge is concerned. The aim of the present study is to estimate the levels of endogenous hormones like Serum Testosterone, Estrogen and Progesterone in their follicular, mid luteal and luteal phases.

### Materials and Method

The present study was conducted in the department of Biochemistry of Saveetha Medical College. 50 diagnosed cases of breast cancer after careful examination of FNAC report and histopathological examination belonging to premenopausal age group <45 years (premenopausal women are taken by the fact that they have continuous 9 menstrual cycles over previous 12 months before the sample collection)<sup>(13)</sup> were included in this study. Out of cases, 41 were having ductal carcinoma and 8 patients were having lobular carcinoma and 1 was having mixed carcinoma (both ductal and lobular). 75 age matched women who have no history of breast diseases were taken as controls. Exclusion criteria included patients on Hormonal replacement therapy, Oral Contraceptive usage, Individuals suffering with benign disorders like Fibroadenoma, Lactating mothers, Hypertension, Chronic illness, Autoimmune diseases, Renal disorders and Liver disorders. The following information were obtained from both the cases and controls like Parity,

Menopausal status, Hereditary information, Life style factors including, Tobacco usage, Alcohol consumption, Dietary habits, Obesity, Hormonal replacement therapy. Informed consent was obtained from all the cases and controls. We have obtained permission from Ethical Clearance Committee for this study.

10ml of fasting blood samples were collected by venipuncture from all cases and controls during

follicular phase (-15 to -2 days), mid cycle phase (-1 to +1 days) and luteal phase (+2 to +15 days), by day in cycle relative to LH peak. Estradiol and Testosterone were estimated by CMIA (Chemi Luminescent Microparticle Immuno Assay) and Total Progesterone by CLIA (Chemi Luminescent Immuno Assay). The data were analysed by using paired t test, independent sample test and Mann-Whitney test using SPSS package.

## Results

**Table 1: Cases and Controls**

	Cases	Controls
Mean age at menarche	13.6	13.5
Mean length of menstrual cycle	29.1	29.2
Mean height (cms)	151	150
Mean weight	66	61
Mean BMI	26.3	24.8
Percentage parous	76.1	90.7
Percentage reporting first degree family history	18.9	3.0
Percentage reporting past use of oral contraceptives	63.6	24.7

**Table 2: T-Test Group statistics**

Parameter	Group	N	Mean	Std. Deviation	Std. Error Mean
Testosterone (ng/ml)	Cases	50	2.478	.9147	.1294
	Controls	75	.412	.2627	.0303
Follicular Estrogen (pg/ml)	Cases	50	130.72	51.323	7.258
	Controls	75	128.24	53.838	6.217
Mid luteal estrogen (pg/ml)	Cases	50	238.68	98.926	13.990
	Controls	75	243.04	104.281	12.041
Luteal estrogen (pg/ml)	Cases	50	131.80	68.752	9.723
	Controls	75	127.45	70.313	8.119
Follicular progesterone (ng/ml)	Cases	50	.747	.3905	.0552
	Controls	75	.579	.4100	.0473
Mid luteal progesterone (ng/ml)	Cases	50	21.488	26.2393	3.7108
	Controls	75	16.345	6.1047	.7049
Luteal progesterone (ng/ml)	Cases	50	17.944	5.7492	.8131
	Controls	75	17.760	5.2722	.6088

**Table 3: Independent Samples Test**

Parameter		Levene's test for Equality of Variances		t- test for Equality of Means						
		F	Sig	T	df	Sig (2-tailed)	Mean difference	Std. error difference	95% confidence interval of the difference	
									Lower	Upper
Testosterone (ng/ml)	Equal Variances Assumed	74.768	.000	18.478	123	.000	2.0655	0.1118	1.8443	2.2868
	Equal Variances Not assumed			15.545	54.428	0.000	2.0655	0.1329	1.7992	2.3319
Follicular Estrogen (pg/ml)	Equal variances assumed	0.110	0.740	0.257	123	0.798	2.480	9.649	-16.620	21.580

