Research in Molecular Medicine (RMM)
Volume 6, Issue no. 4, DOI 10.18502/rmm.v6i4.4803
Production and Hosting by Knowledge E

Research Article

Serum Liver Proteins and 17β-estradiol in Postmenopausal Women with Breast Cancer

Zahra Tahmasebi Fard[1], Fatemeh Rouhollah[1], and Nahid Nafisi[2]

[1]Department of Cell and Molecular Biology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[2]Assistant Professor, Surgery Department, Iran University of Medical Science, Tehran, Iran

Abstract

Background: Breast cancer is a hormone-dependent malignancy that is associated with estrogen and progesterone interactions. The liver is the most important organ to be affected by the metastasis of breast cancer, which causes functional impairment. We compared levels of obesity, 17β-estradiol, and secreted proteins in postmenopausal women with breast cancer but without hepatic symptoms to those in healthy postmenopausal women.

Materials and Methods: We recruited 105 postmenopausal women with breast cancer but without any clinical hepatic symptoms based on a physician's diagnosis, and 105 healthy postmenopausal women. After taking blood samples, we separated the serum and determined the levels of alanine aminotransferase (ALT), enzyme aspartate aminotransferase (AST), sex hormone-binding globulin (SHBG), and 17β-estradiol using an enzyme-linked immunosorbent assay (ELISA). The results were statistically analyzed using SPSS.

Results: The mean ages of the subjects in the cancer and control groups were 60.88 ± 0.85 and 55.56 ± 0.81 years, respectively. The exception ages (p=0.002), body mass index (BMI) values (p=0.033), serum glutamic oxaloacetic transaminase (SGOT) levels/AST levels ($p=3.1*10^{-4}$), serum glutamic pyruvic transaminase (SGPT) levels/ALT levels(p=0.001), SHBG levels(p=0.014), and 17β-estradiol levels(p=0.003) in the serum differed significantly between the groups. Moreover, the mean serum 17β-estradiol (E2) levels and weights were higher in the cancer group than in the control group. Nevertheless, the mean serum levels of synthetic liver enzymes (SHBG, ALT, and AST) were lower in the cancer group than in the control group.

Conclusion: In general, the postmenopausal cancer patients had higher serum estrogen levels and BMIs than their healthy counterparts. Furthermore, the levels of liver enzymes apparently decreased in the cancer group, probably owing to liver malfunction.

Keywords: Breast cancer, estradiol, liver, SGOT, SGPT

Corresponding Author:
Zahra Tahmasebi Fard;
Department of Cell and Molecular Biology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
email: ztahmasebi@riau.ac.ir
Tel: (+98) 912-226-6686;
Fax: (+98) 21-76501812
ztahmasebifard@yahoo.com

Production and Hosting by Knowledge E

Dr. Alireza Rafiei

1. Introduction

Similar to many other malignancies, breast cancer is induced by environmental and hereditary factors, such as DNA damage, genetic mutation, chemicals, family history, age, geographical diversity, radiation, alcohol consumption, smoking, and viral agents [1, 2]. Although some of the risk factors for breast cancer are known, approximately 50–80% of them remain undetermined. Therefore, greater efforts are being made to identify this unknown risk factors [3].

Breast cancer cells spread through the blood stream, causing the liver to become metastatic [4]. A metastatic liver is found in 6–25% of patients with metastatic breast cancer, reducing the survival rate to 1–14 months. Liver metastases can develop with or without clinical symptoms, such as abdominal pain, mass, ascites, jaundice, or weight loss. Liver function tests indicate impaired liver function, which often increases the levels of synthetic proteins in the liver [5].

Estrogen plays a significant role in the development of the epithelium in normal and neoplastic breasts. An increased risk of breast cancer is associated with estrogen level and exposure time in postmenopausal women [6]. Estrogen is mainly synthesized by the ovaries, and there are three types in the blood: estradiol, estriol, and estrone. One of the most effective normal estrogens is 17β-estradiol [7]. However; the estrogen produced by the ovaries is completely different from that produced by the placenta [8].

