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EVALUATION OF SERUM TESTOSTERONE, ESTRADIOL AND PSA LEVELS AMONG PRE- AND POST-MENOPAUSE BREAST CANCER FEMALE PATIENTS

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ABSTRACT

Breast cancer has emerged as the most common malignancy among females during the last few years. In Egypt breast cancer accounts for 37.7% of all new cancer cases among women. Early diagnosis is essential for disease management so research is underway to identify potential serological bio markers. **Objective:** To identify the diagnostic role of total prostate-specific antigen, CA 15-3, testosterone and estradiol levels in breast cancer in women pre and post-surgery. Also, studying the possible effect of diabetes mellitus in breast cancer female patients pre and post-menopause on levels of PSA and other parameters. **Methods:** The current study included 124 female patients with breast cancer and 37 healthy women serving as normal control groups. Serum CA 15-3, PSA, testosterone and estradiol levels were assayed before surgery and three week after surgery and in healthy controls. **Results:** In present study there was very highly significant increase in serum CA 15-3, PSA, estradiol and testosterone levels in all patients group compared to the control. On comparing Pre-surgery patients group to the Post-surgery patients group, it was found that serum CA 15-3, PSA and testosterone levels fall following breast surgery. Concerning diabetic patients, serum CA 15-3, PSA and testosterone levels were non-significantly changed compared to non-diabetic patients in all groups. As evident from the present data, diabetic female patients had lower estradiol level than non-diabetic female patients. **Conclusions:** The present study indicated a clinical significance of measurement of serum PSA in the diagnosis of women with breast cancer, and may be a

useful marker for monitoring the response to treatment. CA15-3 is an important diagnostic, prognostic indicator. Estradiol and testosterone levels may play important roles in the development of breast cancer in older women. A single measurement of bioavailable estradiol and testosterone may be used to estimate a woman's risk for breast cancer. Based on these data we conclude that serum CA15-3 and PSA levels are useful tumor markers for breast cancer patient diagnosis or monitoring.

INTRODUCTION

Breast cancer remains the most common malignancy in women worldwide and is the leading cause of cancer-related mortality in females. More than 1.2 million cases are diagnosed each year, affecting 10-12 % of the female population and accounting for 500,000 deaths per year worldwide. In 2008, breast cancer caused 458,503 deaths worldwide "13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together (World Cancer Report "International Agency for Research on Cancer, 2008).

The incidence rate of breast cancer has been raising both in the developed and developing countries (Notani, 2001) and it is becoming frequent in some developing countries like Egypt and Tunisia (Park, 2000). Carcinoma breast is the second most common cancer among Indian women, and an increasing trend in its incidence has been observed in most of the metropolis with Mumbai topping the list (*Yeole et al*., 2001). In Egypt breast cancer accounts for 37.7% of all new cancer cases among women (Ferlay, 2010).

Breast cancer is mainly a postmenopausal disease, with more than three-quarters of tumors being hormone responsive (Benson *et al*., 2009). Breast cancer is more than 100 times more common in women than breast cancer in men, although males tend to have poorer outcomes due to delays in diagnosis (World Cancer Report "International Agency for Research on Cancer, 2008).

A tumor marker is a substance found in the blood, urine, or body tissues that can be elevated in cancer (Nair and Johnson, 2008).There are many accepted tumor markers used in breast cancer. Tumor markers used in screening, treatment, and surveillance of breast cancer are Cancer Antigen 15-3 (CA 15-3), cancer antigen 27.29 (CA 27.29), carcinoembryonic antigen (CEA), estrogen-receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), urokinase plasminogen activator (UPA), plasminogen activator inhibitor

1 (PAI-1), and certain multiparameter assays for gene expression (Mammoprint, Onco Type DX) (Harris *et al*., 2008).

PSA is a single-chain glycoprotein expressed at high levels in the epithelium of the human prostate gland. The prostatic function of PSA is to liquefy the sperm-entrapping seminal coagulum after ejaculation. The seminal substrates of PSA, a serine protease with chymotrypsin-like specificity, are the major structural constituents of the gel-like coagulum, seminogelin I and II and fibronectin (Lilja *et al*., 1987).

The name "PSA" reflects the initial widespread belief that expression of the protein was restricted to the prostate gland. Over the past 5 years, however, this notion has clearly been dispelled. Numerous studies have shown that PSA is expressed extraprostatically, suggesting that PSA may be functional outside the prostate gland. The periurethral (Skene's) gland was the first female tissue that was suggested to be able to produce PSA (Diamandis and Yu, 1997). This tissue has been referred to as the "female prostate" because its developmental origin is homologous to that of the male prostate. Therefore, it may not be surprising that this gland produces PSA in both males and females. It is now clear that hormonally regulated tissues in females, such as the breast, can produce PSA. PSA is detectable in normal and hyperplastic breast tissue (Yu *et al.*, 1996) and is present in the majority of breast tumors (Diamandis *et al*., 1994) and breast cysts. PSA is released into breast secretions, such as the milk of lactating women (Diamandis *et al.*, 1996) and nipple aspirate fluid (Sauter *et al*., 1998). Mammary PSA is identical in molecular weight and mRNA sequence to seminal PSA (Monne *et al*., 2008). PSA gene expression in breast tumors appears to be under hormonal control because the steroid hormone receptor-positive breast cancer cell lines T-47D and BT-474 can be induced by androgens, progestins, mineralocorticoids, and glucocorticoids to produce PSA (Zarghami *et al*., 1997) in vitro. Detectable circulating levels of PSA, likely originating from breast tissue, are present in the serum of women (Yu and Diamandis, 1998). The PSA concentration in female sera is approximately 1000-fold less than that of males (0.004 mg/liter) (Melegos and Diamandis, 1998).

Testosterone is a steroid hormone from the androgen group and is found in humans and other vertebrates. In humans and other mammals, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. Small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid (Bassil *et al*., 2009). When evaluating testosterone's impact on breast cancer, it is unclear if testosterone is

a singular causative agent or if breast cancer is a result of other hormonal stimulation such as estrogens or synthetic progestins. Several clinical studies have attempted to answer this question with results suggesting that there is more to the equation than testosterone. A pooled analysis of data from some studies found that higher levels of blood testosterone increased breast cancer risk in premenopausal women (Zhang *et al*., 2013).

