Dysregulation of Some Biological Processes in Saudi Breast Cancer Patients

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Abstract: In woman breast cancer is a second leading cause of death following lung cancer and the most common type of cancer. It was confirmed that there is a relationship between breast cancer and dysregulation of some biologicalprocesses, therefore there is a high risk of breast cancer and some biological processes. 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. According to the clinical investigation on the patient there was a high risk of breast cancer in relation to depression, early menarche, age at first birth, menstruation, menopause, marital status, breast feeding, suffering disease and hormone profile. Super oxide dismutase enzyme which protects the body against free radicals was also investigated.

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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 can also be caused by allergic reactions, poor diet, nutritional deficiencies, substances abuse or biochemical imbalance in the body which is considered to be the major contributing factor to stress causing biochemical imbalance in the body that deplete the immune system causing illness which 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].

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 [26] and [27] respectively.

The oxidative stress and mitochondrial DNA damage also play an important role in breast cancer. However, free radical is easily formed when covalent bond between entities is broken and one electron remains with each newly formed atom[28]. Antioxidants are molecules or compounds that act as free radical scavengers. They protect against oxidative stress and cell damage. Superoxide dismutase enzyme(SOD) is one of the antioxidants in the cell. Serum glutamyltransferase GGT, lactate dehydrogenase LDH and superoxide dismutase might be the most sensitive biomarkers in breast carcinoma in early detection of the disease, their activities in patients with breast carcinoma were tremendously increased compared to controls and their activities increased from stage I to stage IV as well as in distant metastasis [29]. However, numerous genetic abnormalities have been found to occur in breast

cancer. Those aberrations may effect both oncogenes and tumour suppressor genes.

Changes in DNA can sometimes cause normal cells to transform to cancer cells by deactivating the tumour suppressor genes. Most DNA mutations that causes 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 [30].

The aim of this study is to confirm a relationship between breast cancer and dysregulation of some biological processes and there is a high risk of breast cancer and menstruation, early menarche, menopause, marital status, age at first birth, breast feeding and suffering disease.

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. Full history data were taken with regards to marital status, number of pregnancies had breast feeding or not, early menarche, late menopause, first child at 35 years of age had received HRT fertility drug or oral contraceptive pills. 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 (Electrochemiluminescenceimmuno 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 [31-36], Cortisol, Progesterone and Estradiol [36-40].

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 [41-44].

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 [45-53].

Statistical Analysis

Chi square test was used to test the existence of a relation between control group and the female patients.

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 ± SD 259.04 ng/ml and progesterone was found to be 13.13 ± SD 20.93 ng/ml and androstenedione was found to be 0.48 ± 0.22 ng/ml. As for SOD enzyme was found to be 13.52 ± 10.47 ng/ml. (See Table 1).

b. Breast cancer patients

Twenty seven women were studied for stress hormones such as prolactin is stress hormone 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 ± SD 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 15.53 ± 10.65 ng/ml (as shown in 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.* Period time (menstruation)**

There was a highly significant difference between breast cancer patientsand control subjects in the period time (menstruation) as shown from Table 2.

Table 2: Period time in control subjects and patients

Period time	Not Defined		End of Month		Mid of Month		1st of Month	
	F	%	F	%	F	%	F	%
Patient (n = 27)	13	48.14	2	7.40	2	7.40	10	37.03
Normal (n = 32)	4	12.50	7	21.87	8	25.00	13	40.62
Total	17	28.81	7	11.86	10	16.94	23	38.98

***2.* Early menarche**

The patients had their period at the age of 12 years while control subjects had it later than that. Women who had early menarche however had a chance of having breast cancer about 66.66 % times than those who had it late. See Table 3.

Table 3. Early menarche in control subjects and patients

Early menarche	Yes		No	
	F	%	F	%
Patient (n = 27)	18	66.66	9	33.33
Control (n = 32)	15	46.87	17	52.13
Total	33	55.93	26	44.07

3. Menopause

It was found that patients had early menopause than control subjects. Women who were menopausal had about 29.62 chances to get breast cancer than those who were not menopausal. See Table4.

Table 4.Menopause in control subjects and patients

Menopause	Yes		No	
	F	%	F	%
Patient (n = 27)	8	29.62	19	70.37
Control (n = 32)	4	12.50	28	87.50
Total	12	20.33	47	82.45

4. Marital status

Most of the patients were widows and divorced and this percentage were higher in patients than in control subjects inpatients however 29.63% were widows and 22.22% were divorces as for controls 3.13% were widows and 9.37% were divorced. See Table 5.

