Studies on risk Factors of Breast Cancer as to be controller in Saudi Women

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Abstract: Breast cancer is uncontrolled growth of breast tissues, the most common type of cancer and the second leading cause of death following lung cancer in females. However there is a high risk of breast cancer and dysregulation of some biological processes and there is a high risk of breast cancer in relation to some controlled factor such as number of pregnancies, oral contraceptive, hormone replacement therapy HRT, fertilizer, any surgeries, suffering from depression, obesity and age. In the current work, twenty seven breast cancer females as well as thirty two control subjects, were assessed their ages ranged between 30 – 70 years. The investigated parameters were carried out on physical examination morphological biopsy and laboratory findings. According to the clinical investigation on the patients, there was a high risk of breast cancer to some controlled factor such as hormone profile, number of pregnancies, oral contraceptive, hormone replacement therapy, fertilizers, any surgeries and age. A positive result was obtained with women who received HRT and fertility drug who had more breast cancer risk than those who did not receive the treatment. Superoxide dismutase enzyme which protects the body against free radicals was also investigated.
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Key words: breast cancer, superoxide dismutase, hormone replacement therapy, oralcontraceptive.

1. Introduction

Breast cancer is uncontrolled growth of breast tissues and is the second cause of death in females in the world. Cancer is a potential invasion to break through normal breast tissues barriers and metastasize to other parts of the body [1]. Information needed for indicating how far cancer has metastasized within the breast tissues is obtained from the results of lymph node biopsy, blood test, bone scan and X-rays. There are four stages of breast cancer. These are Stages I, II, III and IV to determine the extent or severity of the disease [2-7]. Risk factors for breast cancer, which cannot be changed are depression, gender, age, genetic factors, family history, race, early menstruation, late menopause and radiation [6, 8-15].

Other risk factors that can be controlled are Hormone Replacement Therapy (HRT), Oral Contraceptive, Alcohol Consumption, Obesity, Breast Feeding and pregnancy [16-22]. Association between certain psychological factors and breast cancer such as depression, bereavement, emotional loss and emotional repression and life events all thought to alter both immune system and hormonal functioning which may lead to cancer induction or promotion [23]. Other factors caused by allergic reactions are poor diet, nutritional deficiencies, substances abuse or biochemical imbalance in the body which is considered to be the major contributing factor to stress. Biochemical imbalance in the body that weakens the immune system cause illness and creates more stress for the person. Although certain amount of stress is normal feelings in our lives, prolong bouts of stress can lead to exhaustion and minor illness along with minor serious health problems [24]. The stress associated with breast cancer diagnosis and treatment can cause dysregulation of psychological and biological processes. Women with breast cancer develop emotional stress, anxiety and depression [25]. Women with the family history of breast cancer of first degree relative (mother, sister or daughter) have an increased risk of developing breast cancer, if more than the first degree relative has breast cancer the risk is even higher or when the relative developed, breast cancer before the age of 40 in both breasts [13].

Use of HRT increased the risk of breast cancer [26,27]. Birth control pills may slightly increase the risk of breast cancer which is influenced by age, length of the use and other factors [28]. Length of oral contraceptives use before the age of twenty five years and a prolonged use before the pregnancy both were associated with remarkable increased risk for neoplastic growth in the breast [29]. Significant alcohol consumption has been associated with an increased risk of breast cancer [30]. The cells in woman's breast change during pregnancies and during breast feeding. These changes may protect against cancer. As for age, woman who had their first baby in their 20's have less risk of breast cancer than woman who gave their first birth at older age or who never had children. Several months of breast feeding may reduce the risk of breast cancer than in women who have never breast fed [31].