PATIENTS AND METHODS

This study was carried out in General Surgery and Clinical Pathology Departments, Zagazig University Hospitals and Biological Applications Department, Atomic Energy Authority during the period from 2013 to 2015. It was conducted on (124) female patients with breast cancer with mean age ±SE (58.855 ±0.811) years and 37 females age matched healthy women serving as a normal control group with mean age ±SE (55.486 ±1.984) years. A signed informed consent taken from all participants before withdrawal of the samples and their enrollment into this study.

Group (A)

Control: 37 healthy females with Mean age ±SE,(55.486 ± 1.984) were served as control in the present study.

The control group subdivided according to menopause into

A.1- Premenopausal group

Include 12 healthy females their ages less than 50 years.

A.2- Postmenopausal group

Include 25 healthy females their ages more than 50 years.

Patients

The present study included 124 breast cancer female patients were included in the study. They were classified into 4 groups.

Group (B)

Pre-Surgery breast cancer patients (all patients) includes newly diagnosed 124 breast cancer female patients with Mean age ±SE, (58.855 ±0.8112).

This group subdivided according to serum fast glucose level into

B.1- Non-diabetic group

Includes 73 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

B.2- Diabetic group

Includes 51 breast cancer female patients with serum glucose level more than 110 mg/dl.

This group was subdivided according to menopause into

B.1`- Premenopausal group

Include 19 breast cancer female patients their ages less than 50 years.

B.2`- Postmenopausal group

Include 105 breast cancer female patients their ages more than 50 years.

Premenopausal group is subdivided according to glucose level into

B.1`.1- Non-diabetic premenopausal group

Include 14 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

B.1`.2- Diabetic premenopausal group

Include 5 breast cancer female patients with serum glucose level more than 110 mg/dl.

Postmenopausal group is subdivided according to glucose level into

B.2`.1- Non-diabetic postmenopausal group

Include 43 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

B.2`.2- Diabetic postmenopausal group

Include 62 breast cancer female patients with serum glucose level more than 110 mg/dl.

Group (C)

Post-Surgery breast cancer patients include: 76 breast cancer female patients underwent modified radical mastectomy with Mean age ±SE, (58.187 ± 1.114) years.

This group was subdivided according to serum fast glucose level into

C.1- Non-diabetic group

Includes 41 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

C.2- Diabetic group

Includes 35 breast cancer female patients with serum glucose level more than 110 mg/dl.

Post-surgery breast cancer patients was subdivided according to menopause into

C. 1`- Premenopausal group

Include 17 breast cancer female patients their ages less than 50 years.

C.2`- Postmenopausal group

Include 59 breast cancer female patients their ages more than 50 years.

Premenopausal group of post-surgery breast cancer patients is subdivided according to glucose level into

C.1`.1- Non-diabetic premenopausal group of post-surgery breast cancer patients

Include 14 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

C.1`.2- Diabetic premenopausal group of post-surgery breast cancer patients

Include 3 breast cancer female patients with serum glucose level more than 110 mg/dl.

Postmenopausal group of post-surgery breast cancer patients is subdivided according to glucose level into

C.2`.1- Non-diabetic postmenopausal group of post-surgery breast cancer patients

Include 31 breast cancer female patients with serum glucose level less than 110 mg/dl considered non diabetic according to American Diabetes Association.

C.2`.2- Diabetic postmenopausal group of post-surgery breast cancer patients

Include 28 breast cancer female patients with serum glucose level more than 110 mg/dl.

Eligibility criteria

- Breast conserving surgery or Modified radical mastectomy.

- Breast cancer patients who had undergone chemotherapy or radiotherapy before surgery were excluded.
- Stage T1-3, N0-3, M0.
- No history of contralateral breast cancer.
- No serious cardiovascular or pulmonary diseases.

All patients were subjected to the following

Clinical evaluation

Include detailed history taking and clinical examination, particularly stressed upon:

1. Age of diagnosis.
2. Menopausal status

Radiological assessment: Mammography, as well as computed tomographic scan, and abdominal ultrasound.

Histopathological assessment

Tumor size

Which classify the cancer into T1 (≤ 2cm), T2 (> 2cm ≤ 5cm), T4 (any size extent to chest wall and/or skin) (Edge *et al.*, 2010).

Histological grading was evaluated according to Bloom-Richards on breast cancer grading system (Edge *et al.*, 2010).

Clinical staging of breast cancer patients according to TNM classification (Edge *et al.*, 2010).

Laboratory investigations

Sample collection

Five ml venous blood samples were collected from all patients and healthy controls by venipuncture under complete aseptic conditions and delivered into two tubes, one ml of blood was delivered into EDTA vacutainer tubes for CBC, then the samples were allowed to clot at 37°C for 20 minutes, serum was stored at -80°C until assay of carcinoma antigen 15.3, prostate specific antigen, testosterone and estradiol levels.

Biochemical Analyses

1. Determination of serum CA 15-3

Serum carcinoma antigen 15-3 (CA15-3) level determined by micro-particle Enzyme Immunoassay (MEIA) according to the method of Hayes *et al.* (1986).

2. Determination of serum PSA

The total PSA assay determined by micro-particle Enzyme Immunoassay (MEIA) technology according to the method of Chen *et al.* (1995).

3. Assay of serum sex hormones levels

a- Estradiol (E2)

Serum estradiol assayed by radioimmunoassay kit (Siemen Medical Solutions Diagnostics, TKE21-2125, USA).

b- Total testosterone

Serum total testosterone assayed by radioimmunoassay kit (Siemen Medical Solutions Diagnostics, TKTT1-1999, USA).

Statistical Analysis of all the grouped data were statistically calculated using Microsoft Office Excel 2010 software for windows and SPSS version 16.0 for statistical analysis.

RESULTS

(I) All patients

The results represented in table (1), showed significant increase in serum CA 15.3, estradiol and testosterone levels in comparison between control group and all patients group. Whereas PSA was not-detectable in control group, On the other hand, it showed a significant increase in all patients group. Statistical analysis in non-diabetic group revealed a significant increase in serum CA 15.3, PSA, estradiol and testosterone levels when compared to the control group.

Table 1: Mean±SE of biochemical parameters in control and all patients groups.