Table 5. Marital Status in control subjects and patients

Marital Status	Married		Widow		Divorced		Unmarried	
	F	%	F	%	F	%	F	%
Patient (n = 27)	12	44.44	8	29.63	6	22.22	1	3.70
Normal (n = 32)	12	37.50	1	3.13	3	9.37	16	50.00
Total	24	40.67	9	15.25	9	15.25	17	28.81

5. First baby at age 35

The number of Patients who had their child late were found to be more than in control subjects, who had their first child at earlier age. Patients who had first child at the age of 35 had chance of about 22.22 % times to get breast cancer than those who had their first child at earlier age.See Table 6.

Table 6. First baby at age 35 in control subjects and patients

First baby at age 35	yes		No	
	F	**%**	**F**	**%**
Patient (n = 27)	6	22.22	21	77.77
Control (n = 32)	1	3.12	31	96.87
Total	7	11.86	52	88.14

6. Breast feeding

The number of patient who breast fed their babies were less than control subjects. Patients who breast fed their babies had chance of about 48.15 % times to get breast cancer than the other groupas shown in Table 7.

Table 7. Breast feeding in control subjects and patients

	Yes		No	
Breast feeding	**F**	**%**	**F**	**%**
Patient (n = 27)	13	48.15	14	51.85
Control (n = 32)	12	37.50	20	52.50
Total	25	42.37	34	57.62

7. Suffering Disease

The number of patients who had suffering diseasewere found to be more than control subjects. Patients who had suffering disease had chance of about 7.40 % times to get breast cancer than those who had not suffering disease. See Table 8.

Table 8. Suffering Disease in control subjects and patients

Suffering Disease	Yes		No	
	F	**%**	**F**	**%**
Patient (n = 27)	2	7.40	25	92.59
Control (n = 32)	1	3.13	31	96.75
Total	3	5.1	56	95

Discussion

General investigation of the patients:

According to the total number of Saudi breast cancer cases reported since 2000 onwards the increase incidence of this disease has motivated us to study dysregulation of some biological processes and breast cancer in the Western Province of Saudi Arabia (Jeddah). In the current work, investigation showed that eighteen patients out of twenty seven (66.66%) had early menarche that started at the age of 12. Early menarche could increase the risk of breast cancer and women with early menarche have lower levels of circulating sex-hormone binding globulin and higher levels of estradiol, which may be central agent in the development of breast cancer [54].

In our investigation, eight patients out of twenty seven (29.62%) had menopause at 55 or older.Relative risk of breast cancer increased with age at natural menopause. Women with natural menopause at age of 55 or older had twice the breast cancer risk experienced than those who menopause occurred before the age of 55 [55]. Although the early menopause protects the breast from long exposure to estrogen, yet patients in the current study have early menopause, which is probably due to drug, or medication they received for cancer therapy. In our current study eight patients out of twenty seven (29.63%) were widow, six patients out of twenty seven (22.22%) were divorced and one patient out of twenty seven (3.70%) was unmarried. The difference between the two cases is high and there is a strong association between marital status effects on the stress hormones such as cortisol. Variation in cortisol level can have a substantial impact on immune functions causing cancer.

In the current investigation, six patients out of twenty seven (22.22%) had their first child before the age of 35. However, there is only a weak association between increased risk of breast cancer and the age of giving birth.In our work, thirteen patients out of twenty seven (48.15%) were breast feeding babies and fourteen patients out of twenty seven (51.85%) were not breast feeding babies.

Breast feeding might influence the risk of breast cancer by several ways

May cause hormonal changes, such as a decrease in the level of estrogen which may decrease a woman's risk of developing breast cancer. It suppresses ovulation. Women who have fewer ovulatory cycles over the course of their reproductive lives may have a decreased risk of developing breast cancer. It may remove possible carcinogens that are stored in the adipose tissue of the breast. Also it caused physical changes in the cells that line the mammary ducts. These changes may cause cells to become more resistant to a mutation that causes cancer [56].

Suffering diseases showed that patients who had suffering disease had a chance of about 7.40% time to get breast cancer than those who had not suffering diseases.

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 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[57].

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 [58].