By binding to its receptor in the cell cytoplasm, estrogen increases DNA and RNA levels, and raises the target tissue levels of proteins that are responsible for functions such as the development of the female reproductive system and feminine characteristics including breast development and the subtle female voice. After the menopause, almost no estrogen is produced in the body, leading to decreased osteogenesis, which causes postmenopausal osteoporosis in severe cases [9].

The main specific blood protein for binding to testosterone and estradiol is sex hormone-binding globulin (SHBG), which is a 93-kDa dimeric glycosylated protein secreted by the liver [10]. SHBG is essential for regulating the concentration of free steroid hormones in the blood. The broader role of the SHBG glycoprotein is the identification of specific SHBG receptors in the membranes of various tissue types. Studies have shown that the risk of breast cancer is associated with the levels of endogenous estrogens [11]. Other studies have demonstrated that the effect of sex hormones, especially estrogens, on the risk of breast cancer is more dependent on a woman's age than on her menopausal status [12]. In the context of this background, the aim of the present study was to compare the levels of enzymes produced by the liver in

the sera of postmenopausal breast cancer patients with no clinical hepatic symptoms to those of healthy postmenopausal women. We also assessed changes in the serum levels of 17β-estradiol and obesity in these groups to investigate the relationship between obesity and serum estrogen levels in cancer patients and healthy women.

2. Materials and Methods

In the present case-control study, a total of 105 postmenopausal patients were selected by a physician after confirmation of cancer. Each individual was randomly selected from patients with confirmed cancer, based on the results of cancer tests, mammograms, and surgery. Premenopausal patients were excluded from the study. After obtaining a consent form from each participant, we collected age, weight, and other data. The stage of cancer was determined according to the pathological report. The control group comprised 105 postmenopausal women in the same age range as the patients. The controls were included after confirmation of their health by a specialist physician; they were free from cancer according to the results from biochemical tests and mammograms. Written informed consent was obtained from all the participants.

We took a 3–5-mL blood sample from each individual in the cancer and control groups. After blood coagulation, the serum was immediately separated and placed in a freezer at -20˚C until required. We determined the serum concentrations of 17β-estradiol and SHBG (Dia.Metra Srl, Spello PG, Italy) in nanomoles per liter (nmol/L) by enzyme-linked immunosorbent assay (ELISA) using commercial kits. A DIALAB kit (DIALAB, Austria) was also used to measure the levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) enzymes. The serum concentrations of the enzymes were determined as units per liter in accordance with the kit protocol. Information on the status of estrogen receptor (ER) and progesterone receptor (PR) was collected from the patients' pathological records. We evaluated paraffin blocks containing tumor tissues using immunohistochemical techniques with specific antibodies. Other information about the type and grade of cancer was also obtained from the patients' reports.

3. Statistics

We analyzed the results for each protein using IBM SPSS 23. The mean levels of sex hormones (± standard deviation) were calculated for the cancer and control samples. An independent *t* test was used to compare the mean values between the cancer and

control groups. The correlation between hormones and other variables was calculated using a Pearson's correlation coefficient test. The significance level was considered at $\alpha = 0.05$.

4. Results

The mean age of the cancer patients was 60.88 ± 0.85 years (range, 41–79 years), and the mean age of the controls was 55.56 ± 0.81 years (range, 41–76 years). The characteristics of the patients and controls are presented in Table 1.

Based on the findings, age, body mass index (BMI), and the serum concentrations of SHBG, SGOT, SGPT, and 17-estradiol differed significantly between the groups. The mean serum 17β-estradiol level was higher in the cancer group than in the control group. However, the mean serum levels of synthetic hormones of the liver were lower in the cancer group than in the control group. Among the patients, 54 (51.43%) were positive for both ER and PR, 35 (33.33%) only expressed ER at the cell surface, 10 (9.52%) were only positive for PR, and 6 (5.72%) were negative for both ER and PR.