Item	Control (37)	T.patients (124)	Significance
CA 15-3 (U/ml)	$15.6^{b} \pm 0.123$	$190.278^{a} \pm 5.739$	VHS
PSA (ng/ml)	< 0.002 (ND)	1.033 ± 0.030	-
Estradiol (pg/ml)	$89.432^{b} \pm 7.931$	$115.379^{a} \pm 3.423$	VHS
Testosterone (ng/ml)	$0.269^{b} \pm 0.026$	$1.174^{a} \pm 0.034$	VHS

NS: not significant (P-value >0.05) S: significant (P-value<0.05) VHS: very highly significant (P-value <0.001) ND: not detected.

The data obtained in diabetic patient group revealed that serum CA 15-3, PSA, estradiol and testosterone was significantly increased in comparison with that in control group.

The statistical analysis of serum CA 15-3, PSA and testosterone levels in diabetic patient. Meanwhile, serum estradiol level was significantly lower in diabetic patient group than non-diabetic patient group.

Table 2: Mean ±SE of biochemical parameter in control, Non-diabetic and diabetic patients groups.

Item	Control (37)	Total Patients (124)	
		Non-diabetic (73)	Diabetic (51)
CA 15-3 (U/ml)	$15.6^{b} \pm 0.123$	$190.180^{a} \pm 7.723$	$190.418^{a} \pm 8.606$
PSA (ng/ml)	< 0.002 (ND)	$1.020^{a} \pm 0.041$	$1.051^{a} \pm 0.045$
Estradiol (pg/ml)	$89.432^{c} \pm 7.931$	$178.392^{a} \pm 4.457$	$165.950^{b} \pm 5.388$
Testosterone (ng/ml)	$0.269^{b} \pm 0.026$	$1.169^{a} \pm 0.043$	$1.178^{a} \pm 0.057$

Values with the same letter considered insignificant (P>0.05).

Values with the different letters considered significant (P<0.05).

ND: not detected.

Table (3) showed that comparison between control and all patients group according to menopause. The data obtained from premenopausal control group revealed that serum CA 15-3 and PSA levels were non-significantly changed from the postmenopausal control group. The results showed that there was a significant decrease in serum estradiol levels in comparison between postmenopausal control group and premenopausal control group. Contrary, there was a significant increase in serum testosterone level between postmenopausal control group and premenopausal control group.

The obtained results in postmenopausal patient group revealed a significant increase in serum CA 15-3 level in comparison with premenopausal patient group. Statistical analysis of

postmenopausal patient group revealed a significant decrease in serum estradiol when compared to premenopausal patient group. At the same time there were no differences between postmenopausal and premenopausal patient groups in PSA and testosterone levels.

Concerning premenopausal patient group, serum CA 15-3, PSA, estradiol and testosterone levels was significantly increased compared to premenopausal control group.

The statistical analysis of serum CA 15-3, PSA, estradiol and testosterone levels in postmenopausal patient group showed a significant increase in comparison with that in postmenopausal control group.

Table 3: Mean ±SE of biochemical parameter in Premenopausal and Postmenopausal in control and all patients groups.

Item	Control (37)		All patients (124)	
	Premenopausal (12)	Postmenopausal (25)	Premenopausal (19)	Postmenopausal (105)
CA 15-3 (U/ml)	16.3 ^{c} ± 0.145	18.2 ^{c} ± 0.162	161.167 ^{b}±12.219	195.465 ^{a} ±6.296
PSA (ng/ml)	< 0.002	< 0.002	1.149 ^{a} ± 0.053	1.011 ^{a} ± 0.102
Estradiol (pg/ml)	132.325 ^{b}±14.961	45.564 ^{d} ± 2.109	180.063 ^{a} ± 13.552	70.063 ^{c} ± 13.552
Testosterone (ng/ml)	0.179 ^{c}± 0.030	0.311 ^{b} ± 0.033	1.041 ^{a} ± 0.047	1.198 ^{a} ± 0.067

Values with the same letter considered insignificant (P>0.05).

Values with the different letters considered significant (P<0.05).

ND: not detected.

The data obtained from diabetic premenopausal patient group revealed that serum CA 15-3, PSA, estradiol and testosterone levels were non-significantly changed from the non-diabetic premenopausal patient group.

The statistical analysis of serum CA 15-3, PSA, estradiol and testosterone levels in diabetic postmenopausal patient group showed a non-significant change in comparison with that in non-diabetic postmenopausal patient group.

The results represented in table (4), showed significant increase in serum CA 15-3 and testosterone levels in comparison between non-diabetic postmenopausal patient group and non-diabetic premenopausal patient group. While, serum PSA and estradiol levels in non-

diabetic postmenopausal patient group showed a non-significant change in comparison with that of the non-diabetic premenopausal patient group.

Concerning diabetic postmenopausal patient group, serum CA 15-3, PSA and testosterone levels were non-significantly changed compared to diabetic premenopausal patient group. Only serum estradiol in diabetic postmenopausal patient group showed a significant decrease in comparison with that of diabetic premenopausal patient group.

Table 4: Mean±SE of biochemical parameter in Non-diabetic and diabetic patients during Pre and Postmenopausal periods in all patients groups.

Item	All patients (124)					
	Premenopausal (19)			Postmenopausal (105)		
	Non-diabetic (14)	Diabetic (5)	P-value	Non-diabetic (43)	Diabetic (62)	P-value
CA 15-3 (U/ml)	159.907±12.29 *	166.4±34.188	NS	202.155±10.66 *	196.642 ± 7.614	NS
PSA (ng/ml)	1.175 ± 0.052	1.085 ± 0.149	NS	1.042 ± 0.045	0.999 ± 0.050	NS
Estradiol (pg/ml)	188.72 ± 14.532	172.022± 30.654 **	NS	80.522 ± 5.435	65.678 ± 3.147 **	NS
Testosterone (ng/ml)	1.030± 0.042 *	1.072 ± 0.148	NS	1.288 ± 0.063 *	1.200 ± 0.049	NS

NS: not significant (P-value >0.05V)

* Significant difference in comparing non-diabetic premenopausal patients group with non-diabetic postmenopausal patients group.

** Significant difference in comparing diabetic premenopausal patients group with diabetic postmenopausal patients group.

(II) Post-surgery patient

In this study serum CA 15-3 level in Post-surgery patients group was very highly significant decreased compared to that of pre-surgery patients group. The results of serum PSA and testosterone levels in Post-surgery patients group showed a significant decrease in comparison with that of pre-surgery patients group. On the other hand, no statistically significant change was recorded for serum estradiol level in post-surgery patients group compared to that found in pre-surgery patients group.

