RELEATIONSHIP BETWEEN ANTIOXIDANTS GLUTATHIONE, GLUTATHIONE - S-TRANSEFERASE AND ALPHA-L- FUCOSE SUGAR AS TUMOR MARKERS IN BREAST CANCER PATIENTS

A Thesis
Submitted to the College of Medicine, University of Babylon, in Partial Fulfillment of the Requirements for the Degree of Master in Clinical Biochemistry

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بسم الله الرحمن الرحيم

قالوا سُبحانكَ لا عِلمَ لنا الاّ ما علّمتنا انكَ أنتَ العليمُ الحكيمُ

صدق الله العظيم
سورة البقرة : الآية (32)

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Dedication
To…..
My Mother …….. Father
and
Memory of My Late Brother Ali

To …….
All the Members of My Family

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Abstract

Glutathione (GSH), Glutathione- S- transferase (GST) and total alpha-L- Fucose (TF) 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 , GST and TF at the period (1/12/2006 to 1/12/2007). All studied patients samples were female with mean age (51.16) years old , 56% had family history and 44% had no family history, 52% from rural areas and 48% from urban areas , 63% were obese and 37% were non obese and 52% were exposed to chemicals and 48% not exposed to them .

The result of the study revealed that serum The result of the study revealed that serum GSH concentration decreases in breast cancer patients, while GST activity and TF concentration increases in the same patients. The relation between GSH concentration and GST activity is inverse relation .The relation between TF concentration and GST activity is inverse relation too, while there is no relation between GSH and TF concentration.

Stage II is the most common stage with breast cancer patients in which there were 71 cases of stage II.

The most common of age is the range age from 40-49 years old. So this study recommend to used these bio marker to early detecting of the disease and bringing special equipment to measure it .

Abbreviation

Abbreviation	Details
GSH	Reduced glutathione
GST	Glutathione-S- transferase
TF	Total Fucose
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
HPLC	High performance liquid chromatography
DTNB	5,5 dithiobis (2-nitro benzoic acid)
TCA	Tri chloroacetic acid
$EDTANa_2$	Ethylene diamine tetra acetic acid- di sodium
CDNB	1-chloro 2,4-dinitro benzene
DNA	Deoxy ribonucleic acid
TNM	Tumor, Nodes , Metastasis
ADP	Adenosine di nucleotide
ATP	Adenosine tri nucleotide
GDP	Guanosine di nucleotide
DW	Distilled water
Conc.	Concentration

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Introduction

1.1 The Breast

The breast is a collection of glands 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 size and shape of breasts vary widely from person to person of each breast is the nipple and the darker-colored ring surrounding the nipple is the areola (2).

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 (3).The breast also contains connective tissue called fascia which cover the milk glands and speared them. There are blood vessels, which supply the breast with nutrients and oxygen and nerves which give it sensation. (4).

Under beneath each breast is of two large flat mostly the pectorals muscles, which help to move the arms and under these muscles, ribs to the myocardium (5). A UK national clinical trail is current underway to try to as certain if there is a survival advantage with radiotherapy and to identify which patients are at highest risk of local relaps and thus would benefit most from postoperative breast irradiation. Currently those thought to be at highest risk include those with extensive carcinoma in situ (or of course invasive cancer) at the margins of excision patients under 35 years, and those with multifocad disease

The axillary tail of breast is of considerable surgical importance. In some normal cases it is palpable, and in a few it can be seen premenstrual and during lactation.

A well developed axillary tail is sometimes mistaken for a mass of enlarged lymph nodes or lipoma.

The lobule is the basic structural unit of the mammary gland. The number and size of lobules varies enormously they are most numerous in young women. From 10 to over 100 lobules empty via ductules into lactiferous duct is lined by a spiral arrangement of contractile myoepithelial cells and is provided with aterminal ampulla (lactiferous sinus) – reservoir for milk or abnormal discharages.

Ligaments of cooper are hallow conical projections of fibourous tissue filled with breast tissue, the apices of the cones being attached firmly to the superfacial fascia and thereby to the skin overlying the breast. These ligaments account for the dimpling of the skin overlying a carcinoma, or other lesions of the breast accompanied by coetaneous edema.

The areoal contains involuntary muscle arranged in concentric ring as well as radially in the subcutaneous tissue. The areoral epithelium contains numerous sweat glands and sebaceous glands, the latter of which enlarge during pregnancy and serve to lubricate the nipple during lactation.