The correlation between variables was evaluated using the Pearson's correlation coefficient test. There were significant negative correlations between the 17β-estradiol levels and the ER ($P = 1.2 \times 10^{-6}$) and PR ($P = 1.8 \times 10^{-4}$) levels. However, there was a significant positive correlation between the estrogen level and the cancer grade (P = 0.006) (Table 2). Moreover, there were signification positive correlations between the serum SGPT and SHBG levels (P = 0.003) and the SGOT levels (P = 0.002) as well as the expression level of ER in both groups (P = 0.036) (positive correlation coefficients).

Based on the findings, there was a significant positive relationship between BMI and cancer grade (P = 0.046). In contrast, there was a significant negative correlation between BMI and ER ($P = 1.1 \times 10^{-5}$). The age variable only had a significant negative correlation with the SGOT level (P = 0.020).

5. Discussion

In the present study, the mean age (P = 0.002), BMI (P = 0.033), and serum levels of 17-beta-estradiol (P = 0.003) were significantly higher in the breast cancer group, whereas the mean levels of SHBG (P = 0.014), SGOT ($P = 3.1 \times 10^{-4}$), and SGPT (P = 0.001) were significantly lower in the breast cancer group.

The mean serum level of the 17β-estradiol hormone in the breast cancer group was 53.55 ± 1.76 nmol/L, whereas the mean level of 17β-estradiol in the non-cancerous group

TABLE 1: Clinical characteristics and biochemical indices of postmenopausal women in the cancer and control groups.

Variable	Range	Mean ± Std Error Difference		P-value
		Case	Control	
Age (years)	40–50	15 (14.29%)	29 (27.62%)	**0.002**
	50–60	30 (28.57%)	43 (40.95%)	
	60–70	42 (40%)	22 (20.95%)	
	> 70	18 (17.14%)	11 (10.48%)	
	Mean ± Std Error Difference	60.88 ± 0.85	55.56 ± 0.81	
BMI (kg/m2)	25 ≥	39 (37.14%)	79 (75.24%)	**0.033**
	> 25	66 (62.86%)	26 (24.76%)	
	Mean ± Std Error Difference	25.66 ± 0.28	24.09 ± 0.21	
Serum E2 (nmol/L))	Post Menopause Mean ± Std Error Difference	53.55 ± 1.76	47.29 ± 1.24	**0.003**
SHBG (nmol/L)	Concentration Mean ± Std Error Difference	40.91 ± 2.46	52.30 ± 3.78	**0.014**
SGOT/AST (U/L)	Concentration Mean ± Std Error Difference	21.27 ± 0.56	24.51 ± 1.15	**3.1×10^{-4}**
SGPT/ALT (U/L)	Concentration Mean ± Std Error Difference	18.40 ± 0.63	27.49 ± 2.41	**0.001**
Estrogen receptor	Positive	89 (84.76%)		
	Negative	16 (15.24%)		
Progesterone receptor	Positive	64 (60.95%)		
	Negative	41 (39.05%)		
Type of cancer	Invasive Lobular Carcinoma (ILC)	35 (33.33%)		
	Invasive Ductal Carcinoma (IDC)	43 (40.95%)		
	Ductal Carcinoma in Situ (DCIS)	19 (18.10%)		
	Lobular Carcinoma in Situ (LCIS)	8 (7.62%)		
Grade of cancer	grade I	19 (18.10%)		
	grade II	17 (16.19%)		
	grade II/III	25 (23.81%)		
	grade III	28 (26.67%)		
	metastasis	16 (15.23%)		

BMI = body mass index (BMI); SHBG = sex hormone-binding globulin; SGOT = serum glutamic oxaloacetic transaminase; AST = aspartate aminotransferase; SGPT = serum glutamic pyruvic transaminase; ALT = alanine aminotransferase

was 47.29 ± 1.24 nmol/L; the serum levels of 17β-estradiol differed significantly between the breast cancer and non-cancerous groups (P = 0.003).