Table 5: Mean ±SE of biochemical parameter in pre-surgery and post-surgery patients groups.

Item	Pre-surgery (76)	Post-surgery (76)	P-value	Significance
CA 15-3 (U/ml)	183.222 [a] ±7.762	36.5 [b] ± 1.319	0.0001	VHS
PSA (ng/ml)	1.065 [a] ±0.037	0.928 [b] ± 0.038	0.014	S
Estradiol (pg/ml)	71.743 [a] ±4.961	124.240 [a] ± 2.417	0.302	NS
Testosterone (ng/ml)	1.202 [a] ±0.035	1.072 [b] ± 0.040	0.013	S

NS: not significant (P-value >0.05) S: significant (P-value<0.05) VHS: very highly significant (P-value <0.00105)

Values with the same letter considered insignificant (P>0.05)

The data obtained in diabetic pre-surgery patients group revealed that serum CA 15-3 level was non-significantly changed from the non-diabetic pre-surgery patients group. The results of serum estradiol and PSA levels in diabetic pre-surgery patients group showed a significant decrease in comparison with that of non-diabetic pre-surgery patients group. There was a highly significant increase in the serum testosterone level in diabetic pre-surgery patients group when compared with its level in non-diabetic pre-surgery patients group.

Concerning diabetic post-surgery patients group, serum CA 15-3, PSA, estradiol and testosterone levels were non-significantly changed compared to non-diabetic post-surgery patients group.

Statistical analysis in non-diabetic post-surgery patients group revealed a significant decrease in serum CA 15-3 and testosterone levels when compared to the non-diabetic pre-surgery patients group. The result of serum PSA level, in non-diabetic post-surgery patients group was non-significantly changed in comparison with that in non-diabetic pre-surgery patients group. There was a highly significant increase in the serum estradiol level in non-diabetic post-surgery patients group when compared with its level in non-diabetic pre-surgery patients group.

In this study serum CA 15-3 level in diabetic post-surgery patients group was significantly decreased compared to that of diabetic pre-surgery patients group. The data of serum PSA and testosterone levels in diabetic post-surgery patients group showed a non-significant change in comparison with that in diabetic pre-surgery patients group. There was a highly significant increase in the serum estradiol level in diabetic post-surgery patients group when compared with its level in diabetic pre-surgery patients group.

Table 6: Mean ±SE of biochemical parameter in Non-diabetic and diabetic patients groups, pre- and post-surgery.

Item	Pre-surgery (76)		P-value	Post-surgery (76)		P-value
	Non-diabetic (41)	Diabetic (35)		Non-diabetic (41)	Diabetic (35)	
CA 15-3 (U/ml)	187.656+11.458 *	186.229+10.451 **	0.893 NS	34.8 ± 1.686 *	36.755 ± 2.069 **	0.298 NS
PSA (ng/ml)	1.059+0.045	1.036+0.065	0.003 HS	1.013 ± 0.042	0.794 ± 0.065	0.015 S
Estradiol (pg/ml)	73.594+6.592 *	65.842+6.507 **	0.001 VHS	169.813 ± 3.940 *	160.778 ± 3.199 **	0.801 NS
Testosterone (ng/ml)	1.199+0.046 *	1.233+0.066	0.006 HS	1.055 ± 0.049 *	1.043 ± 0.069	0.652 NS

NS: not significant (P-value >0.05) S: significant (P-value<0.05) HS: highly significant (P-value<0.01) VHS: very highly significant (P-value <0.001.)

*: Significant difference in comparing non-diabetic pre-surgery with non-diabetic post-surgery patients.

****:** Significant difference in comparing diabetic pre-surgery with diabetic post-surgery patients.

The data obtained from postmenopausal pre-surgery patients group revealed that serum CA 15-3 and PSA levels was non-significantly changed from the premenopausal pre-surgery patients group, whereas, serum estradiol level in postmenopausal pre-surgery patients group showed a highly significant decrease compared to premenopausal pre-surgery patients group. There was a significant increase in the serum testosterone level in postmenopausal pre-surgery patients group when compared with its level in premenopausal pre-surgery patients group.

The results represented in table (7), showed a very highly significant increase in serum CA 15-3 in comparison between postmenopausal post-surgery patients group and premenopausal post-surgery patients group, whereas, serum estradiol level in postmenopausal post-surgery patients group showed a significant decrease compared to premenopausal post-surgery patients group. The serum PSA and testosterone levels in postmenopausal post-surgery patients group was non significantly changed in comparison with that in premenopausal post-surgery patients group.

Statistical analysis in premenopausal post-surgery patients group revealed a significant decrease in serum CA 15-3 when compared to premenopausal pre-surgery patients group. Whereas, no statistically significant change was recorded for serum PSA, estradiol and testosterone levels in premenopausal post-surgery patients group compared to the premenopausal pre-surgery patients group.

Concerning postmenopausal post-surgery patients group, serum CA 15-3, PSA and testosterone levels was significantly decreased compared postmenopausal pre-surgery patients group. The data of serum estradiol level in postmenopausal post-surgery patients group showed a non-significant change in comparison with that in postmenopausal pre-surgery patients group.

Table 7: Mean ±SE of biochemical parameter in Premenopausal and Postmenopausal in pre-surgery and post-surgery patients groups.

Item	Pre-surgery (76)		P-value	Post-surgery (76)		P-value
	Premenopausal (17)	postmenopausal (59)		premenopausal (17)	Postmenopausal (59)	
CA 15-3 (U/ml)	163.576+13.131 *	189.27+8.965 **	0.155 NS	27.612 ± 2.577 *	39.105 ± 1.361 **	0.0001 VHS
PSA (ng/ml)	1.132+0.058	1.043+0.043 **	0.302 NS	1.032 ± 0.074	0.897 ± 0.044 **	0.147 NS
Estradiol (pg/ml)	102.541+14.958	62.474+4.069	0.001 HS	160.504 ± 8.329	70.525 ± 1.993	0.042 S
Testosterone (ng/ml)	1.029+0.051	1.251+0.043 **	0.014 S	0.943 ± 0.061	1.109 ± 0.048 **	0.081 NS

NS: not significant (P-value >0.05) S: significant (P-value<0.05) VHS: very highly significant (P-value <0.001)*: Significant difference in comparing premenopausal pre-surgery with premenopausal post-surgery patients

**Significant difference in comparing postmenopausal pre-surgery with postmenopausal post-surgery patients

In this study serum CA 15-3 and PSA levels in non-diabetic premenopausal post-surgery patients group was significantly decreased compared non-diabetic premenopausal pre-surgery patients group. While, serum estradiol and testosterone levels in non-diabetic premenopausal post-surgery patients group showed a non-significant change compared to the non-diabetic premenopausal pre-surgery patients group.