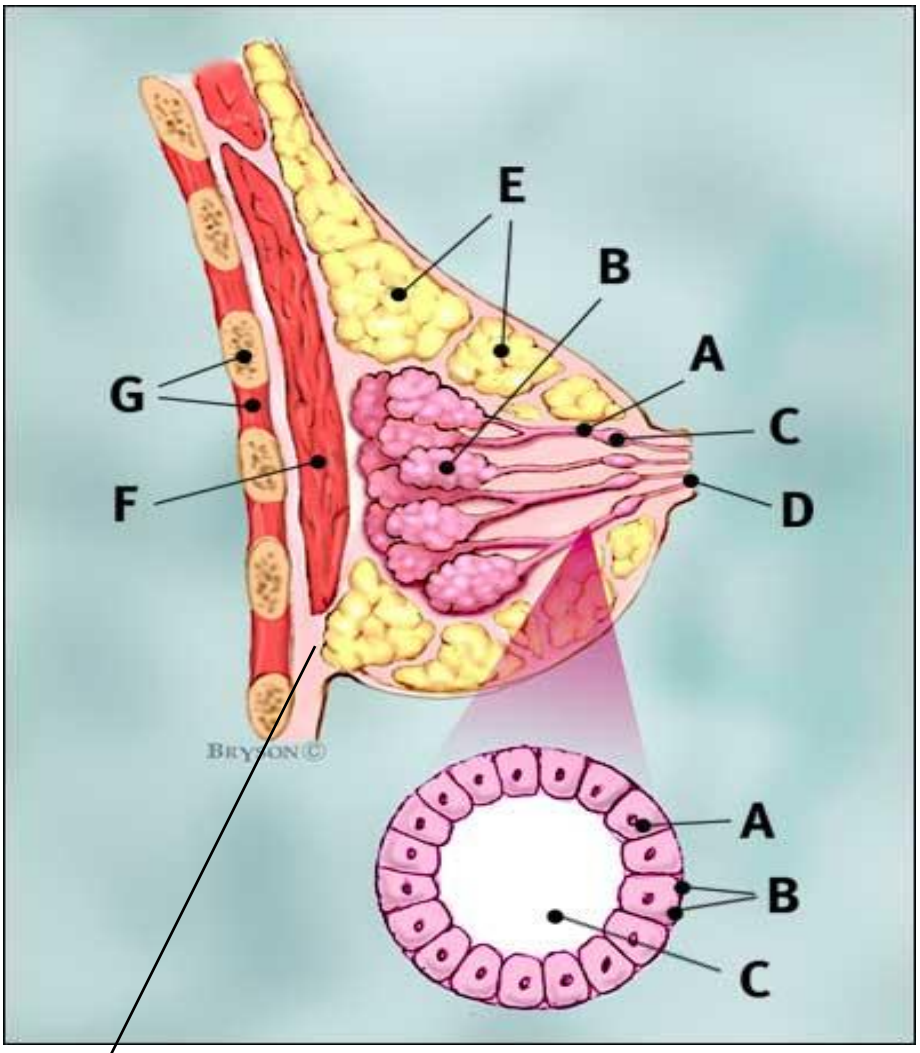
The nipple is covered by thick skin corrugations near its apex lie the orifices of the lactiferous ducts. The nipples contain smooth muscle fibers arranged concentrically and longitudinally; thus it is an erectile structure which points outwards.

Lymphatic 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 (6).

- 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 .

1.2 Breast Anatomy



Breast profile:

Enlargement:

A- Ducts **B-** Lobules

A- Normal duct cells

B- Basement membrane

C- Dilated section of duct to hold milk **D-** Nipple

C- Lumen (center of duct)

E- Fat **F-** Pectoralis major muscle

G- Chest wall/rib cage

Fig (1) shows the breast anatomy. (7)

1.3 Breast Cancer

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 world wide (8, 9).

1.3.1 Clinical Presentation :

Breast cancer usually present 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 presentation 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 nests. The prognosis is related to the underlying tumor . (9)

1.3.2 Staging and Grading of Breast Tumor :

There are two basic methods for classifying cancers

1. Grading according to the histological or cellular characteristics of the tumor, which involves the microscopic examination of cancer cell to determine their level of differentiation and the number of mitosis, it is classified as grades I, II, III. IV divides by the Colombia clinical Classification CCC. (10)

2. Staging according to the clinical spread of disease and related to the progress of disease.

Stage I: tumor <2 cm, no nodes

Stage II: tumor 2-5 cm and moveable axillary nodes

Stage III: chest wall or skin fixation and for fixed axillary nodes.

Stage IV: Metastases. (11, 12)

The T N M (Tumor, Nodes, Metastases) System (13) .

1.4 Treatment of breast cancer:

The treatment of breast cancer will largely depend upon clinical stage of the disease at presentation, including not only classic TNM staging but also other tumor characteristics such as tumor grade.

Treatment of early breast cancer will usually include surgery with or without radiotherapy. System therapy such as chemotherapy or hormone therapy is added if there are adverse prognostic factors such as lymph node invasion, indicating a high likelihood of metastasis relapse. At the other end of the spectrum, locally advanced or metastasis disease is usually treated by

systematic therapy to palliate symptoms, with surgery playing a much smaller role. Local control is achieved through surgery and or radiotherapy (6).

1.4.1 Surgery:

Surgery still has a central role to play in the management of breast cancer but there has been a gradual shift towards more conservative techniques, backed up by clinical trials which have shown equal efficacy between mastectomy and excision followed by radiotherapy this followed a change in the model of breast cancer spread , which no longer thought of a centrifugal anatomical spread but rather that it's the presence of haumatogenous metastasis's which predetermines the outcome of the disease . It was initially hoped that avoiding mastectomy would help alleviate the considerable psychological morbidity associated the with breast cancer, but in many studies have shown that over 30% of women develop significant anxiety and depression following both radical and conservative surgery. After mastectomy they tend to worry a bout the effect of operation on their appearance and relationships, where as after conservative surgery they may remain fearful of recurrence. (6)

1.4.2 Radiotherapy

Radiotherapy to chest wall after mastectomy has been largely abandoned except in cases of extensive local disease with infiltration of the chest wall. It is conventional to combine conservative surgery with radiotherapy to the remaining breast tissue. However there is currently doubt as to whether all

patients undergoing conservative surgery should receive radiotherapy, which is not without morebidity (and even long- term mortality) from inadvertent irradiation (6).