There is evidence of a relationship between hormone levels and breast cancer. Breast cancer rarely occurs in men, as breast tumors are often estrogen-dependent. Fertility

Table 2: Pearson's correlation coefficients among 17-beta estradiol, SHBG, SGOT, SGPT, and age, BMI cases and controls combined and estrogen and progesterone receptors, type of cancer and grade of cancer for case group alone.

Correlation coefficients								
	Age	BMI	N	ER	PR	Type of cancer	Grade of cancer	N
Estradiol (E2)	-0.023	0.281	210	-0.606	-0.422	0.842	0.268	105
	P = 0.737	**P = 1.1×10^{-5}**		**P = 1.2×10^{-6}**	**P = 1.8×10^{-4}**	**P = 2.4×10^{-5}**	**P = 0.006**	
SHBG	-0.001	-0.146	210	0.08	0.203	-0.08	-0.143	105
	P = 0.993	**P = 0.034**		**P = 0.419**	**P = 0.038**	**P = 0.418**	**P = 0.146**	
SGOT	-0.041	-0.074	210	0.064	0.048	-0.061	-0.006	105
	P = 0.557	**P = 0.286**		**P = 0.517**	**P = 0.626**	**P = 0.536**	**P = 0.952**	
SGPT	-0.16	-0.001	210	0.205	0.05	0.098	0.032	105
	P = 0.020	**P = 0.991**		**P = 0.036**	**P = 0.612**	**P = 0.319**	**P = 0.745**	

SHBG = sex hormone-binding globulin; SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; BMI = body mass index; ER = estrogen receptor; PR = progesterone receptor

history, including age of menstruation and menopause, is another significant factor. Biological and epidemiological evidence also indicates a relationship between the level of hormones and breast cancer after the menopause [13]. Most studies have shown a positive relationship between sex hormones and the risk of breast cancer in post-menopausal women, and an inverse relationship between SHBG and the risk of cancer. Women who have higher levels of androgens and serum estrogens after menopause are nearly twice as likely to develop breast cancer compared to others [14].

In the present study, we found significant differences in the mean levels of SHBG between the breast cancer and non-cancerous groups (P = 0.014), but the mean level of SHBG in the breast cancer group (40.91 ± 2.46 nmol/L) was lower than in the control group (52.30 ± 3.78 nmol/L). SHBG is a plasma glycoprotein that is biologically linked to active estrogens and androgens. The affinity of this protein for its ligand is four to five times higher than that of albumin. Changes in the serum levels of SHBG can affect plasma distribution, and influence accessibility to target cells and tissues [15]. The level of liver-produced SHBG, unbound to steroids at the plasma surface, is 50% in women and approximately 20% in men. Several factors can control the level of SHBG; changes occur in the serum within a few days or weeks, rather than a few minutes or hours. A study by Hryb et al. (2002) showed that SHBG was secreted by tissues and cells containing rabbit sex hormone-binding globulin (RSHBG), which suggests the local regulation of SHBG signaling through autocrine/paracrine mechanisms [16].

In the present study, the BMI and estrogen levels were higher in the cancer group than in the control group; however, the cancer group had a lower mean SHBG level. The risk of breast cancer increases with increasing BMI in postmenopausal women. A meta-analysis revealed that the risk increased by 3% for every 1 kg/m^2 of BMI. Although the mechanism of this relationship is unknown, estrogen production is probably increased by the action of aromatase in adipose tissues and by reductions in the levels of serum SHBG [17].

The authors of a study by the Endogenous Hormones and Breast Cancer Collaborative Group on more than 6000 postmenopausal women concluded that the serum levels of sex hormones and several factors including age, BMI, smoking, and alcohol consumption are associated with the risk of breast cancer in postmenopausal women [18]; we report similar findings in the present study.

Our results revealed significant differences in both ER and PR expression levels and serum estrogen concentrations between the two groups. The present study also showed that the expression level of ER was higher than that of PR. In agreement with these findings, Fourkala et al. (2012) found that the serum levels of androgens and estrogens are associated with breast cancer in postmenopausal women. An increase in the levels of ER (not androgen receptor) is associated with the risk of disease 2 years before diagnosis [19]. Moreover, Lim et al. (2014) reported that estrogen levels in Asian women differ from those in European women, and this could affect the activity of ER and the risk of breast cancer [20]. Adly et al. (2006) concluded that the level of serum estrogen is associated with the risk of breast cancer in postmenopausal women. However, the level of androgens does not affect the risk of breast cancer [21].