Statistical analysis in diabetic premenopausal post-surgery patients group revealed a significant decrease in serum CA 15-3 level when compared to the diabetic premenopausal pre-surgery patients group. The result of serum PSA, estradiol and testosterone level in diabetic premenopausal post-surgery patients group was non-significantly changed in comparison with that in diabetic premenopausal pre-surgery patients group.

Table 8: Mean ±SE of biochemical parameter in Non-diabetic and diabetic patients during Premenopausal period, in pre and post-surgery patients groups.

Item	Pre-surgery		Post-surgery	
	Premenopausal (17)		Premenopausal (17)	
	Non-diabetic (14)	Diabetic (3)	Non-diabetic (14)	Diabetic (3)
CA 15-3 (U/ml)	159.907±12.29 *	131.667+10.929 **	25.842±2.0383 *	35.867±11.561 **
PSA (ng/ml)	1.175 ± 0.052 *	1.095+0.272	0.986 ± 0.082 *	1.247 ± 0.113
Estradiol (pg/ml)	188.72±14.532	113.456+38.157	174.071 ± 9.735	150.857±16.314
Testosterone (ng/ml)	1.030 ± 0.042	1.171+0.235	0.909 ± 0.051	1.099 ± 0.277

*Significant difference in comparing non-diabetic premenopausal pre-surgery with non-diabetic premenopausal post-surgery patients

**Significant difference in comparing diabetic premenopausal pre-surgery with diabetic premenopausal post-surgery patients

The results represented in table (9), showed significant decrease in serum CA 15-3 and PSA levels in comparison between non-diabetic postmenopausal post-surgery patients group and non-diabetic postmenopausal pre-surgery patients group. The result of serum estradiol and testosterone levels in non-diabetic postmenopausal post-surgery patients group was non-significantly changed in comparison with that in non-diabetic postmenopausal pre-surgery patients group.

Statistical analysis in diabetic postmenopausal post-surgery patients group revealed a significant decrease in serum CA 15-3, PSA and testosterone levels when compared to the diabetic postmenopausal pre-surgery patients group. While serum estradiol in diabetic postmenopausal post-surgery patients group showed a significant increase in comparison with that of diabetic postmenopausal pre-surgery patients group.

Table 9: Mean±SE of Biochemical parameters in in non-diabetic and diabetic patientsduring postmenopausal period, pre-surgery and post-surgery patients groups.

Item	Pre-surgery		Post-surgery	
	Postmenopausal (59)		Postmenopausal (59)	
	Non-diabetic (31)	Diabetic (28)	Non-diabetic (31)	Diabetic (28)
CA 15-3 (U/ml)	191.942+13.385 *	191.429+12.204 **	38.958± 1.824 *	38.936±2.029 **
PSA (ng/ml)	1.098+0.036 *	0.956+0.083 **	0.948±0.059 *	0.846±0.066 **
Estradiol (pg/ml)	69.957+7.054	54.058+3.298 **	78.830±2.706	64.446±2.786 **
Testosterone (ng/ml)	1.305+7.051	1.255+0.057 **	1.124±0.063	1.083±0.071 **

* Significant difference in comparing non-diabetic postmenopausal pre-surgery with non-diabetic postmenopausal post-surgery patients.

**Significant difference in comparing diabetic postmenopausal pre-surgery with diabetic postmenopausal,post-surgerypatients.

Table 10: the grade and TNM for pre-surgery breast cancer patients.

Pre-surgery (124)									
Item	**Total Pre-surgery (124)**	**Non-diabetic (73)**	**Diabetic (51)**	**Premenopausal**			**Postmenopausal**		
				Total Premenopausal (19)	**Non-diabetic (14)**	**Diabetic (5)**	**Total Postmenopausal (105)**	**Non-diabetic (43)**	**Diabetic (62)**
Grade									
grade II	75	61	37	18	14	4	82	33	44
grade III	49	12	14	1	-	1	23	10	18
TNM									
T1N0M0	60	30	30	2	1	1	47	22	31
T1N1M0	16	9	7	6	5	2	11	3	7
T2N1M0	31	20	11	8	8	-	17	6	13
T2N2M0	-	-	-	-	-	-	-	-	-
T2N3M0	12	10	2	1	-	1	11	4	3
T3N1M0	-	-	-	-	-	-	-	-	-
T3N2M0	5	4	1	2	-	1	19	8	8

Table 11: the grade and TNM for post-surgery breast cancer patients.

	Post-surgery (76)								
Item	**Total Post-surgery (76)**	**Non-diabetic (41)**	**Diabetic (35)**	**Premenopausal**			**Postmenopausal**		
				Total Premenopausal (17)	**Non-diabetic (14)**	**Diabetic (3)**	**Total Postmenopausal (59)**	**Non-diabetic (31)**	**Diabetic (28)**
Grade									
grade II	60	32	28	16	14	2	44	22	22
grade III	16	9	7	1	-	1	15	9	6
TNM									
T1N0M0	28	20	8	5	5	-	23	15	8
T1N1M0	18	9	9	2	1	1	16	9	7
T2N1M0	21	10	11	7	6	1	12	5	7
T2N2M0	-	-	-	-	-	-	-	-	-
T2N3M0	4	1	3	-	-	-	4	1	3
T3N1M0	-	-	-	-	-	-	-	-	-
T3N2M0	5	1	4	3	2	1	4	1	3

DISCUSSION

Breast cancer is the most frequently diagnosed cancer in women worldwide (Jemal *et al*., 2011). Statistically, breast cancer is the most common female malignancy among Egyptian women as recorded by National Cancer Institute (NCI), Egypt. Breast cancer represents about 38% of all reported cancer cases in Egyptian females, with an average age of 49.6 per 100,000 populations, with higher incidence in urban areas compared to rural area (Dey *et al*., 2010).