1.4.3 Chemotherapy:

Chemotherapy typically involves the use of several antineoplastic (anti cancer) drugs to treat cancer, though some people are treated with single medications. While the drugs in this family are toxic to cancer cells, many are also toxic to healthy cells, which give rise to numerous side effects. A few drugs used in chemotherapy enhance immune function, while some alter hormonal activity, one anticancer drug, Methotrexate, is also used to treat severe cases of rheumatoid arthritis (14).

1.4.3.1 Classification of chemotherapy drugs:

1-***Alkylating agents*** **:**

- Cisplstin
- Cyclophosphamide.

2- Antineoplastic antibiotics:

- Bleomycin
- Dactinomycin (For injection)
- Mitomycin (For injection)
- Pentostation

3- Antimetabolites:

- Fluorouacil
- Methotexate
- Thioguanine (Tabloid) .

4- Hormonal agonistsl antagonists:

- Anstrozole
- Tamoxifen

5- Mitotic inhibiters:

- Etoposide
- Teniposed
- Vinblastine

6- Immuno modulators:

- Aldesleukin (Proleukin for injection)
- Levamisole (Ergamisol)

7***- Miscellaneous Antineoplastics:***

- Docetaxel (Taxotere for injection)
- Interferon alpha
- Paclitaxel

1.5 Tumor Markers

Tumor Markers can be defined as biologic substances synthesized and released by cancer cell 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. (15, 16)

However, the characteristics of an ideal tumor marker from analytical requirements point of view should follow the following requirements:

- High analytical sensitivity.
- High analytical specificity.
- Accuracy.

- Precision.
- Rapid
- Turn a round time.
- Easy to measure at a low cost (10).

1.5.1 Application of tumor markers:

- Detections: screening in a 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 extent of disease.
- Localization: Nuclear scanning of injected radio.
- Active antibodies.
- Therapy: cytotoxic agents directed maker contain cells (15, 17)

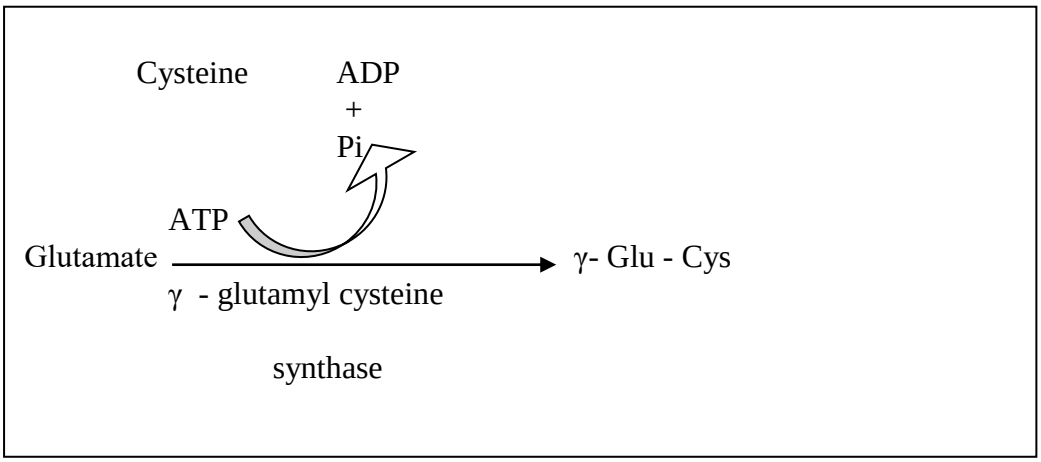
1.6 Reduced Glutathione

Reduced Glutathione (GSH) or commonly Glutathione , It is a tri- peptide 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(18).

Glutathione is involved in detoxification .It binds to toxins such as heavy metals, solvents and pesticides. Glutathione transform them to the form can be excreted in urine or bile. Glutathione is also an important anti- oxidant; dietary Glutathione take from fruits raw vegetable has been associated with protection against some forms of cancer (19 - 23).

1.6.1 Synthesis of glutathione:

The first step in the synthesis of glutathione is the formation of peptide linkage between the γ- carboxyl group of glutamate and the amino group cysteine in a reaction catalyzed by (γ-glutamyle cysteine synthase) Formation of this peptide bond requires activation of the γ-carboxyl group which is achieved by ATP (Adenosine tri phosphate) as in equation (1)



Equation (1)