	Equal variances not assumed			0.260	108.573	0.796	2.480	9.557	-16.462	21.422
Mid luteal estrogen (pg/ml)	Equal variances assumed	0.420	0.518	-0.234	123	0.816	-4.360	18.656	-41.288	32.568
	Equal variances not assumed			-0.236	108.912	0.814	-4.360	18.459	-40.945	32.225
Luteal estrogen (pg/ml)	Equal variances assumed	0.036	0.849	0.342	123	0.733	4.353	12.725	-20.834	29.541
	Equal variances not assumed			0.344	106.780	0.732	4.353	12.667	-20.758	29.465
Follicular progesterone (ng/ml)	Equal variances assumed	0.199	0.656	2.296	123	0.023	0.1687	0.0735	0.0233	0.3141
	Equal variances not assumed			2.319	108.631	0.022	0.1687	0.727	0.0245	0.3129
Mid luteal progesterone (ng/ml)	Equal variances assumed	1.651	0.201	1.635	123	0.105	5.1427	3.1448	-1.0824	11.3677
	Equal variances not assumed			1.362	52.555	0.179	5.1427	3.7772	-2.4348	12.7202
Luteal progesterone (ng/ml)	Equal variances assumed	.467	.496	.184	123	.854	.1840	.9982	-1.7918	2.1598
	Equal variances not assumed			.181	98.784	.857	.1840	1.0157	-1.8315	2.1995

**Table 4: Non Parametric Tests Mann-Whitney Test Ranks**

Parameter	Group	N	Mean Rank	Sum of Ranks
Testosterone (ng/ml)	Cases	50	99.88	4994.00
	Controls	75	38.41	2881.00
	Total	125		
Follicular Estrogen (pg/ml)	Cases	50	64.79	3239.50
	Controls	75	61.81	4635.50
	Total	125		
Mid luteal estrogen (pg/ml)	Cases	50	62.52	3126.00
	Controls	75	63.32	4749.00
	Total	125		
Luteal estrogen (pg/ml)	Cases	50	64.69	3234.50
	Controls	75	61.87	4640.50
	Total	125		
Follicular progesterone (ng/ml)	Cases	50	73.19	3659.50
	Controls	75	56.21	4215.50
	Total	125		
Mid luteal progesterone (ng/ml)	Cases	50	69.49	3474.50
	Controls	75	58.67	4400.50
	Total	125		

Luteal progesterone (ng/ml)	Cases	50	64.78	3239.00
	Controls	75	61.81	4636.00
	Total	125		

**Table 5: Test statistics**

	Testosterone (ng/ml)	Estrogen (pg/ml)	Mid luteal estrogen (pg/ml)	Luteal estrogen (pg/ml)	Follicular progesterone (ng/ml)	Mid luteal progesterone (ng/ml)	Luteal progesterone (ng/ml)
Mann-Whitney U	31.000	1785.500	1851.000	1790.500	1365.500	1550.500	1786.000
Wilcoxon W	2881.000	4635.500	3126.000	4640.500	4215.500	4400.500	4636.000
Z	-9.295	-.451	-.121	-.426	-2.569	-1.636	-.449
Asymp. Sig. (2-tailed)	.000	.652	.904	.670	.010	.102	.654

## Discussion

From our study it was found that there was statistical significant increase in the levels of testosterone and also in the levels of follicular progesterone in cases when compared with controls. No statistical differences were observed in the levels of follicular, mid luteal and luteal estrogen and mid luteal, luteal progesterone levels between cases and controls.

Our study shows that premenopausal women who have increased levels of serum testosterone are more prone for breast cancer. Our study was correlating and supports the previous studies where positive associations between testosterone<sup>(19)</sup> and androstenedione<sup>(12)</sup> levels and breast cancer risk were observed. Breast cancer risk is increased among women who have an ovarian androgen excess, chronic anovulation, and an associated reduction of luteal-phase progesterone production.<sup>(21,22)</sup> This is supported by case control studies that showed breast cancer patients have higher plasma or urinary concentrations of testosterone or its urinary metabolites<sup>(22,23)</sup> than cancer free control subjects.<sup>(21,24,25)</sup>

In our study, no statistical significant difference was observed in the levels of mid luteal and luteal progesterone whereas statistical significant difference was observed in the levels of follicular progesterone. Progesterone may either decrease breast cancer risk, by mitigating the estrogen-induced proliferation of breast epithelial cells,<sup>(26,27)</sup> or increase risk because of the higher breast cell proliferation in the luteal phase<sup>(28)</sup> and the increased risk associated with estrogen-plus progesterone hormone replacement therapy.<sup>(29-31)</sup> Some studies have observed suggestive<sup>(19)</sup> or statistically significant<sup>(13,15)</sup> inverse, although not linear, associations between luteal progesterone levels and breast cancer risk.