Egyptian breast cancer patients are characterized by high mortality rate (20.1 per 100,000) compared to USA (14.7 per 100,000) (Salhia *et al*., 2011). It should be noted that breast cancer in the Arab region has shown to present a decade earlier than western countries (Najjar *et al*., 2010).

The major objectives in staging breast cancer are to estimate the prognosis and determine the need for adjuvant therapy. Currently, the preferable system for staging of breast carcinoma is the tumour-node-metastasis (TNM) system adopted by the American Joint Committee (AJC) on cancer, ensuring a uniform grading system worldwide (Amin *et al*., 2017).

Despite intense research and prevention efforts, breast cancer remains the most prevalent cancer in women. The best current diagnostic regimen (mammogram, breast self-exam, and physical exam) still misses a significant percentage of early cancers. New detection methods to identify patients with the disease must be developed. Advances in laboratory techniques may bring value to known markers previously thought worthless for the diagnosis and follow up of breast cancer.

No single marker in clinical use is sufficiently sensitive and specific to detect breast cancer. Therefore, multiple markers will be required to optimize our ability to detect disease at its earlier stage which is the major step in avoiding metastasis that, in most cases, the cause of death and to increase the patients chances of full recovery(Martin *et al*., 2015).

In the present study, it was observed that significant increase in serum CA 15.3 in comparison between control group and all patients group. This result is in harmony with that reported by Mohd *et al*. (2016) who found that an increase in serum CA 15-3 concentrations in breast cancer patients compared to the control group. Cancer antigen (CA) 15-3 is the product of MUC-1 gene, and mucins are aberrantly over-expressed in many adenocarcinomas

in an under-glycosylated form and are then shed into the circulation. CA15-3 has been shown to be an independent predictor of first recurrence as well as a powerful prognostic indicator in patients with advanced breast cancer (Park *et al*., 2008). Elevated pre-operative CA15-3 level is directly related to tumour burden and independent prognostic factors for breast cancer. It could be considered for clinical use such as predicting patient outcome and determining adjuvant treatment for better outcome (Hiba *et al*., 2013).

The current results confirm previous observation of Mohd *et al*. (2016) who found that mean serum CA 15-3 levels in patients before surgery were significantly higher compared with those of CA 15-3 after surgery. The authors also found that elevated preoperative serum levels of CA 15-3 were significantly correlated with the presence of metastatic disease. Similar results are obtained by Antonella *et al.* (2013) who found that on comparing preoperative serum CA 15-3 level to the postoperative level, it was found that Ca 15-3 level falls following breast surgery. These findings suggest that CA 15-3 has definitive prognostic role in breast carcinoma. Even with normal preoperative CA 15-3 values, post-operative CA15-3 values are important to detect progression of disease, recurrence or metastasis. If postoperative CA 15-3 level remains stable or increases, it indicates chances of recurrence (Mohd *et al*., 2016).

CA 15-3 is the most widely used serum biochemical tumor marker in breast cancer (Macis *et al*., 2009). Assay of CA 15.3 is a relatively convenient and noninvasive method for evaluating prognosis in newly diagnosed breast cancer patients (Duffy, 2011).The results of this study found that non-significant change in CA 15-3 levels in diabetic and non-diabetic groups. However, analysis indicated that patients with type 2 diabetes have a slightly elevation in CA15-3 levels compared with non-diabetic patients. Diabetes can be considered as a risk factor for breast cancer independent of obesity and age. But the biological mechanisms are still unclear (Edward, 2010).The unwarranted connection between diabetes mellitus (DM) and breast cancer has gained new ground in recent years. DM is diagnosed in the age group of 30+ years with possible exposure to predisposing factors like hyperisulinemia and obesity at younger age. Furthermore, 12% of the breast cancer cases are diagnosed in the young females aged 20-34 years (Arif *et al*., 2011). Genetic predisposition and environmental factors such as high fat diet accompanied with sedentary life style constitute increased breast cancer risk. Thus, metabolic abnormalities including obesity and type 2 diabetes (T2DM) are positively associated with the breast cancer risk (Krebs *et al*.,

2006 and Larsson *et al*., 2011). Diabetic condition induces changes in several hormonal systems, including insulin, insulin like growth factors, estrogens and other cytokines that may affect the breast cancer risk. Characteristics of T2DM including insulin resistance and the resultant hyperinsulinemia are strongly correlated with postmenopausal as well as pre-menopausal breast cancer risk (Marghoob *et al*., 2014).

PSA is a valuable tumor marker used for diagnosis and management of prostate cancer. Recently, PSA has been found in various female tissues and body fluids. Female breasts both normal and abnormal, including cancerous tissues, can produce PSA, and this production is regulated by androgens and progestins.

The present study demonstrated that serum PSA level was not-detectable in control group, On the other hand, it showed a very highly significant increase in all patients group.

These observations are in agreement with Prakruti *et al*. (2011) and Fawzi *et al*. (2013) this could be attributed to disrupted hormonal balance in such women leading to aberrant expression of hormone-dependent genes like PSA, which is normally under hormonal control and up-regulated by androgens and progesterone (Narita *et al*., 2006).

The marked rise in serum total PSA in all patients group is in agreement with various authors who have registered a rise in serum total PSA and free PSA in women with tumorous growths in breast in comparison to those with no breast pathology (Prakruti *et al*., 2011). Thus PSA, considered to be highly tissue specific have been recommended to be an ideal tumour marker for breast malignancies. Prominent rise of TPSA and FPSA in breast tumour cases as well as the significant fall in both parameters after surgical removal of tumour tissue establishes breast tissue to be the source of PSA. The significant positive association between free and total PSA with serum testosterone strengthens the regulatory role of testosterone on the production of PSA, sharing the common HRE (hormone response element) in DNA with progestins and glucocorticoids (Magklara *et al*., 2000).

The association of PSA with the disease process of breast tumour may be explained by its versatile biological role such as PSA acts as mitogen to breast tissue through of TGF-β (transforming growth factor-β), a known mitogen of breast tissue (Killian *et al*., 1993). PSA degrades fibronectin and laminin, the cellular matrix proteins, thereby facilitating local invasion (Webber *et al*., 1995). It increases IGF-1, a proven mitogen of breast tissue (Cohen

et al., 1994). It degrades IGFBP-3, which normally induces apoptosis in breast tissue and its degradation by PSA stimulates tumour progression (Oh *et al*., 1995). However, the connection between PSA expression and the pathophysiology of breast cancer remains to be established. Many authors have observed that PSA possesses anti-angiogenic properties and breast cancer cases having higher levels of PSA shows better prognosis (Fortier *et al*., 1999).