Stressors can also activate the sympathetic – adrenal medullary (SAM) axis as well as Hypothalamic - pituitary - adrenal (HPA) axis and therefore provoke the release of pituitary and adrenal hormones such as catecholamine's (adrenaline and nor adrenaline), adrenocorticotropic hormones (ACTH), cortisol, growth hormones and prolactin. These stress hormones can induce quantitative and qualitative changes in immune function most immune cells however have receptors for stress hormones which are associated with hypothalamus-pituitary adrenal and hypothalamus adrenal medulla HPA and SMA axis [32] and [33] respectively. Therefore an association between breast cancer incidence and certain psychosocial factors such as depression, emotional loss and repression and life events are through to alter both immune system and hormonal functioning, which may conduct cancer induction or promotion [25].

Changes in DNA can sometimes cause normal cells to transform to cancer cells by deactivating the tumour suppressor genes. Most DNA mutations that cause breast cancer are not inherited, but occur during a woman's life and maybe caused by different factors such as exposure to radiations, diet and smoking [10]. Breast cancer oncogene such as BRCA1 and BRCA2 at the normal cases they are tumour suppressor genes which help repair damaged DNA (a process that also prevent tumour development). Mutations in BRCA1 and BRCA2 have higher risk of developing both breast and ovarian cancer than in women who do not have this genetic mutation. Currently BRCA1 mutation account for about 5% of all breast cancer cases [11].

Acquired mutations during a person's life time present in certain cells and are called somatic mutation, they are not inherited. Somatic mutation however occurs when DNA replicates itself during cell division. DNA replication errors result in multiple copies of a gene on the chromosome known as gene amplification. Gene amplification however result in tumour formations. Amplification of Her-2/neu oncogene causes cell division and formation of cancerous cells. Over expression of Her-2/nue oncogene is found in about 25% of breast cancer [12]. Many other gene associated with breast cancer include P53, Ataxia telongiectasia (AT), growth arrest DNA damage GADD and Retinoblastoma tumour RB [34].

The aim of this study is to confirm a relationship between breast cancer and some controlled factors dysregulation and also to confirm that there is a high risk of breast cancer in relation to number of pregnancies, oral contraceptive, hormone replacement therapy HRT, fertilizers, any surgeries, depression and age.

2. Materials and Methods

Subjects:

Two groups, the control group (n=32) and patients group (n=27) were studied in the current work. The patients were voluntarily participating in this work. Their ages ranged between 30 – 70 years. Subjects were fully clinically examined. Number of pregnancies, contraceptive, hormone replacement therapy HRT, fertilizer, any surgeries, depression and age. Blood samples of the patients were collected from outpatient clinic of National Guard Hospital and King Abdulaziz University Hospital, Jeddah. The experimental work was conducted at the biochemistry lab of King Abdulaziz University Hospital, Jeddah, under sterile conditions.

The blood samples were collected by venepuncture. Centrifugation was carried out at room temperature for 10 – 15 minutes at 1200 – 1600 x g. Serum was then separated and carefully transferred to plastic tubes and stored at 2-8^{o}C for 24 hours prior to assay. Specimens held for longer time were aliquoted and frozen once at -20^{o}C.

Materials

- Elecsys (Electrochemiluminescence immuno assay) kits were used for hormonal assay (Prolactine, Cortisol, Progesterone and Estradiol).
- ELISA (Enzume Linked Immunosorbet Assay) kits were used for androstendione hormone assay and for superoxide dismutase enzyme assay.

Elecsye kits were used for determination of Prolactin hormone [35,36], Cortisol, Progesterone and Estradiol [37,38].

The kit is an electrochemiluminescent immunoassay, which is based on a competitive test used two monoclonal antibodies specifically directed against human hormone. The chemiluminescent reaction that occurs leads to emission of light from the mouse monoclonal ruthenium complex. The light is measured by photomultiplier and was related to the amount of hormone present in the specimen.

Results were determined via calibration curve. The curve was generated by 2 point calibration and a master curve provided via the reagent barcode, all were plotted by Elecsys instrumentation. The analyser automatically calculates the analysed concentration of each sample.

Determination of Androstenedione hormone human Androstenedione kit was used in an enzyme – linked immuno–sorbent assay (ELISA) based on the competitive principle and the microtiter plate separation. The concentration of antigen in blood serum was found to be inversely proportional to its optical density measured by ELISA detector. The plate was read on a microwell plate reader at 450 nm [39,40].