Conclusion

Risk factor for breast cancer such as depression, gender, age, genetic factor, family history, race early menarche, menstruation late menopause, marital status, age of first birth, breast feeding and suffering disease and circulating sex hormone binding globulin may be the central agents in the development of breast cancer.Highfemale's hormone 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 screen for some oncogenes such as BRCA1, BRCA2 and Her-2. Mammograms and general check up should also be taken into considerations every two years.Control

SOD levels and reduces free radicals level by taking some antioxidants such as vitamin A, C, E as well as vitamin D. the researchers however concluded that a high intake of antioxidants might help to prevent breast cancer [59]. Relaxation improve overall health, changing lifestyle might reduce risk of breast cancer.

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References

[1] **Marbella, A. M. and Layde, P. M.,** Racial trends in age specific breast cancer mortality rates in US women. *Am. J. Pub. Health*; **91** (1):118-21(2001).

[2] **Singletary, S. E.,** Revision of the American Joint Committee on Cancer Staging System for Breast Cancer. *J. Clin. Oncol*.; **20** (17):3628-3636 (2000).

[3] **Ptchefasky, A. S., Shaber, G. S., Schwartz, G. F., Fieg, S. A. and Ner-Linger, R. E.,** The pathology of breast cancer detected by mass population screening. *Cancer*; **40**:1659-1670 (1977).

[4] **Rossen, P. P., Senic, R., Schollenfeld, D. and Ashikari, R.,**Noninvasive breast cancer- Frequency of unsuspected invasive and implications for treatment. *Ann. Surg.;***189**:33-7 (1979).

[5] **Carter, D.,** Intraducatal papillary tumors of the breast- A study of 78 cases. *Cancer*; **39**:1689-1692 (1977).

[6] **Carolyn, M. K.,** Breast cancer: Strategies for living (second edition). New York, NY: *McGraw-Hill, Inc*., pp: 7-9 (2001).

[7] **Bunnell, C. A.,** Breast cancer: staging and prognosis. *Clin. Oncol.,***74**: 715-725 (2003).

[8] **Joshi, M. G., Lee, A. K. and Loda, M.,** Male breast carcinoma: An evaluation of prognostic factors contributing to a poorer outcome. *Cancer*, **77**: 490-498 (1996).

[9] **Ries, L. A. G., Eisner, M. P., Kosary, C. L., Hankey, B. F., Miller, B. A. and Clegg, L**. (Eds), SEER cancer statistics review.Bethesda, MD*: Natl. Cancer Inst*. 1975- 2000 (2003).

[10] **King, M. C.,** A novel BRCA2-bindig protein and breast and ovarian tumorigenesis. *N. Engl. J. Med.*; **350** (12):1252-3 (2004).

[11] **Foulkes, W. D., Metcalfe, K., Sun, P., Hanna, W.M., Lynch, H. T., Ghadirian, P., Tung, N., Olopade, O. I., Weber, B. L., McLennan, J., Olivotto, I. A., Begin, L. R. and Narod, S. A.,** Estrogen receptor status in BRCA1- and $BRCA_2$ - related breast cancer: the influence of age grade, and histological type. *Clin. Cancer Res*., 15; **10** (6):2029-34 (2004).

[12] **Ueda, Y., Wang, S., Dumont, N., Yi, J. Y., Koh, Y. and Arteaga, C. L.,** Overexpression of HER2 (erbB2) in human breast epithelial cells unmaska transforming growth factor beta-induced cell motility. *J. Biol. Chem.*, **279** (23): 24505-13 (2004).

[13] **Loman, N., Johannsson, O., Kristoffersson, U., Olsson, H. and Borg, A.,** Family history of breast and ovarian cancer and BRCA1 and BRCA2 mutations in a population-based series of early-onset breast cancer. *J. Natl. Cancer Inst.*, **93** (16):1215-1223 (2001).

[14] **Hotes, J. L., Mclauylin, C. C., Frith, R., Roney, D., Cormier, M., Fulton, P., Holowaty, E., Howe, H. L., Kosary, C. and Chen, V. W.,** Cancer in North American, 1996-2000. Volume one: Incidence. Volume 2: Mortalty. Sphingfield IL: North American Association of Central Cancer Registries pp, 233-256 (2003).

[15] **Goss, P.E. and Sierra, S.,** Current perspectives on radiation-induced breast cancer. *J. Clin. Oncol*.,**16** (1): 338-347 (1998).

