The Relationship between Antioxidants Glutathione, Glutathione - S-Transeferase as Tumor Markers in Breast Cancer Patients

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Abstract

Glutathione (GSH), Glutathione- S- transferase (GST) in patients with proved breast cancer have been estimated to find the possibility of using such parameters as a biomarker in the diagnosis of breast cancer patients compared to control.

Sera of (100) breast cancer patients has been taken to estimate the levels of GSH and GST. All studied patients samples were female with mean age (51.16) years old

The result of the study revealed that serum GSH concentration and GST activity decrease in breast cancer patients; however the relation between GSH concentration and GST activity is reversible. Stage II is the most common stage with breast cancer patients where there were 71 cases of stage II. The most common of age is the range age from 40-49 years old. So this study recommends the use of these bio marker for early detecting of the disease and bringing special equipment to measure it.

الخلاصه

تمت دراسة التغيرات في الكلوتاثيون المختزل وانزيم الكلوتاثيون – اس – ترانسفريز في مصل دم مرضى سرطان الثدي والبالغ عددهم 100 مريض وتم اخذ العينات من المرضى في مستشفى مرجان التخصصي خلال الفترة (الاول من كانون الاول عام 2006 لغاية الاول من كانون الاول عام 2007) .اظهرت النتائج بانخفاض معنوي لقيم الكلوتاثيون وانزيمه المشار اليه في مرضى سرطان الثدي علما بان العلاقة بين تركيز الكلوتاثيون وفعاليه الانزيم هي علاقه عكسيه وسجلت الدراسة وجود اكبر نسبه من سرطان الثدي من الدرجة الثانية وبحدود71 حاله .

كما بينت الدراسة إن أكثر نسبة للإصابة بمرض سرطان الثدي يقع ضمن المدى العمري من 40–49 سنة ويليه من 50–59 سنة . وعليه توصي هذه الدراسة باستخدام هذه الدالات البايوكيميائية للاستدلال المبكر على وجود المرض وتوفير الأجهزة الخاصة لقياسها .

Introduction

The breast is a collection of glands of fatty tissues. Breast tissue runs from about collarbone down to the lowest rib, and from the breast bone to the area under the arm[1]. The breast is made up of several types of tissue mostly fat within this fatty tissue the milk glands spread out in a wagon-wheel pattern[2]. The breast also contains connective tissue called tascia which cover the milk glands and sport them. There are blood vessels, which supply the breast with nutrients and oxygen and nerves which give it sensation[3]. Lymphatics of the breast drain predominantly into the axillary and internal mammary lymph nodes. The axillary nodes receive approximately 75% of the drainage and are arranged in the following groups [4]:

- lateral, a long the axillary vein;

- anterior, a long the lateral thoracic vessels;
- central, embedded in fat the centre of the axilla ;
- posterior, along the subscapular vessels ;
- inter pectoral, few nodes lying between the pecctoralis major and minor muscles;
- a pical .

Breast cancer remains a common and frequently fatal disease, the most commonly diagnosed cancer in women and second ranking cause of cancer death in Eastern Mediate area region, North America and Europe. More than 1.2 million women are diagnosed with breast cancer annually worldwide [5,6].

Breast cancer usually occurs as a mass that persists throughout the menstrual cycle. A nipple discharge occurs in 10 % and pain only 7%. Less common presentations include inflammatory carcinoma with diffuse indurations of the skin of the breast which confers an adverse prognosis. Increasingly women present as a consequence mammography screening.

Around 4% will have auxiliary nodal disease, the likelihood rising with the size of the primary tumor. The involvement of auxiliary nodes by tumor is the strongest prognostic predictor. Diston metastases are frequently present at the presentation of the commonest sites of spread are : bone 70% , lung 60% , liver 55%, pleura 40%, adrenal, 35 %, skin 30% and brain 10-20 % .

Paget's disease of nipple accounts for 1% of all cases and present with arelatively of aczemamateans change in the nipple with itching, burning, oozing or bleeding. There may be a palpable underlying lump. The nipple contains malignant cell singularly or nusts. The prognosis is related to the underlying tumor [6].

Tumor markers can be defined as biologic substances synthesized and released by cancer cells or substances produced by the host in response to cancerous tissue. Tumor Markers can be present in the circulation body cavity fluids, cell membranes, serum and cytoplasm or nucleus of the cells.