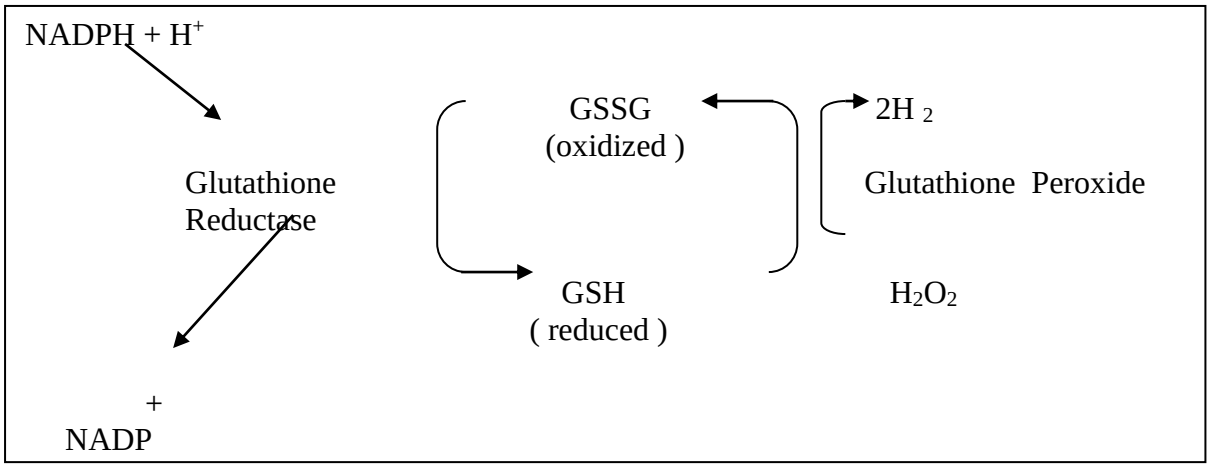
In the second step, which is catalyzed by glutathione synthase, ATP activates the carboxyl group of cystiene to enable it to condense with the amino group of glycine as in equation (2)

Equation (2)

The reduced form of glutathione serve as sulfhydryl buffer that maintains the residues of hemoglobin and other red cell proteins in the reduced state (24).

1.6.2 The function of glutathione:

GSH usually mediates the intracellular reduction of hydrogen peroxide by the intracellular NADPH (reduced Nicotinamide adenine dinucleotide phosphate) which is produced by pentose phosphate shunt a part of carbohydrate metabolism (25,26).The enzyme that catalyze the reaction is glutathione reductase. (25) as in equation (3).



Equation (3)

Glutathione maintains proper oxidation – reduction (redox) potential inside cells. Redox affects the oxidation state of sulfur in enzymes and thus affects the rates of biochemical reaction in the cells (26).

Acts as a cofactor for glutathione –S-transferase GST in detoxicative pathway (27,28)

As substrate for gamma- glutamyl trans peptidase enzymes which are located on the outer cell surface which transfer the glutamine moiety from GSH to other amino acids for subsequent uptake into the cell (29,30).

Scavenges peroxides and oxidizing free radicals directly and also serve as the basis for the antioxidant network (25, 26).

The GSH transferases are large group of isoenzymes that conjugate GSH with fat – soluble substances as the major liver detoxification.(31,32)

Enzymes Collectively known as GSH trans hydrogenases use GSH as a cofactor to reconvert dehydroascorbate to ascorbate , ribonucleotide to

deoxyribonucleotides , and for a variety of –S-S- $\rightleftharpoons$ -SH- inter- conversion (33).

Through its significant reducing power, GSH also makes major contributions of the recycling of other anti oxidants that have become oxidized. This could be basis by which GSH help to conserve lipid phase anti oxidants such as alpha – tocopherol (Vitamin E) ,and perhaps also the carotenoids (34) .

GSH. Stors and transport cystein throughout the body. It regulates the cell cycle and DNA, play role in synthesis of proteins and gene expression and proteolysis. GSH Protects thyroid cells from self_ generated hydrogen peroxide, and Participates in bile production.

1.6.3 The factors and conditions are known to cause decrease in intracellular glutathione concentrations:

These factors can be divided into three groups (35)

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) Rise of export of GSH from cells.
3) Factors lead to loss of GSH from the scavenging pathway.

This group includes the genetic defects (36), elevated adrenaline secretion (30, 37) due to various stress, deficient diet (38) or fasting (39), surgical trauma (40, 41) burns (42) and morphine (43)

- The second group is comprised of toxins that conjugate GSH and remove it from the body (44), 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. These include strenuous or extended exercise (45),infections (producing leukocyte activation)(46), toxin that produce oxidizing free radicals during phase I detoxication by cytochrome P450 enzymes(47, 49), ionizing radiation, iron overload, (50) cardiac arrest, head trauma, and hemorrhage (51) .

1.6.4 Hereditary Deficiency of glutathione

With regard to the essentiality of GSH for the survival of the whole organism, substantial information is available from studies on hereditary GSH depletion in the human, , and from experimental depletion and repletion of GSH in animal models and cell cultures . Inherited deficiency of the enzyme gamma- glutamylcysteine synthetase first of the two enzymes necessary for GSH synthesis, has been described in two human siblings. They exhibited generalized GSH deficiency, hemolytic anemia, spinocerebellar degeneration, peripheral neuropathy , and aminoaciduria, and severe neurological complications as they moved into their fourth decade of life . Their red cell GSH was less than 3% of normal , muscle GSH less than 25% , and their white cell GSH less than 50 % normal . One of them may have been hypersensitive to antibiotics, having developed psychosis after a single dose of sulfonamide for a urinary tract infection.

Deficiency in GSH synthetase, the second enzyme of GSH synthesis, also is associated with hemolytic tendency and defective central nervous system function. This condition is complicated by metabolic consequences of an excess of 5- oxoproline, formed as a "spillover" from the accumulation of gamma- glutamyl cysteine after its normal synthesis by the first enzyme and its lack of conversion to GSH by the second enzymes (52) .