[16] **Olsson, H.L., Ingvor, C. and BladSlorm, A.,** Hormone replacement therapy containing Progezlin and given continuously increase breast carcinoma risk in Sweden. *Cancer*, **97** (6):1387-1392. (2003).

[17] **Newcomb, P. A., Titus, E. L. and Egan, K. M.,** Postmenopausal estrogen and progesterone use in relation to breast cancer risk. C*ancer Epidemiol. Biomarker Prer*., **11** (7): 593-600 (2002).

[18] **Kumle, M., Weiderpass, E., Braaten, T., Persso, I., Adami, H. O. and Lund, E.,** Use of oral contraceptives and breast cancer risk: TheNorwegian-Swedish Women`s Lifestyle and Health Cohort study. *Cancer Epidemiol. Biomarker Prer*.,**11** (11):1375-1381 (2002).

[19] **Mansfield, C. M.,** A review of the etiology of breast cancer, *J. Natl. Med. Assoc*., **85**:217-221 (1993).

[20] **Smith Warner, S. A.,** Alcohol and breast cancer in women. JAMA, **279** (7): 535-539 (1998).

[21] **Calle, E. E., Rodriguez, C., Walker Thurmond, K. and Thun, M. J.,** Overweight, obesity and mortality from cancer in a prospectively studies cohort U. S. adult. *N. Engl. J. Med.*, **348** (17):1625-1638 (2003).

[22] **Beral, V.,** Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study, **362**: 419-27 (2003).

[23] **Gammon, M. D. and John, E. M.,** Recent etiologic hypothesis concerning breast cancer. *Epidemiol. Rev*., **15**: 163-168 (1993).

[24] **Chopra, D.,** Stress in Alternative Medicine. The burton Goldberg group, Future Medicine Publishing, INC., (1997).

[25] **Epping, J. E., Compas, B. E. and Osowiecki. D.,** Psychological adjustment in breast cancer: Processes of emotional distress. *Health Psychology*; **18**:315-326 (1999).

[26] **Rabin**, Stress, Immune function and health: *The Connection*, **3**:23-37 (1999).

[27] **Ader, R., Felten, D.L. and Cohen, N.,** PNI 3rd edn. ACad*The Connection*; **3**: 23-37 (2001).

[28] **Karlsson, J.,** Introduction to Nutraology and Radical Formation. In: Antioxidats and Exercise. Illinois. Human Kinetics Press, pp, 1143 (1997).