Application of tumor markers: [7]

- Detections: screening in symptomatic persons
- Diagnosis: Differentiating malignant from benign conditions.
- Monitoring: predicting effect of therapy and detecting recurrent cancer.
- Classification: choosing therapy and predicting tumor behavior (prognosis).
- Staging: Defining extend of disease.
- Localization: Nuclear scanning of injected radio.
- Active anti antibodies.
- Therapy: cytotoxic agents directed maker contain cells

Reduced Glutathione (GSH) or commonly Glutathione is a tripeptide composed of the amino acids glutamic acid , cystein and glycin (glutmyl cycteinyl glycine) present in most cells of the body, bile, epithelial-lining fluid of the lungs and much smaller concentrations in blood[8]. Glutathione is involved in detoxification and binds to toxins such as heavy metals, solvents and pesticides. Glutathione transforms them to the form can be excreted in urine or bile. Glutathione is also an important anti-oxidant. The dietary glutathione taken from fruits raw vegetable has been associated with protection against some forms of cancer[9].

The factors and conditions which are known to cause decrease in intracellular glutathione concentrations can be divided into three groups[10].

The first group is made up of those that

1) Lower the rate of GSH synthesis or rate of reduction of GSSG to GSH.

2) Raise of export of GSH from cells.
3) Lead to loss of GSH from the scavenging pathway.

The second group is comprised of toxins that conjugate GSH and remove it from the body [11], such as organ phosphate pesticides, halogenated, furniture oil an acetaminophen and some types of inhalation anesthesia.

The third group is comprised of conditions that raise the production rates of reactive oxygen species high enough to produce oxidative stress, causing cells to export GSSG..

Exogenous Causes of GSH Depletion

Cigarette smoke contains thousands of different chemical species , and a single puff of cigarette smoke contains trillions of free radicals. [12] Cigarette smoke literally burns the antioxidant vitamins C and E, as well as other nutrient. The cigarette tars are long lived free radical generators and potent carcinogens.

Many pharmaceutical products are oxidant capable of depleting GSH from the liver, kidneys, heart, and other tissues. The popular over the counter drug acetaminophen is a potent oxidant. It depletes GSH from the cell of the liver , and by so doing renders the liver more vulnerable to toxic damage. The halogenated hydrocarbons (halocarbons) are portent oxidant. Halocarbons are ubiquitous, being used in the plastic industry, as industrial and dry cleaning solvent , as pesticides and herbicides, and as refrigerant. The chlorofluocarbons that currently threaten the ozone layer are one type of halocarbon[11].

Strenuous aerobic exercise can deplete antioxidants from the skeletal muscles, and sometimes from the other organs. Exercise increases the bodies, oxidative burden by calling on the tissues to generate more energy. Making more ATP requires using more oxygen, and this in turn results in greater production of oxygen free radicals[12].

Studies on GSH status with advancing age have been few, but to date there does appear to be a correlation between age- associated GSH depletion and poor health.

Glutathione-S-transferaseuses (GST) are ubiquitous multi-functional enzymes. GSTs are thought to play a physiological role in initiating the detoxification of potential alkylating agents, including pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with the SH group of glutathione, there by neutralizing their electrophilic sites and rendering the products more water –soluble.[13]

The variants of GST and breast cancer

Scientists have shed new light on the genetic bases of breast cancer risk with the finding that variation in xenobitic metabolizing enzymes and the known breast cancer genes BRCA1 and BRCA2 have synergistic effects. Polymorphisms in the enzymes GSTI1, GSTM1 and GSTPI were linked to breast cancer risk, but only if all three were mutated. The researchers then looked at how breast cancer risk was influenced by different combinations of mutations when all three GST enzymes were mutated that increased the risk of breast cancer [14]

Materials and Methods

Patients and control group

One hundred patients with diagnostic of breast cancer were subjected to the present study as well as one hundred age matched apparently healthy females as control group. The patients were visitors to Marjan Teaching Hospital in Hilla city. Blood samples withdrawn from control and patients (5 ml vein) were allowed to clot for 15-minutes and serum obtained for

analysis after centrifugation for ten minutes at 3000 xg.

Chemical Materials

All common laboratory chemicals were obtained from the Firms, Fluka, Hopkins and Williams, Sigma Chemicals, Merck and used as supplied without further purification.

Methods

Determination of serum reduce glutathione

All analytical methods such as, photometric enzymatic flourometric, and HPLC methods used to determine tissue homogenate , erythrocytes , and serum glutathione (GSH) depend on the action of the sulfhydryl groups.[15]

Enzymatic assay of glutathione-S-transferase

The determination was done according to the method described by Habig W.H. et al[16].

Results and Discussion

Serum GSH

The mean of reduced glutathione in blood serum had shown a decrease in its patient with breast cancer in comparison to those of the control group.