Our study revealed an inverse relationship between the serum level of SHBG and 17β-estradiol. Serum estrogen concentrations appear to be associated with decreasing SHBG concentrations and the risk of breast cancer in postmenopausal women. This finding was in accordance with the results of the Fortunati et al. (2010) study that indicated SHBG activity in breast cancer cells, leading to cross-reactivity with estradiol-activated pathways, which ultimately inhibited several effects of estradiol in breast cancer cells. Generally, the results identified the unique ability of SHBG to regulate the unbound part of estrogen and interact with estradiol pathways to inhibit the growth and proliferation of breast cancer cells. Therefore, SHBG can reduce the risk of neoplasm development following exposure to estrogen [22].

In the present study, there was a significant difference in the levels of liver transaminases between the patients and controls. However, the results showed a reduction in the mean SGPT and SGOT levels in the patients, compared to the controls. The selected

postmenopausal patients had no clinical symptoms associated with liver damage. Therefore, liver damage is unlikely to be associated with liver dysfunction in patients that experience increased secretion of serum enzymes. Liver function impairment or damage is more common in breast cancer than in other cancers. Previous reports have suggested that increased doses of anti-cancer drugs, including doxorubicin, are associated with increased levels of serum transaminases and bilirubin. A comparison of the average levels of SGPT and SGOT in patients with and without hepatotoxicity revealed significant differences in all liver function tests [23].

In conclusion, the results of the present study suggest a relationship between 17β-estradiol levels and BMI in postmenopausal women. There was a significant difference between the groups in terms of adipose tissue as a source of estrogens. Furthermore, there were higher expression levels of ER and PR in the cancer group, which were significantly associated with the estrogen level. In agreement with other reports, the present study revealed an inverse relationship between the mean serum levels of estrogen and those of SHBG in postmenopausal women. Because muscular obesity is more androgenic, it may be associated with a decline in SHBG synthesis. In the present study, there was no relationship between age and serum estrogen and SHBG levels. Moreover, the mean levels of liver transaminases were lower in the patients than in the controls; tumor-suppressing drugs might negatively affect liver function.

Acknowledgments

We would like to thank the research departments of the Islamic Azad University, Tehran Medical Sciences Branch, who provided financial assistance. This paper is based on an approved research project. We would also like to express our appreciation of the help provided by all those who participated in sample collection. Furthermore, the authors would like to express their gratitude to all the patients who participated in this study (IR.IAU.TUM.REC.1397.007).

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

[1] Hortobagyi GN, de la Garza Salazar J, Pritchard K, Amadori D, Haidinger R, Hudis CA, et al. The global breast cancer burden: variations in epidemiology and survival. Clin Breast Cancer. 2005;6(5):391-401.

[2] Kaminska M, Ciszewski T, Lopacka-Szatan K, Miotla P, Staroslawska E. Breast cancer risk factors. Przeglad Menopauzalny. 2015;14(3):196-202. doi:10.5114/pm.2015.54346.

[3] Lawson JS, Tran D, Rawlinson WD. From Bittner to Barr: a viral, diet and hormone breast cancer aetiology hypothesis. Breast Cancer Res. 2001;3(2):81-5.

[4] Weinrich M, Weiß C, Schuld J, Rau BM. Liver Resections of Isolated Liver Metastasis in Breast Cancer: Results and Possible Prognostic Factors. HPB Surg. 2014;2014:6 pages. Article ID 893829. http://dx.doi.org/10.1155/2014/893829.

[5] Wyld L, Gutteridge E, Pinder SE, James JJ, Chan SY, Cheung KL, et al. Prognostic factors for patients with hepatic metastases from breast cancer. BJC. 2003;89(2):284-90. doi:10.1038/sj.bjc.6601038.