The results of serum PSA in Post-surgery patients group showed a significant decrease in comparison with that of pre-surgery patients group. This means that surgical removal of breast cancer resulted in a marked and significant decline of pre-surgical value of serum PSA. This result is in agreement with the findings of several authors (Blacl *et al*. (2000); Hautmann *et al*. (2000); Dash *et al*. (2011) and Fawzi *et al*. (2013) indicating breast tissue to be the source of PSA in females, as it is one of the major hormone_responsive organs in the female body (Dash *et al*., 2011).

Fawzi *et al*. (2013) reported that the reduction in TPSA after surgery is not sharp as that of FPSA. (The percentage reduction in the median of TPSA was 34.6% as compared to 82.4% in the median of FPSA.) This is an indication that the major component of total PSA is PSA_ACT which is not produced by the tumor cells but more likely by normal breast tissue (Melegos *et al*., 1996). The sharp decrease in the level of FPSA after surgery may strongly indicate that this fraction (i.e. FPSA) is produced by breast tumors (Blacl *et al*., 2000). It is likely that the tumor is producing FPSA which is incapable of binding to serine protease inhibitors such as ACT (Melegos and Diamandis, 1996). The breast tumor may produce an endopeptidase which causes a post-translationar modification (internal cleavage) of PSA produced by the breast, thus preventing complex formation with ACT and increasing the proportion of FPSA (Dash *et al*., 2011). In other words, PSA could occur in the free form either because it is in a tissue compartment in which it is not exposed to protease inhibitors, thus retaining its proteolytic activity, or because the PSA is nicked and thus enzymicaity inactive and unable to interact with the protease inhibiiors (Parish, 1998).

Margot *et al*. (2011) observed that an increase in total PSA levels in the serum of all patients in comparison to the control group. They speculate that the slight increase in total PSA in the serum of women with breast cancer, benign breast disease, or uterine fibroids is the result of a disrupted hormonal balance in these women, triggering the aberrant expression of hormone-dependent genes such as PSA. The observation that total PSA is only slightly decreased in breast cancer patients after surgery is an indication that a major component of total PSA (in

this case PSA-ACT) is not produced by the tumor cells but more likely by normal breast tissue. Alternatively, free PSA decreases more dramatically after surgery, strongly indicating that this fraction is produced by breast tumors. It has been reported that the majority of breast tumor PSA occurs in the free form (Diamandis *et al*., 1994 and Giai *et al*., 1995), whereas free PSA and PSA-ACT appear to exist in approximately equal proportions in breast cyst fluid (Diamandis *et al*., 1994). It has been established that prostate cancer patients have a greater fraction of PSA complexed to ACT, whereas they report that a significant proportion of breast cancer patients have a higher percentage of free PSA. The mechanism behind this apparent discrepancy is unknown at present, mainly because the differential generation of PSA molecular forms in benign or malignant prostatic or mammary tissue is not yet clear. However, it must be kept in mind that although breast and prostate cells both produce PSA, the two tissues differ in a multitude of ways. For example, prostatic and mammary tissue (healthy, hyperplastic, or malignant) respond differently to hormonal proliferation and differentiation signals (*e.g.*, estrogen *versus* androgen effects) (Lilja *et al*., 1991).

Our study demonstrates a significant difference in the PSA levels between pre-surgery patients with breast cancer and control group. However, it does not agree with the findings of Jungchan *et al*. (2015) who reported that there was no significant difference in serum PSA level between pre-surgical breast cancer patients and control groups.

Our findings are not in accordance with other studies which did not demonstrate any significant differences in the PSA levels between patients with benign breast disease and breast cancer patients. They have proven superior discriminatory role of tissue PSA level or PSA concentration in nipple aspirate fluid for diagnostic and prognostic purposes (Yu *et al*., 1998 and Black *et al*., 2000). Based on these observations it seems unlikely that the PSA levels in the serum of breast cancer patients are significantly different from the PSA levels in the serum of normal women (Nikhil *et al*., 2014).

Concerning diabetic patients, serum PSA level was non-significantly changed compared to non-diabetic patients in all groups. Results from epidemiologic studies on the relationship between diabetes and the level of PSA are often confusing. Some studies suggest a lower level of PSA among diabetics. This relationship was investigated in the health professionals follow-Up study in the united states. The basis of this relationship is unclear, however it may reflect hormonal changes associated with diabetes, may be low testosterone level (Livija *et al*., 2010).

Estrogens appear to hold the key to understanding of breast cancer (Parkin *et al*., 2001). Estradiol, the most potent endogenous estrogen, is the important secretory product of the ovary which represents the principal source of estrogen for breast cancer in premenopausal women. After the menopause, most of the circulating estradiol is derived from estrone that produced by the peripheral conversion of androstenedione, the precursor of testosterone (Ray *et al*., 2000).

Estrogen is an important steroid hormone involved in regulating the differentiation and proliferation of normal breast epithelial cells. In the present study, the all patients group exhibited marked elevation of serum estradiol compared to the control group. The present data support the findings of Essam (2000); Ju-Yeon et al. (2013) and Zsuzsanna *et al*. (2013) who indicated that breast cancer is considered as the outcome of a complex interplay amongst genetic, hormonal, and environmental factors. In addition to modulating growth factor genes, estrogen may increase the production of proteases such as pro-cathepsin D which can enhance the invasiveness of tumour cells (Ray *et al*., 2000).. Polyamines have also been found to play a role in estrogen-regulated breast cancer cell growth. The bcl-2 is a key protein involved in the control of apoptosis, and studies have indicated estrogenic regulation of bcl-2 (Shah *et al*., 2001). Steroid hormone receptors are also considered to play a potential oncogenic role due to recognition that steroid hormone receptors have structural homology with the avian erythroblastosis viral oncogene v-erb-A which has been shown to transform avian erythrocytes (Ray *et al*., 2000). Recent research, studying the molecular mechanism of transcriptional regulation of target genes by steroid receptors has revealed a very complex network of protein-protein interactions in addition to protein-DNA interactions necessary for proper function of steroid hormones. Disruption in this intricately regulated mechanism can disturb steroid receptor signaling. Specifically, mutations in the ER as also altered receptor expression have been found in breast cancer, and are associated with cancer progression and hormonal resistance (Hopp and Fuqua, 2002).