Table 1 The mean of glutathione of breast cancer with healthy control group

Group	No. of samples	Mean mg/dl	S D mg/dl	significance
Patients	100	0.333064	±0.2857	P<0.01,
Control	100	2.3451	±0.755122	p<0.05

According to t-test of two sample means, there was a significance difference between the mean of GSH in serum blood of patients and mean of GSH in serum blood of controls which $p < 0.01$, $p < 0.05$,That difference can be related to continuous consume pH on of GSH pool that found in serum blood in those patients with cancer in order to compete the oxidation stress occurring in the tumor cell ,Also GSH is required to carry out an immune response since it's needed by the lymphocytes to multiply in order to develop a strong immune response for killing cancer cell. [17]

GSH directly reduces the radicals that are critical to anti tumor activity. On the other hand GST catalyzes the reaction between GSH and either hydrophobic or electrophilic compounds that consume more GSH.[8]

GSH plays an important role maintaining normal balance between oxidation and anti oxidation, in cancer that balance being shifted towards oxidation side because the GSH as an intercellular antioxidant consumed by the cells trying to regulate the cells vital functions such as the synthesis and repair DNA, synthesis of proteins , the activation and regulation of enzymes[17].

GST activity in serum blood:

Glutathione-S-transfers enzyme had shown an increase in patients with breast cancer in comparison with control.

Table 2 The mean, of GST in contrast patients with control.

Group	No. of samples	Mean GST U/L	S D U/L	significance
Patients	100	2.4058	±0.2315	P<0.01, p<0.05
Control	100	0.3174	±0.2765	

According to t-test, there was significant difference between the mean activity of serum blood of patients and mean GST in serum blood of controls $p < 0.01$, $p < 0.05$.

GST catalyzes the formation of the either conjugation between glutathione and xenobiotics which the bodies of patients contain many free radicals and medicals substances such as chemotherapy compounds, all that cause increasing GST activity[18]. That means the conversion to GSH concentration is high and enzyme activity is small.

Table 3 The correlation between GSH and GST

	Mean	SD	R. correlation
(GSH) mg/dl	0.333064	±0.2857	
GST active U/L	2.4058	±0.23159	-0.732

The correlation is significant at the level 0.05 and 0.01 (2 – tailed), thus the relation between GSH concentration and GST activity is reversible, which means a decrease in GSH concentration is related to an increase in GST activity. $R < 0.05$, $R<0.001$. [19]

Contraceptive drugs effect on GSH and GST level in serum blood:

Table 4 The ratio of patients, were taken contraceptive drugs.

Group	Percentage
Patients were not taken contraceptive drugs.	41%
Patients were taken contraceptive drugs.	59%

The previous table shows that the percentage of patients with breast cancer who have taken contraceptive drug is more than the patients who have not taken contraceptive drugs.

Table 5 The mean of GSH concentration in both patients who were taken contraceptive drugs and were not taken contraceptive drug.

Group	Mean of GSH mg /dl	SD mg /dl	Significance
Control	2.3451	±0.755122	
41%	0.5421	±0.2758	p>0.01, p>0.05
59%	0.16636	±0.159	P<0.01, p<0.05

According to t-test, there was no significant difference between those patients who have not taken contraceptive drugs and healthy control which P. Value more than 0.05 and 0.01.

The comparison between patients were taken contraceptive drugs and healthy control shows there were significance difference between them which $P < 0.01$, $P < 0.05$.

Table 6 The mean GST activity in both who have taken and who have not taken contraceptive drugs.

Group	Mean of GST activity	SD U/L	significance
Control	0.3174	±0.23159	
41%	2.277	±0.8679	$P > 0.01$, $P < 0.05$
59%	2.4916	±0.8803	$P < 0.05$, $P < 0.01$

According to t-test, there was no significant different between patients who did not take contraceptive drugs and healthy at level 0.01 which is $P > 0.01$. At level 0.05 there was a significant difference which is $P < 0.05$ between patients who have taken contraceptive drugs and healthy control.

There was a significant difference between patients who have taken contraceptive drugs and healthy control which $P < 0.05$, $P < 0.01$.Also, there was significant difference between patients were taken contraceptive drugs and healthy controls, which $P < 0.05$, $P < 0.01$.