Superoxide Dismutase (SOD) determination ELISA detection based on the competition between

the protein of the enzyme and the antibody used against it, using the microliter plate separation. The absorbance at 450 nm was read using a plate reader [41,42,43].

Statistical Analysis

Mean value, SD, P-value were used to test the existence of a relation between control group and the female patients.

3. Result

The present study was carried out on 32 female control subjects and 27 female breast cancer patients. Blood samples were taken and serum was prepared for determination of androstenedione, cortisol, estradiol, prolactin, progesterone hormones and SOD enzyme. The investigated parameters were carried out on physical examination, morphological, biopsy and laboratory finding.

All patients were at treatment and at the duration occurrence of the disease. The general descriptions of the clinical parameters of the control subjects and for the patients are represented in Table 1.

1- General description of the subjects

Hormonal investigation

a. Control subjects

Thirty two women were studied for stress hormones such as prolactin and cortisol the mean ± SD was found to be 329.11± 133.48 ng/ml and 292.22± 153.11 ng/ml for the two hormones respectively. Other female hormones such as estradiol were found to be 131.180 ± 259.04 ng/ml and progesterone was found to be 13.13 ± 20.93 ng/ml and androstenedione was found to be 0.48 ± 0.22 ng/ml. As for SOD enzyme was found to be 15.53 ± 10.47 ng/ml. (See Table 1).

b. Breast cancer patients

Twenty seven women were studied for stress hormones such as prolactin and cortisol the mean ± SD was found to be 417.34 ± 368.00 48 ng/ml and 359.63 ± 230.76 ng/ml for these hormones respectively. Other female hormones such as estradiol were found to be 547.66 ± 813.06 ng/ml, progesterone was found to be 1.95 ± 5.00 ng/ml and androstenedione was found to be 0.34 ± 0.14 ng/ml. As for SOD enzyme was found to be 13.52 ± 10.00 ng/ml.(Table 1).

Table 1. Comparison between control subjects and breast cancer patients. The means, standard deviation, and P-value of studied parameters for control subjects (n=32) and breast cancer patients (n=27)

Parameters	Normal			Patients			*P*-value	Significance
	Mean	±	SD	Mean	±	SD		
Age	32.13	±	9.06	47.81	±	9.22	0.000	**H. Sig**
Weight	65.50	±	8.47	63.15	±	10.64	0.368	NS
Height	158.11	±	4.97	156.00	±	6.27	0.176	NS
No. of Pregnancies	1.47	±	1.87	2.96	±	2.34	0.009	**H. Sig**
Androstenedion	0.48	±	0.22	0.34	±	0.14	0.005	**H. Sig**
SOD	15.53	±	10.47	13.52	±	10.00	0.479	NS
Estrogen	131.80	±	259.04	547.66	±	813.06	0.014	**Sig**
Progesterone	13.13	±	20.93	1.95	±	5.00	0.009	**H. Sig**
Prolactin	329.11	±	133.48	417.34	±	368.00	0.212	NS
Cortisol	292.22	±	135.11	359.63	±	230.76	0.169	NS

2. Comparison between patients and control subjects in the investigated parameters:

1. Number of pregnancies

When patients and controls were divided into stages according to number of the pregnancies i.e. Less than 3 pregnancies and 3 pregnancies or more it was found that less than 3 pregnancies got a chance of about 40.74% to get breast cancer and 3 pregnancies or more got about 59.26 % to get breast cancer, however there was no difference in the numbers of pregnancies. (Table 2).

Table 2. No. of Pregnancies Pills in control subjects and patients

	Less than 3		3 or more	
No. of pregnancies	**F**	**%**	**F**	**%**
Patient (n=27)	11	**40.74**	16	**59.26**
Control (n=32)	9	**28.12**	23	**71.88**
Total	20	**33.80**	39	**66.20**

2. **Oral Contraceptive Pills**

The number of patients and control subjects who received oral contraceptive pills was equal. Woman who received oral contraceptive pills had a chance of about 22.22% times to get breast cancer than those who did not receive oral contraceptive pills (Table 3).