Human hereditary GSH deficiency states are not necessarily lethal ,probably because some GSH obtained from diet ,with laboratory animals it is possible to precisely control GSH in the diet.

The investigators found that dietary ascorbte can protect against the tissue damage that typically results from depletion of GSH (53).

Redox phenomena are intrinsic to life processes , and GSH is a major pro-homeostatic modulator of intracellular sulfhydryl (-SH) groups on proteins (54).Many important enzymes(e.g., adenylate cyclase, glucose-6- phosphatase, pyruvate kinase, the transport Ca ATPases) , and at least eight participating in glucose metabolism, are regulatable by redox balance as largely defiend by the balance of (2 – SH = -S-S-) .

1.6.5 Exogenous Causes of GSH Depletion

Cigarette smoke contains thousand of different chemical species , and a single puff cigarette smoke contains trillions of free radicals(55).Cigarette smoke literally burns the antioxidant vitamins C and E, as well as other nutrient. The cigarette tars are longlived 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 hydrocrons (halocarbons) are portent oxidant. Halocarbons are ubiquitous, being used in the plastic industry, as industrial and dry cleaning solvent , as persticides and herbicides, and as refrigerant. The chlorofluocarbons that currently threaten the ozone layer are one type of halocarbon. (44)

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 (31, 55).

Studies on GSH status with advancing age have been few, but to date there dose appears to be a correlation between age- associated GSH depletion and poor health. Lang and collaborators compared blood GSH concentrations between the healthy young and healthy elderly subjects. The40 young subjects (20 -39) years of age) had a blood GSH level 17 % higher on average than 60 elderly Subjects (60-79 years) .

Julius (56) measured GSH in 33 person of age 60-79 years. Higher GSH concentrations were associated with good health, regardless of age; subjects with chronic disease had lower mean GSH concentrations than those free of disease.

Combination of antioxidants given as supplements seem to offer the most promise for achieving clinical breakthroughs. At times, the administration of massive amounts of ascorbate (orally or intravenously) or of sulfhydryls (GSH and NAC (N – Acetyl Cysteine orally and intravenously) will be life saving (55,56).

1.6.6 Interaction of chemotherapy with glutathione

Chemotherapy can injure cancer cells by creating oxidative damage. As a result, some oncologists recommended that patients avoid supplementing antioxidants if they are under going chemotherapy (57).

A modification from of vitamin A has been reported to work synergistically appears to animals with chemotherapy in test tube research. Vitamin C appears to increase the effectiveness of chemotherapy in animals and with human breast cancer cell in test tube research (58) .In a double- blind study, Japanese researches found that the combination of Vitamin E , Vitamin C and N-acetyl cysteine (NAC) are antioxidants protected against Chemotherapy-induced heart damage without interfering with the action of the chemotherapy (59).

Glutathione (GSH) is the main antioxidant found within cells, is frequently depleted in individuals on chemotherapy and / or radiation.

Preliminary studies have found that intravenously injected glutathione may decrease some of the adverse effects of chemotherapy and radiation, such as diarrhea (61).

1.6.7 Dietary sources of glutathione:

Daily intake of fruit and vegetables boots glutathione, some studies have found out that fruit and vegetables increase erythrocyte glutathione peroxidase activity and resistance of plasma lipoproteins to oxidation more efficiently than do the Vitamins and mineral contained in fruit and vegetables. The study investigated the relative influence of nutritive and nonnutritive factors in fruit and vegetables on glutathione peroxidase activity increased only in the group that received 600 gm fruit and vegetables a day the researchers and concluded that fruit and vegetables contain both nutritive and non nutritive factors that might contribute to redox (Antioxidant and per oxidation(62).

1.7 Glutathione – s-transferase

Glutathione-S-transferases ((GSTs)) are ubiquitous multi-functional enzymes (63) .GSTs are though 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 . Clutathione conjugate are thought to be metabolized further by cleavage of the glutamate and glycine residues ,followed by acetylation of the resultant free amino group of cysteinyl residues,to produce the final product, a mercapturic acid . The mercapturic acid,i.e S-alkylated derivatives of N- acetyl cysteine, are the excreted (64).

Glutathione- S- transferases((GSTs)) catalyze the formation of thioether conjugated between glutathione and reactive xenophobic compounds. Their major biological function is believed to be defense against electrophilic chemical species many of which are formed by cellular oxidative reactions catalyze by cytochromes P450 and other oxides (65).