A recent study suggests a link between high dose combined oral contraceptive that were discontinued in most countries years ago and increase risk of breast cancer among women with a strong family history of the disease. High- dose pills have not been available in most countries for more than a decade. If these are still available, women should use two – dose pills instead, especially if they have family history of breast cancer.[7]

Our study revealed that 63% breast cancer patients were obese while only 37% of them were non obese or with normal body weight; there were many research which suggested that the increase in breast cancer risk faced by obese postmenopausal women may largely be due to higher levels of estrogens circulating in their bodies. The study showed that the average concentration of estrogens in obese women was between 50% and 219% higher than in thin women , and the risk of breast cancer increased by 18% with each increase in the body mass index (a measurement of weight and height used to indicate obesity). [12]

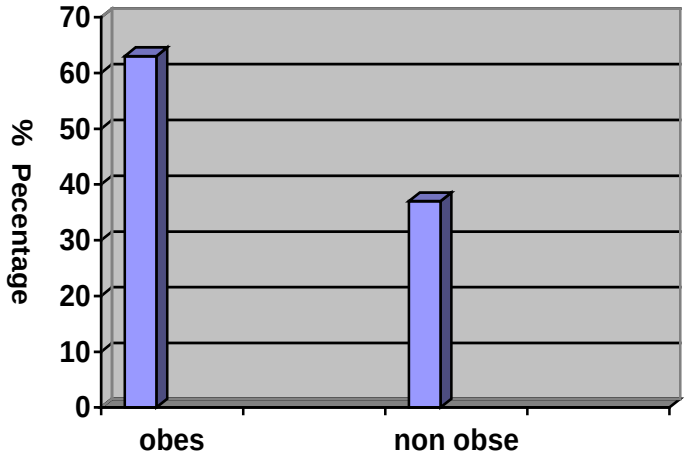

Fig 1 The percentage of obese and non obese patients.

Residence area effect in breast cancer patients

The rural patients residents formed 48% of total breast cancer patients which is nearly the same for the urban area which formed 52% of the total patients.

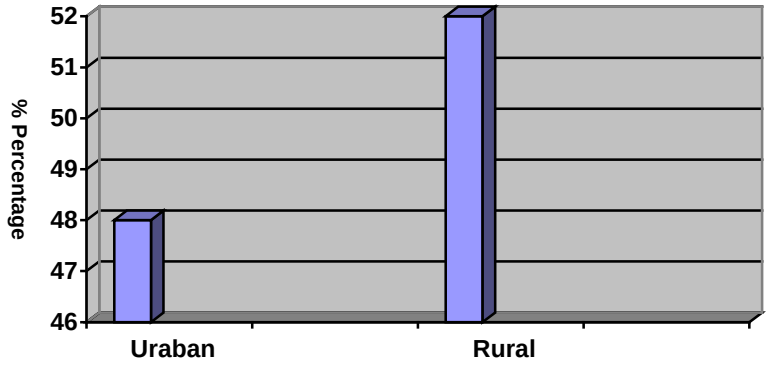

Figure 2 The percentage of urban and rural patients.

Urban areas suffer from air pollution from exhaust of the oil refineries and car engines. In the rural there is much more chance for the availability of pure air by the presence of trees, palms and other different types of plants. On contrary to these good conditions and natural facilities to face the cancerous agents a problem arises here which is the use organophosphate agents (insecticide and pesticides) to protect their farms.

From harmful insects or pests, but the side effects of these agents are very harmful which induces cancer, especially due to the wrong way of applying these agents to the plants by making solution by their hands on the plant .

These results revealed that the incidence of breast cancer are more increased in rural area than in urban area, taking into consideration that about 59% of the patients sample in our study are farmers or living at the country places expecting low living standard associated with low social facilities and increased rates of pollution of all kinds. This reflects badly on the antioxidants level rising

the possibility of cancer affection. Not to mention the harmful effect was on the environment and people who lived in Iraq, since most of these areas being by U.S. and U.K. planes and cruise that hit Iraqi targets with more than 970 radioactive bombs and missile using uranium depleted (UD 238). Thus consequently the incident rate of leukemia and other cancer in Iraq have grown by more than 600%, the white death- leukemia 0, the desert dust carries death; studies indicate that more than 40% of the population around Basra will get cancer; truly we are living through another Hiroshima. Unfortunately most of the leukemia and cancer victims are not soldiers. They are civilians.

Exposure to chemical effect on the patients with breast cancer

Breast cancer patients with positive exposure to chemicals (tobacco, aromatic hydrocarbon, organophsphate agents and uranium depleted UD238) were 52% while those with negative exposure to chemicals were 48% as in the table 7.

Table 7 The percentage of exposure to chemicals in patients with breast cancer.

Group	Percentage
Patients (+ ve Exposure)	52%
Patients (- ve Exposure)	48%

Conclusions

1- The breast cancer affected the GSH levels by decreasing its level while GST increased proportionally.
2- Increasing the level of GSH in control serum in contrast with patients of breast cancer, while there was proportionally increasing in GSH level in patients did not take contraception drugs in contrast with patients who took contraception drugs.
3- Family history affected the levels of breast cancer.
4- Obesity affected the levels of breast cancer.
5-Stage and age were affected on the breast cancer patients.

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