The obtained results of this study demonstrated that there was a significant decrease in serum estradiol level between postmenopausal and premenopausal in control group and also in patient group. Concerning premenopausal patient group serum estradiol level was significantly increased compared to premenopausal control group. The statistical analysis of serum estradiol level in postmenopausal patient group showed a significant increase in comparison with that in postmenopausal control group.

It has been believed that breast cancer has a hormonal origin. In particular, because of its profound stimulatory influence on breast ductal epithelium, it was thought that estradiol must play a central role (Essam, 2000).

Several studies have evaluated serum E2 concentration in breast cancer patients and normal controls. These studies have suggested that postmenopausal breast cancer patients have higher endogenous E2 levels than normal controls (Wu *et al*., 1999). From a hormonal point of view, it has been suggested that some benign breast diseases and breast cancer may share common epidemiological factors or even represent different stages of one process. Because benign breast diseases occur earlier in life than breast cancer, it may be rewarding to focus on the influence of hormonal status. There was a significant reduction in serum estradiol level in postmenopausal breast cancer patients than premenopausal cases. These findings are greatly supported by those reported by Wu *et al*. (1999) who obtained a 23% reduction in serum E2 levels among postmenopausal breast cancer women. Also serum E2 levels were highly significantly increased in both pre- and postmenopausal women with breast cancer than their corresponding women with benign breast diseases, which in accordance with the results of Berrino *et al.*, (1996). Yue *et al.*, (1998) reported that E2 stimulates the growth of breast tumor cells in both pre- and postmenopausal women. Following the menopause, the levels of E2 breast tumor tissues are similar to those from tumors obtained prior to cessation of ovarian function, even though plasma E2 levels are 10–50 fold lower in postmenopausal than in premenopausal women (Essam, 2000).

As evident from the present data, diabetic female patients had lower estradiol level than non-diabetic female patients. Sullivan and Maric say that women with diabetes tend to have too little estrogen and/or too much testosterone (a low E:T ratio). This lack of estrogen is associated with increased kidney disease and worse outcomes. They also point out that the diabetic women reach menopause earlier than non-diabetics, suggesting lower baseline E levels (Sullivan and Maric, 2009).

Lianne, (2017) evaluated on average, women with type 1 diabetes starts their menstruation later than women who do not have diabetes. They are more likely to experience menstrual complications before age 30. Type 1 diabetes women tend also tend to have longer menstrual cycle, and longer and heavier menstruations than women who do not have type 1 diabetes. This means that they also have a higher tendency to experience alter menarche, fewer pregnancies, more stillbirths, and earlier menopause than women without diabetes.

Testosterone exhibits important physiological effects in women, being both a precursor hormone for ovarian and extragonadal estrogen biosynthesis and acting directly via androgen receptors(AR) throughout the body (Simpson *et al*., 2000). The present investigation demonstrated that significant increase in serum testosterone level in comparison between control group and all patients group.

The present results are consistent with those of other prospective studies of the relation between sex-steroid hormone levels and the risk for breast cancer in women (Jane *et al.*, 1999 and Joanne *et al*., 2012). Sources of testosterone in postmenopausal women include direct secretion from the ovary and from the precursor hormones, androstenedione or dehydroepiandrosterone sulfate. Testosterone could influence the risk for breast cancer directly or indirectly (as a source of estradiol). Androgen receptors have been identified in human breast cancer cells, although in vitro activation of the androgen receptor tends to suppress the proliferation of breast cancer cells. In three studies, the association between levels of total testosterone and breast cancer was not independent of levels of bioavailable estradiol (Hankinson *et al.*, 1998).

Studies of many authors generally show that after menopause women with higher levels of estrogen and testosterone in their blood have a risk of breast cancer that is double that of women with the lowest levels (Fourkala *et al*., 2012; Schernhammer *et al*., 2013 and Zhang *et al.*, 2013).

When evaluating testosterone's impact on breast cancer, it is unclear if testosterone is a singular causative agent or if breast cancer is a result of other hormonal stimulation such as estrogens or synthetic progestins. Several clinical studies have attempted to answer this question with results suggesting that there is more to the equation than testosterone. A study that followed 508 postmenopausal women receiving testosterone in addition to usual hormone therapy, evaluated the role of testosterone in hormone replacement therapy. Clinical studies have provided conflicting results when looking for a clear correlation between testosterone blood levels and breast cancer in postmenopausal women (Adly *et al.*, 2006). Dimitrakakis *et al.* (2004). reported that there aren't any unbiased trials of sufficient size and duration to evaluate the effect of testosterone in breast cancer. A review of published studies did not find an adverse effect from estrogen/testosterone therapy when evaluating testosterone's effect on breast cancer. In addition, one study concluded that testosterone may decrease the risk of breast cancer when conventional hormone therapy (i.e. estrogen and

progesterone) includes testosterone. Another study looked at androgen receptor antagonist in primates and concluded that endogenous androgens (such as testosterone) inhibit mammary proliferation, thus potentially decreasing its impact on breast cancer (Janna *et al.,* 2007).

Prakruti *et al*. (2011) evaluated a significant positive correlation of serum testosterone with both serum total PSA and serum free PSA, explaing the stimulatory effect of androgens on production of PSA (Narita *et al.,* 2006). Steroid receptor cell lines like BT 474, T-47D and MCF-7 when stimulated by androgens, progestins and glucocorticoids, produced PSA in the culture medium. This provides evidence that androgens, progestins and glucocorticoids share the same HRE in DNA and that HRE is probably associated with the PSA gene and upregulates its expression (Magklara *et al.,* 2000).

CONCLUSIONS

The prominent increase in serum levels of PSA in breast cancer patients before surgery, as well as the significant fall in its level after surgery, may establish the serum PSA to be used as a tumor marker for diagnosis of breast cancer and may be a useful marker for monitoring the response to treatment. Results of this study support the hypothesized association of serum estradiol and testosterone to breast cancer risk, which needs to be explored in additional studies.

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