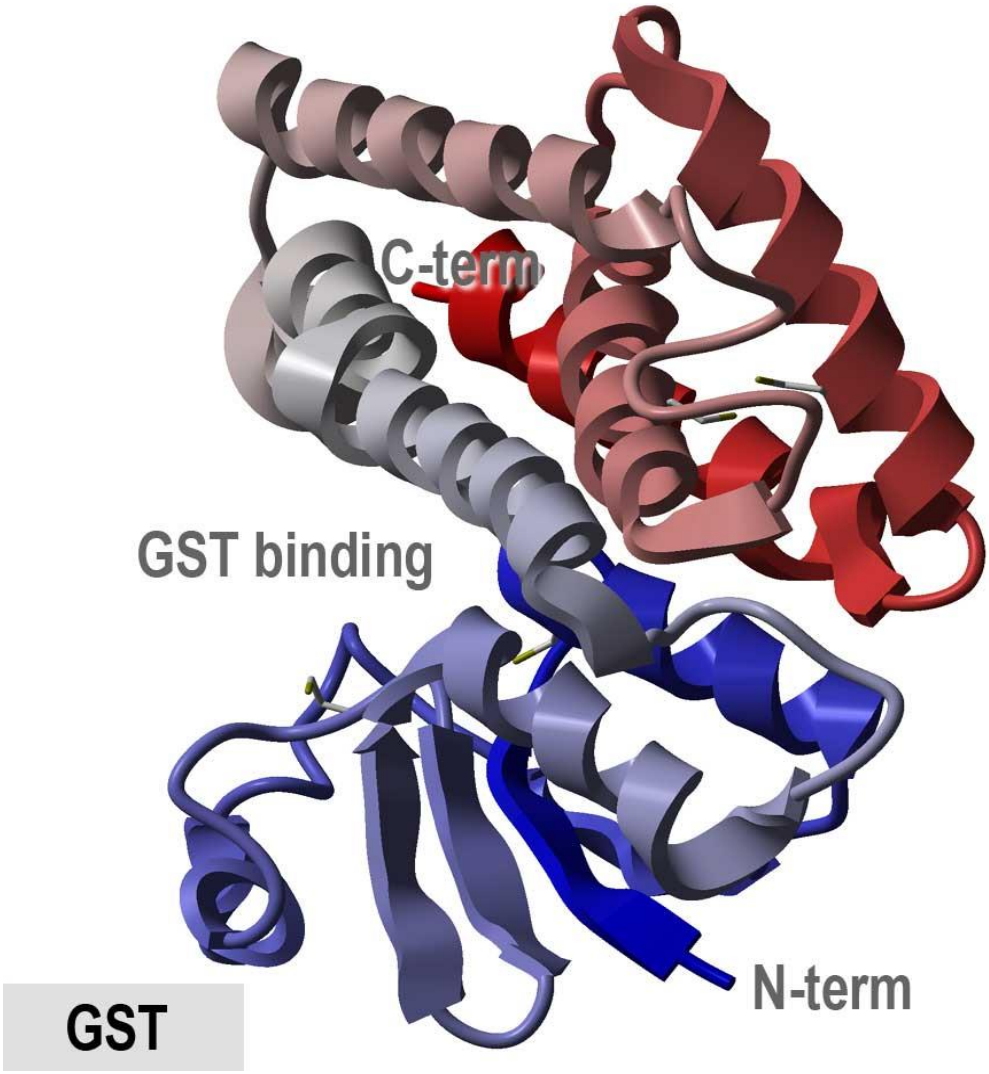


Fig (2) shows glutathione -S-transferase molecule

1.7.1 Classification of GSTs

Four structural classes of cytosolic GSTs have been identified in mammals based on primary structures: Alpha, Mu, Pi and Theta. The enzymes exist as dimmers of approximately 25 KDa subunits from a single structural class.

Recombinant human GSTs are all over produced in E-coli and purified by affinity chromatography. GST A1_1 is found in only a few tissues of the body, including kidneys ,lung and liver ;GST M1-1is also restricted to a few tissues including the liver; GST P1-1 is abundant in most types of tumor cells as well as being widely distributed through out the body .(65-68)

There is anther class of GST known as GST Zeta.

This class gene is associates with the development of sporadic breast cancer, within tested population, possible; however, that other factors such as exposure to certain carcinogens or lifestyle may influence this result (69).

Attempts have been made to classify such enzyme activities on the basis of the carbon skeleton of the electrophilic or the specific leaving group involved (2), hence the formerly common use of the terms aryl, alkyl, and epoxide transferase.

Transferases B and C were found to be homogenous by the criterion of sodium dodecyl sulfate – gel electrophoresis;a single band with a molecular weight of about 25 000 dalton was found for each enzyme . In mixing experiments,, the subunits of transferase A and transferase C migrated together and appeared to be identical by this criterion. Transferase B was also examined by sedimentation equilibrium analysis, a straight line was obtained when the logarithm of protein concentration was plotted. Sedimentation equilibrium

analysis at two concentrations of transferase B, 50 and 100 microgram per ml, yielded a molecular weight of 47000 dalton .

Comprise cytosolic enzymes, a fifth rather distinct form is microsomal all cytosolic Glutathione-S- transferases are found to be homo-or hetero- dimeric enzymes (from within the same class) with a relative molecular weight 50000 dalton .

The primary structure of the human microsomal glutathione S – transferase gene (GST12) was determined by genomic cloning The gene structure of GST12 spans 12.8 kd and consists of four exons and three introns . The coding sequence resides on exons 2,3 and 4.All introns commenced with nucleotides GTAA at the 5 {prime} boundary and ended with nucleotides AG at the 3{prime} } boundary ,in agreement with the proposed consensus sequence for intron spliced donor and acceptance sites. The presence of an in-phase stop codon and an upstream false start codon in the 5 {prime} untranslated region was confirmed.