[29] **Seth, R. K., Kharb, S. and Kharb, D. P.,** Serum biochemical markers in carcinoma breast. *Ind. J. Med. Sci*., **57**: 350-354 (2003).
[30] **Dianne, C. D.,** Microscopy research and technique. History and Cell Biology of Breast Cancer; **59** (1): 68-83 (2002)
[31] **Tietz, N. W.,** Clinical Guide to Laboratory Test. 3rd ed. Philadelphia, pp **512**. (1995).
[32] **Frantz, A. G.,** Prolactin. *New Engl. J. Med*., **298**: 201-207 (1978).
[33] **Mülle, E. E.,** Prolactin-Lowering and –Releasing Drugs, Mechanism of Action and Therapeutic Applications. *Drugs*, **25**:399-432 (1983).
[34] **Pontiroli, A. E.,** Clinical, Endocrine, Roentgenographic and Immune Characterization of Hyperolactinemic Women. *Int. J. Fer*., **32**: 81-85(1987).
[35] **Dericks-Tan. J. S. E., Siedentopf, H. G. and Taubert, H. D**., Discordant Prolactin Values obtained with Different Immuno assays in an infertile Patient. *J. Lab. Med.*, **21** (9):465-470 (1997).
[36] **Bablok, W., A** General Regression Procedure for Method Transformation. *J. Clin. Chem. Clin. Biochem.,* **26**:783-790 (1988).
[37] **Johnson, M. R., Carter, G., Grint, C. and Lightman, S. L.,** Relationship between ovarian steroids, gonadotrophins and relaxin during the menstrual cycle. *Acta. Endocrinol*.,**129**: 121-125 (1993).
[38] **Guillaume, J., Benjamin, F., Sicuranza, B., Wang, C. F., Garcia, A. and Friberg, J.,** Maternal serum levels of estradiol, progesterone and h-Choriongonadotropin in ectopic pregnancy and their correlation with endometrial histologic findings. *Surg. Gynecol. Obstet*.; **165**: 9-12(1987).
[39] **Passing, H. and Bablok, W.,** A New Biometrical Procedure for Testing the Equality of Measurements from Two Different Analytical Methods. *J. Clin. Chem. Clin. Biochem.,***21**: 709-720 (1983).
[40] **Passing, H. and Bablok, W.,** Comparison of Several Regression Procedures for Method Comparison Studies and Determination of Sample Sizes. *J. Clin. Chem. Clin. Biochem.;***22**: 431-445 (1984).
[41] **Berel, E. and Bermudez, J. A**., Androstenedione ELISA Kit. *J. Steroid Biochem.,***13**: 89 (1980).
[42] **Hummer, L.,** Androstendione ELISA kit. *Scand. J. Clin. Lab. Invest*.; **43**: 301 (1983).
[43] **Swinkles, L. M.,**Androstenedione ELISA Kit. *Am. Clin. Biochem*.;**23**: 354 (1988).
[44] **Check, J. H.,** Falsely elevated steroidal assay levels related to heterophile antibodies against various animal species. *Gynecol. Obstet. Invest*.; **40**: 139-140 (1995).
[45] **Malstrom, B., Andreason, L. and Reinhammer, B.,**The Enzymes. Boyer, P., editor. XIIB, Academic Press, New York, **533** (1957).
[46] **Sandstrom, J., Nilsson, P. and Karlsson, K.,** 10-Fold increase in human plasma extracellular superoxide dismutase content caused by a mutation n heparn-binding domain. *J. Biol. Chem*.; **269** (29): 19163-19166 (1994).
[47] **Marklud, S.,** Distribution of CuZn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. *Acta. Physiol. Scand. Suppl*.; **492**: 19-23(1980).
[48] **Sunet, E., Xu, H. and Liu, Q.,** The mechanism for the effect of selsnium supplementation on immunity. *Biol. Trace Elem. Res*; **48** (3): 231-238 (1995).
[49] **Maier, C. M. and Chan, P. H.,** Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. *The Neuroscientist*; **8** (4) 323-334(2002).
[50] **Liu, D.,** The roles of free radicals in amyotrophic lateral sclerosis. *J. Mol. Neuro*., **7**: 159-167 (1996).
[51] **Beckman, J. S. and Koppenol, W. H**., Nitric oxide, superoxide and peroxynitrite: The good, the bad and the ugly. *Am. J. Physiol*., **271**: C1424-C1437(1996).
[52] **Mattiazz, M., D`Aurelio, M. and Gajewski, C. D.,** Mutated human SOD1 causes dysfunction of oxidatie phosphorylation in mitochondria of transgenic mice. *J. Biol. Chem.*, **277** (33) 29626-29633 (2002).
[53] **MacMillan-Crow, L. A., Crow, J. P., ad Kerby, J. D**., Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc. Natl. Acad. Sci*. USA; **93**: 11853-11858 (1996).
[54] **Bunnell, C. A.,** Breast cancer staging nd Prognosis ClinOncol:, **74**: 715-725 (2003)
[55] **Trichopaulos D., Mac Mahon, B and Cole, P.,..**Menopause and breast cancer ris**...**J Natl Cancer Inst; **48** (3): 605 – 13 (1972)
[56] **Jernstorm, H., Lubinski, J., Lynch, H. T,Ghadirian, P, Neuhausen, S., Isaacs C., Weber, B. L., Horsman, D., Rosen, B.,Foulkes, W. D. and Friedman, E,.** Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers**. J.** *Natl Cancer Inst***. 21, 96** (14): 1094-8 (2004)
[57] **Cauley, J. A., Lucas, F. L. and Kuller, L. H**., Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. *Ann. Intern. Med.,***130**: 270-277 (1999).
[58] **Apter, D. and Vihko, R.,** Early menarche a risk factor of breast cancer.indicates early onset of ovulary system. J. Clin Endocrinal Metab., **57** (1): 82- 6 (1983)
[59] **Kaczmarski, M. J., Wojicicki, L., Samochowiee, T. and Dutkiewicz. J.,** The influence of exogenous antioxidants and physical exercise on some parameters associated with production and removal of free radicals. *Pharmazie*; **54**: 303-306 (1999).

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