Table 3. Oral Contraceptive Pills in control subjects and patients

Oral	yes		No	
	count	%	count	%
Patient (n=27)	6	22.22	20	74.07
Control (n=32)	6	18.75	16	50
Total	12	22.03	46	77.97

3. **Hormone Replacement Therapy (HRT)**

The number of patients who received HRT were more than control subjects. Woman who received HRT had a chance of about 48.15 % to get breast cancer than those who did not receive that treatment. (Table 4)

Table 4. HRT in control subjects and patients

HRT	Yes		No	
	F	%	F	%
Patient (n = 27)	13	48.15	14	51.85
Control (n = 32)	1	3.13	25	65.63
Total	14	33.89	39	66.11

4. **Fertilizers**

The number of patients who received fertilizers was more than those who did not receive fertilizers. These female patients had about 22.22% times chance to get breast cancer more than those who did not take any fertilizer. (Table 5)

Table 5. Medication for fertilization in control subjects and patients

Medication for fertilization	yes		No	
	F	%	F	%
Patient (n=27)	6	22.22	21	77.77
Control (n=32)	0	0	32	100
Total	6	15.25	50	84.75

5. **Any surgeries**

Patients who had surgeries had a chance of about **7.41** % times to get breast cancer than those who had not any surgeries. (Table 6)

Table 6. Any Surgeries in control subjects and patients

Any Surges	yes		No	
	count	%	count	%
Patient (n = 27)	2	7.41	25	92.59
Control (n = 32)	7	21.87	25	78.13
Total	9	12.25	50	84.75

6. **Suffering Depression**

Patients who suffered from depression were more than those who did not suffer from depression and had chance of about 92.59 % times to get breast cancer than those who did not suffer from depression. (Table 7)

Table 7. Suffering from Depression in control subjects and patients

Suffering Depression	No		Yes	
	F	%	F	%
Patient (n = 27)	2	7.40	25	92.59
Control (n = 32)	5	15.63	27	84.38
Total	7	11.86	52	88.14

7. **Age**

When patients and controls were divided according to ages i.e. there were two categories less than 35 and 35 or more years. Female patients who were 35 or more years had a chance of about 96.29 % times to get breast cancer more than those who were less than 35. (Table 8)

Table 8. Age in control subjects and patients

Age	Patient		Control	
	count	%	count	%
Less than 35	1	3.70	23	71.87
35 or more	26	96.29	9	28.12
Total	27	100	32	100

4. Discussion

General investigation of the patients

The increase incidence of this disease has motivated us to study the controlled risk factors of breast cancer in the Western Province of Saudi Arabia (Jeddah). In the current work, the investigation showed that number of pregnancies has no effect on developing breast cancer, women who

had less than 3 pregnancies got a chance of about 40.74% to get breast cancer and those who had 3 pregnancies or more got about 59.26% to get breast cancer (Table 2). Women who received oral contraceptive pills had a chance of 22.22% to get breast cancer than those who did not receive any oral contraceptive (Table 3). Also in hormone replacement therapy however it is worth mentioning that in general it is not necessary for women to get breast cancer if they take oral contraceptive. Women who receive HRT had a chance of about 48.15% to get breast cancer than those who did not receive that treatment (Table 4). Prolonged HRT may lead to increased risk for the activation of ovaries in women with maternal history of breast cancer or women who had previously undergone HRT. Also in women with prior benign breast disease or those who had menopause later than the age of 55. The number of patients who received fertilizers was more that those who did not receive fertilizers. Those female patients had about 22.22% times a chance to get breast cancer (Table 5). Therefore a positive result was obtained with women who received HRT and fertility drug.