In Italy at the general hospital of Venice, a study aiming to select a validated panel of tests to assess the GST / GSH system in a clinical setting was implicated. Matched blood and tissue samples (normal and malignant) from cancer patients (non- urological cancers) were investigated .GSH levels and GSH activity were higher in cancer tissues than in matched normal tissues in both malignancies. Moreover a strong Correlation was found between the GSH level in whole blood and GST activity in cancer tissue.

The finding regarding the GSH level in blood indicates that circulating GSH could have a clinical relevance as a surrogate marker of GST activity in tumor tissue (68, 69).

1.7.2 The variants of GST and Breast cancer

Scientists have shed new light on the genetic basses of breast cancer risk with the finding that variation in xenobitic metabolizing enzymes and the know 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, there searcher report.

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 (70).

1.8 The Fucose

The science of glycobiology is exploding as various sugars and complex carbohydrates being recognized for their importance as more than just energy sources. (71). One of those sugars, fucose is found in wide variety of natural substances from many sources and occurs in abundance in glycoproteins and glycolipds in animals and humans .Fucose – conjugating glycoproteins and glycolipds are now know to be important in cell – cell communications involve in both disease and normal functions as receptors on cells surfaces, fucose glycoconjugates become an essential part of disease processes, such as cancer inflammation, and immunity (72).

Fucose abundant in human breast milk and certain mushrooms fucose influences brain development. Animal studies using fucose to indicate that the sacchride may also help to improve the brains ability to create long term memories (73, 74).

Fucose is an immune modulator as well, inhibiting tumor growth and its spread are enhancing cellular communication, high concentrations of fucose are found at the junctions between nerves, in the kidney and tests, and outer layer of skin (75).Fucose metabolism is abnormal in cystic fibrosis, and cancer and during episode of shingles which is caused by aherps virus. Studies suggest the sugar is active against other herps viruses including herp I are cytomegalovirus .The sacchirde also guards against respitory tract infections and inhibit allergic reaction, (70-75).

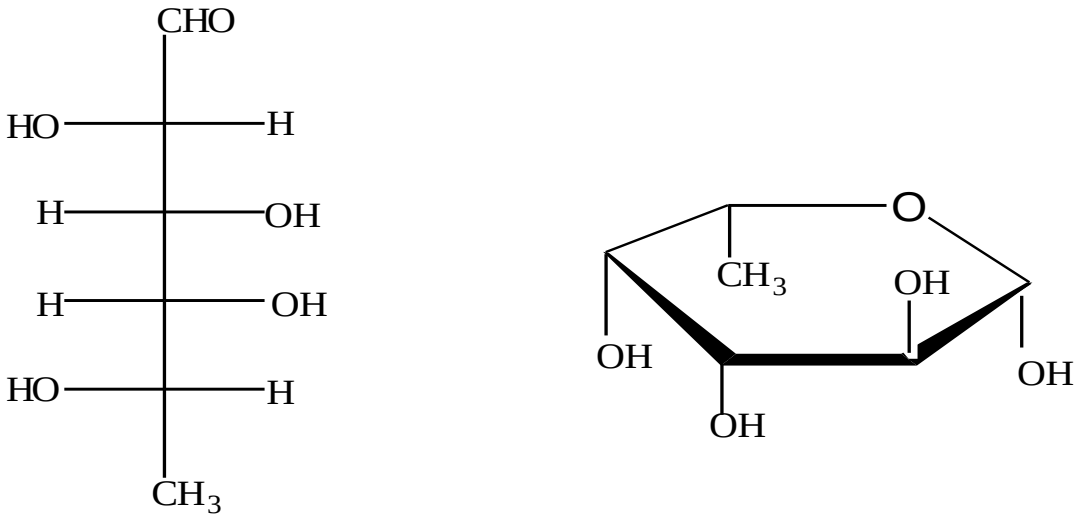


Fig (3) the structure of alpha –L- fucose

1.8.1 Metabolism of fucose

Fucose metabolism is important for formation glycoproteins and glycolipids (74, 75). Endogenous fucose is produced in the sugar- nueleotide form (GDP- Fucose) from GDP- mannose via a dehydratase and epimerase- reductase enzyme. Exogenous (i.e.dietary) fucose is converted to fucos-1-phosphate by fucokinase and then to GDP-fucose by a pyrophsphaerylase enzyme. Inhibitors of fucokinase lower fucose incorporation into glycoproteins (76).