No statistical significance was observed in the levels of follicular, mid luteal and luteal estrogen levels between cases and controls in our study. The positive association between circulating estrogen levels and breast cancer risk is well established in postmenopausal women.<sup>(8,9)</sup> It is known that breast cancer typically arises in luminal epithelial cells of the mammary gland.<sup>(32)</sup> That estrogen activation of ER

results in transcription of various genes like Estrogen receptor  $\alpha$  and  $\beta$ , that are involved in cellular proliferation, exposure to estrogen correlates with risk for breast cancer (risk increasing with duration of exposure).<sup>(33)</sup> Estrogen plays a role in inflammation signaling pathways by repressing production of IL-6 through an estrogen receptor-dependent mechanism.<sup>(34)</sup> IL-6 is also known to increase the expression of aromatase in breast cancer cells, thereby enhancing the conversion of androgens to estrogens<sup>(35)</sup> and also thought to increase the activity of the 17- $\beta$ -hydroxysteroid dehydrogenase, which converts estrogen to estradiol, a process that may contribute to the increased concentration of estrogen around breast tumors.<sup>(36)</sup>

The limitations of our study include non-estimation of FSH, LH and their association with estrogen, progesterone and testosterone levels. Women with menstrual cycles who will vary largely both intra and inter individually may also influence the levels of endogenous hormones. We conclude that in future more studies are required to diagnose the risk of breast cancer in very early stages to reduce the mortality and morbidity in breast cancer patients.

## Acknowledgements

We sincerely acknowledge the subjects who were included in the study and gave their full cooperation throughout the study. We also thank the authors from where the literature has been reviewed and cited.

## Ethical Approval

This study was carried out at clinical biochemistry lab of Saveetha Medical College and Hospital, Chennai, after obtaining the approval from the Institutional Ethical Committee.

## References

1. Harsh Mohan, Systemic pathology, The Breast, Text Book of Pathology, 2010; 6th edition: 754.
2. Vinay Kumar, Abul K. Abbas, Jon C. Aster, Systemic Pathology: Disease of Organ systems, The Breast, Robbins and Cotran Pathologic Basis of Disease, 2015:9th edition: 1043.