Suffering from other diseases such as having any surgeries patients who had surgeries had a chance of about 7.41% times to get breast cancer than those who had no surgeries (Table 6). Also patients who suffered from depression were more than those who did not suffer from depression who had a chance of about 92.59% to get breast cancer than those who did not suffer from depression (Table 7). Stress however is cancer puzzle, the direct cause in human and the true relation between stress and cancer remains elusive, to get conclusive results from such a mixed bag factors is difficult, stress may trigger lifestyle responses that affect the health. Responses however are hard to interpret into data that can be examined in scientific study [44].

A model of gene environment interaction reveals altered mammary gland gene expression and increase tumour growth after experienced social isolation [45]. In the other hand, stress levels may however affect breast cancer risk due to hormonal effects on the immune system. Women with high stress level are less likely to develop breast cancer, this is because prolonged every day stress may activate certain stress hormones (e.g. cortisol), chronic stress (Dysthymia) which decrease estrogen levels over the long term decrease the risk of breast cancer [32].

Women reporting 40% stress (high levels were less likely to develop breast cancer than women developing low levels of stress, therefore for every increased level of stress women were 80% less to develop breast cancer stress, however, had a significantly decreased risk of developing breast cancer in comparison with low stress levels. Daily stress however seem to stop breast cancer. The finding is in contrast with past work, which showed that stress double the risk [46]. The explanation for this finding may be due to sustained levels of high stress which effects levels of the female hormone Estrogen which by time influence the development of breast cancer.

When patients were divided according to ages i.e. there were two categories, less than 35 and 35 or more. Female patients who were 35 or more had a chance of about 96.26% times to get breast cancer more than those who were less than 35 years (Table 8).

Hormonal Investigation

In the current study, hormones have high significant values between breast cancer patients and control subjects in androstenedione, estrogen and progesterone while there were no significant difference in prolactin and cortisol hormones (Table 1).

Estrogen and Breast Cancer risk

The effect of ovarian hormones, such as estrogen, on breast cancer risk was first shown a 100 years ago when researchers found that in women with breast cancer ovariectomy improved their chances of survival. Recent studies have shown that women who had ovariectomy early in life have a very low risk to get breast cancer. Recently many researchers have investigated the possible relationship between exposure to estrogen and breast cancer risk. Women who have higher levels of estrogen circulating in their bodies developed breast cancer more than women without breast cancer. Another recent study showed that women who had been treated for breast cancer and having higher levels of estrogen in their bodies, had a recurrence of the disease sooner than women treated for breast cancer and had lower levels of estrogen [47].

Effects on other hormones that stimulate cell division

Estrogen therefore can indirectly stimulate cell division by its instruction to target cell to make receptors for other hormones which may stimulate breast cells to divide. For example with progesterone, estrogen affects the receptor levels of the female hormone progesterone. Progesterone however also acts as a chemical messenger that tells breast cells to grow [48].

SOD enzyme

High cortisol and low SOD, which is caused by excessive stress, result in a higher production of T-helper lymphocytes-type 2 (TH2), relative to type 1 helper lymphocytes. Both are important for normal immune function but imbalances, such as an excess of TH2 relative to TH1, are associated with disease

states. Breast cancer appears to be associated with an excess of TH2 lymphocytes, which is caused by high cortisol and low SOD. The high level of cortisol cause decrease of natural killer cells NK which are capable of lysing a wide variety of tumour and thought to be important in the body's defense mechanism against tumours metastasis [49].

Conclusion

High female's hormones such as estrogen should be controlled by anti-estrogen drugs such as Tamoxafin and Megace drug. Members with family history of breast cancer should be screened for some oncogenes such as BRCA1, BRCA2 and Her-2. Control SOD levels and reduce free radicals level by taking some antioxidant such as vitamin A, C and E. The researchers however concluded that a high intake of antioxidants (from supplements or fruits and vegetables) might help to prevent breast cancer [50]. Risk factors should be taken into considerations especially with women who have family history of breast cancer or with prior benign breast diseases. Relaxation and reducing the stress might reduce the risk of breast cancer. Improve overall health, changing lifestyle and enjoy it as she could. The healing effect of forest was also documented by Karjalainer [51] also other natural green setting can reduce stress, improve moods, reduce anger and aggressiveness and overall strength and happiness.

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