[6] Russo J, Russo IH. The role of estrogen in the initiation of breast cancer. J Steroid Biochem Mol Biol. 2006;102(1-5):89-96. doi:10.1016/j.jsbmb.2006.09.004.

[7] Burger HG. Androgen production in women. Fertil Steril. 2002;77(4):S3-5. doi:10.1016/S0015-0282(02)02985-0.

[8] Cavalieri E, Rogan E. Catechol quinones of estrogens in the initiation of breast, prostate, and other human cancers: keynote lecture. Ann N Y Acad Sci. 2006;1089:286-301.

[9] Almeida M, Laurent MR, Dubois V, Claessens F, O'Brien CA, Bouillon R, et al. Estrogens and androgens in skeletal physiology and pathophysiology. Physiol Rev. 2017;97(1):135-87.

[10] Haghighi N, Tahmasebifard Z, Nafisi N. Investigation of association between E326K in SHBG gene and breast cancer in Iranian females. Journal of Fasa University of Medical Sciences. 2015;5(2):289-95.

[11] Shimada N, Iwasaki M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H, et al. Genetic polymorphisms in estrogen metabolism and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. J Hum Genet. 2009;54(4):209-15.

[12] Ralph DA, Zhao LP, Aston CE, Manjeshwar S, Pugh TW, DeFreese DC, et al. Age-specific association of steroid hormone pathway gene polymorphisms with breast cancer risk. Cancer. 2007;109(10):1940-8.

[13] Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol Biomarkers Prev. 1996;5(7):533-9.

[14] Lamar CA, Dorgan JF, Longcope C, Stanczyk FZ, Falk RT, Stephenson HE Jr. Serum sex hormones and breast cancer risk factors in postmenopausal women. Cancer Epidemiol Biomarkers Prev. 2003;12(4):380-3. PMID:12692114.

[15] Lawenda, BD, Mondry TE, Johnstone PA. Lymphedema: a primer on the identification and management of a chronic condition in oncologic treatment. CA Cancer J Clin. 2009;59(1):8-24.

[16] Hryb DJ, Nakhla AM, Kahn SM, St George J, Levy NC, Romas, et al. Sex hormone-binding globulin in the human prostate is locally synthesized and may act as an autocrine/paracrine effector. J Biol Chem. 2002;277(29):26618-22.

[17] Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. JNCI. 2003; 95(16):1218-26. PMID:12928347.

[18] Endogenous Hormones and Breast Cancer Collaborative Group, Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, et al. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. BJC. 2011;105(5):709-22. doi:10.1038/bjc.2011.254.

[19] Fourkala EO, Zaikin A, Burnell M, Gentry-Maharaj A, Ford J, Gunu R, et al. Association of serum sex steroid receptor bioactivity and sex steroid hormones with breast cancer risk in postmenopausal women. Endocr Relat Cancer. 2012;19(2):137-147. doi: 10.1530/ERC-11-0310.

[20] Lim VW, Li J, Gong Y, Jin A, Yuan JM, Yong EL, et al. Serum estrogen receptor bioactivity and breast cancer risk among postmenopausal women. Endocr Relat Cancer. 2014;21(2):263-73.

[21] Adly L, Hill D, Sherman ME, Sturgeon SR, Fears T, Mies C, et al. Serum concentrations of estrogens, sex hormone-binding globulin, and androgens and risk of breast cancer in postmenopausal women. Int J Cancer. 2006;119(10):2402-7.

[22] Fortunati N, Catalano MG, Boccuzzi G, Frairia R. Hormone-binding globulin (SHBG), estradiol and breast cancer. Mol Cell Endocrinol. 2010;316(1):86-92.

[23] Damodar G, Smitha T, Gopinath S, Vijayakumar S, Rao YA. An evaluation of hepatotoxicity in breast cancer patients receiving injection doxorubicin. Annals of Medical and Health Science Research. 2014;4(1):74-9. doi: 10.4103/2141-9248.126619.