3. Wei-Chiang Hsiao, Kung-Chia Young, Shoei-Loong Lin and Pin-Wen Lin. "Estrogen receptor- $\alpha$  polymorphism in a Taiwanese clinical breast cancer population: a case-control study" *Breast cancer Research*, 2004; Vol 6: No3:180-185.
4. Globocon WHO 2013, Global Health Estimates, WHO 2013.
5. GLOBOCAN 2008, Global Health Estimates WHO 2008.
6. An assessment of the burden and care of cancer patients. New Delhi: ICMR; National cancer registry program, Jan 3, 2014, Review of data from the National cancer Registry program of ICMR.
7. Raja Sekhar Katikireddy, Siva Nageswara R Aosuadara Setty. "The incidence of common cancers in south Indian region -A Hospital based cross sectional study" – Research article. *Scope Med, IJCRR*.2013;5(23):37-43.
8. Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 2002;94:606–16.
9. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. *J Natl Cancer Inst* 2004;96:1856–65.
10. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, Akhmedkhanov A, Afanasyeva Y, Kato I, et al. Postmenopausal levels of estrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study. *Br J Cancer* 2004;90:153–9.
11. Kaaks R, Rinaldi S, Key TJ, Berrino F, Peeters PH, Biessy C, et al. Postmenopausal serum androgens, estrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. *Endocr Relat Cancer* 2005;12:1071–82.
12. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A prospective study of endogenous hormones and breast cancer. *Cancer Detect Prev* 1994;18:79–85.
13. Kaaks R, Berrino F, Key T, Rinaldi S, Dossus L, Biessy C, et al. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* 2005;97:755–65.
14. Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol Biomarkers Prev* 2000;9:575–9.
15. Micheli A, Muti P, Secreto G, Krogh V, Meneghini E, Venturelli E, et al. Endogenous sex hormones and subsequent breast cancer in premenopausal women. *Int J Cancer* 2004;112:312-8.
16. Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol Biomarkers Prev* 2000;9:575–9.
17. Rosenberg CR, Pasternack BS, Shore RE, Koenig KL, Toniolo PG. Premenopausal estradiol levels and the risk of breast cancer: a new method of controlling for day of the menstrual cycle. *Am J Epidemiol* 1994;140:518–25.
18. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A prospective study of endogenous hormones and breast cancer. *Cancer Detect Prev* 1994;18:79–85.
19. Thomas HV, Key TJ, Allen DS, Moore JW, Dowsett M, Fentiman IS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. *Br J Cancer* 1997;75:1075–9.
20. Wysowski DK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. *Am J Epidemiol* 1987;125:791–9.
21. Grattarola R. The premenstrual endometrial pattern of women with breast cancer. A study of progestational activity. *Cancer* 1964;17:1119–22.
22. Grattarola R. Androgens in breast cancer. I. Atypical endometrial hyperplasia and breast cancer in married premenopausal women. *Am J ObstetGynecol* 1973;116:423–8.
23. Secreto G, Zumoff B. Abnormal production of androgens in women with breast cancer. *Anticancer Res* 1994;14:2113–7.
24. Secreto G, Toniolo P, Pisani P, Recchione C, Cavalleri A, Fariselli G, et al. Androgens and breast cancer in premenopausal women. *Cancer Res* 1989;49:471–6.
25. Secreto G, Toniolo P, Berrino F, Recchione C, Di Pietro S, Fariselli G, et al. Increased androgenic activity and breast cancer risk in premenopausal women. *Cancer Res* 1984;44:5902–5.
26. Mauvais-Jarvis P, Kuttent F, Gompel A. Antiestrogen action of progesterone in breast tissue. *Horm Res* 1987;28:212–8.
27. Doisneau-Sixou SF, Sergio CM, Carroll JS, Hui R, Musgrove EA, Sutherland RL. Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. *Endocr Relat Cancer* 2003;10:179–86.
28. Pike MC, Spicer DV, Dahmouch L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev* 1993;15:17–35.
29. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. *Lancet* 1997;350:1047–59.
30. Chlebowski RT. Breast cancer risk reduction: strategies for women at increased risk. *Annu Rev Med* 2002;53:519–40.
31. Chen WY, Hankinson SE, Schnitt SJ, Rosner BA, Holmes MD, Colditz GA. Association of hormone replacement therapy to estrogen and progesterone receptor status in invasive breast carcinoma. *Cancer* 2004;101:1490–500.
32. Anderson E: "The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis" *Breast Cancer Research*, 2002;4:197-201.
33. Key TJ, Pike MC: "The role of estrogens and progestogens in the epidemiology and prevention of breast cancer" *Eur J Cancer Clin Oncol*, 1988;24:29-43.
34. Martha L. Slattery, 1Karen Curtin, 1Richard Baumgartner, 2Carol Sweeney, 1Tim Byers, 3Anna R. Giuliano, 4 Kathy B. Baumgartner, 2 and Roger R. Wolffl. "L6, Aspirin, Nonsteroidal Anti-inflammatory Drugs, and Breast Cancer Risk in Women Living in the Southwestern United States" *Cancer Epidemiol Biomarkers Prev*, American association of cancer research: 2007:16-4.
35. Purohit A, Ghilchik MW, Duncan L, et al. "Aromatase activity and interleukin-6 production by normal and malignant breast tissues" *J Clin Endocrinol Metab*, 1995;80:3052–8.
36. Robinson EK, Sneige N, Grimm EA. "Correlation of interleukin 6 with interleukin 1ain human mammary tumours, but not with oestrogen receptor expression" *Cytokine*, 1998;10:970-6.