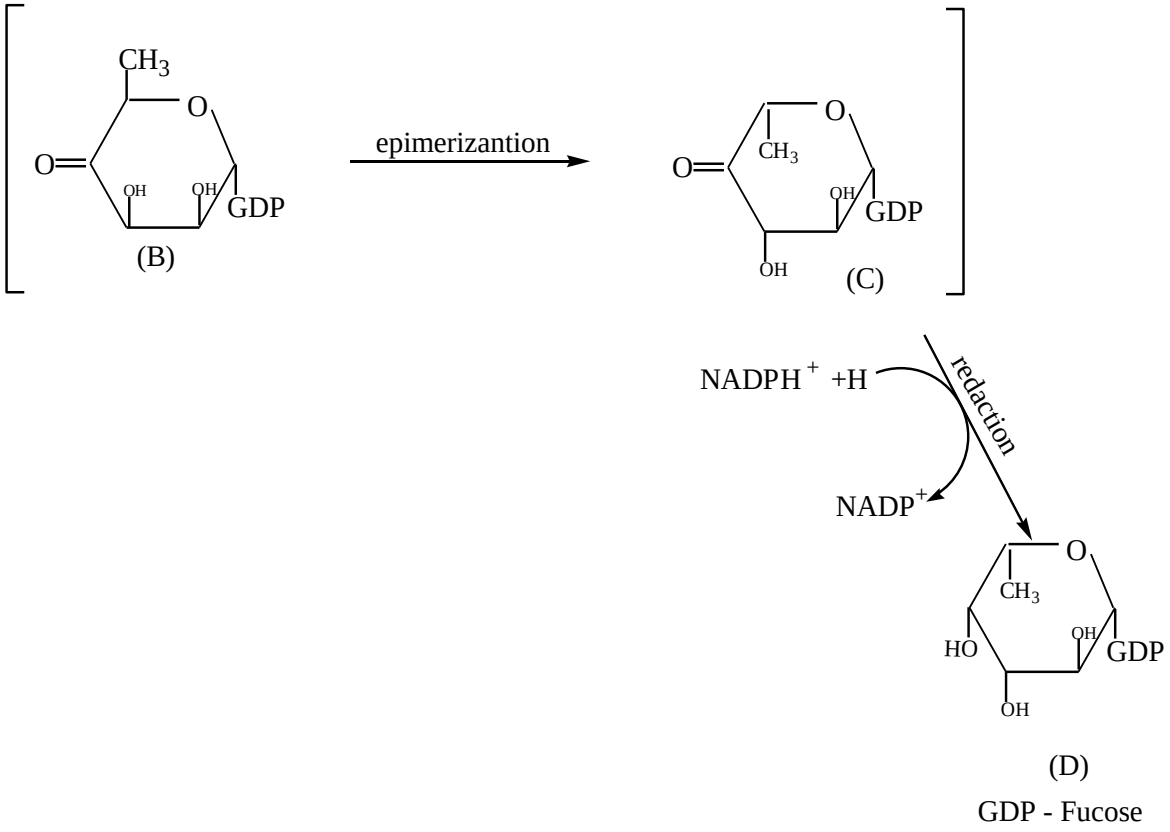


Fig (4) bio synthesis of alpha –L- fucose

Metabolism in humans, a large portion of injected (i.e. By passes intestine) fucose is oxidized, suggesting less dependence on intestinal bacteria (77). Exogenous fucose can be incorporated directly into fucose- containing proteins and other macromolecules with little or no metabolism to other sugars (78, 79).

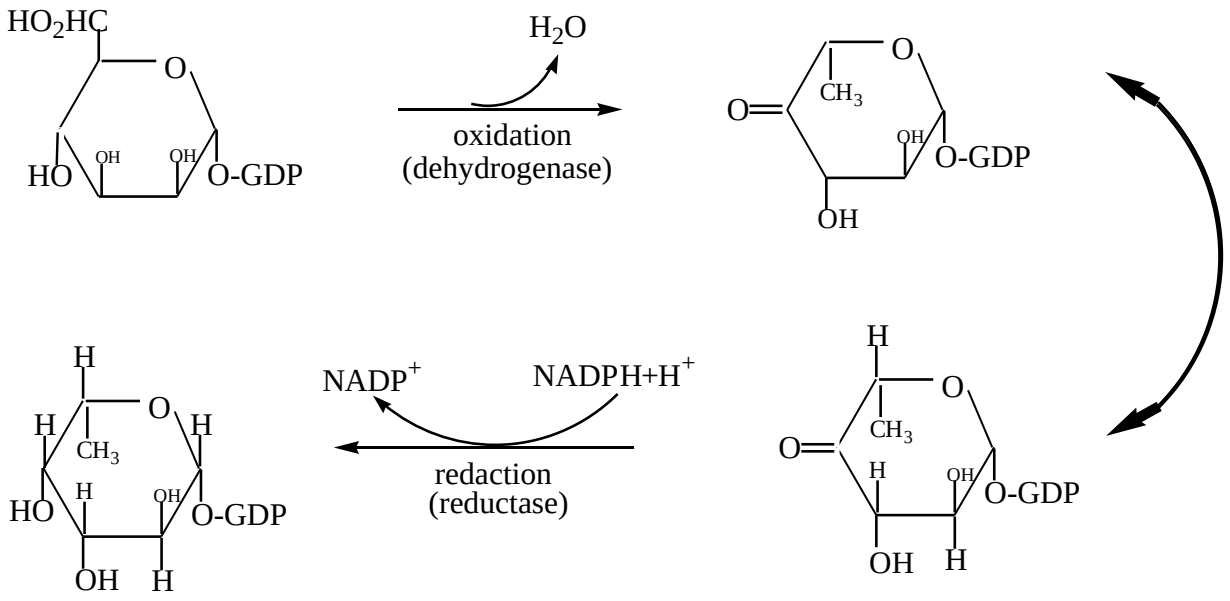


Fig (5) GDP-L- fucose (biosynthesis of endogenous alpha –L- fucose)

A deficiency in fucosidase will lead to disease termd fucosidosis due to accumulation of fucose-containing mucopoly-sacchride and glycolipids (80). Fucose metabolism also appears to be altered in various other diseases.

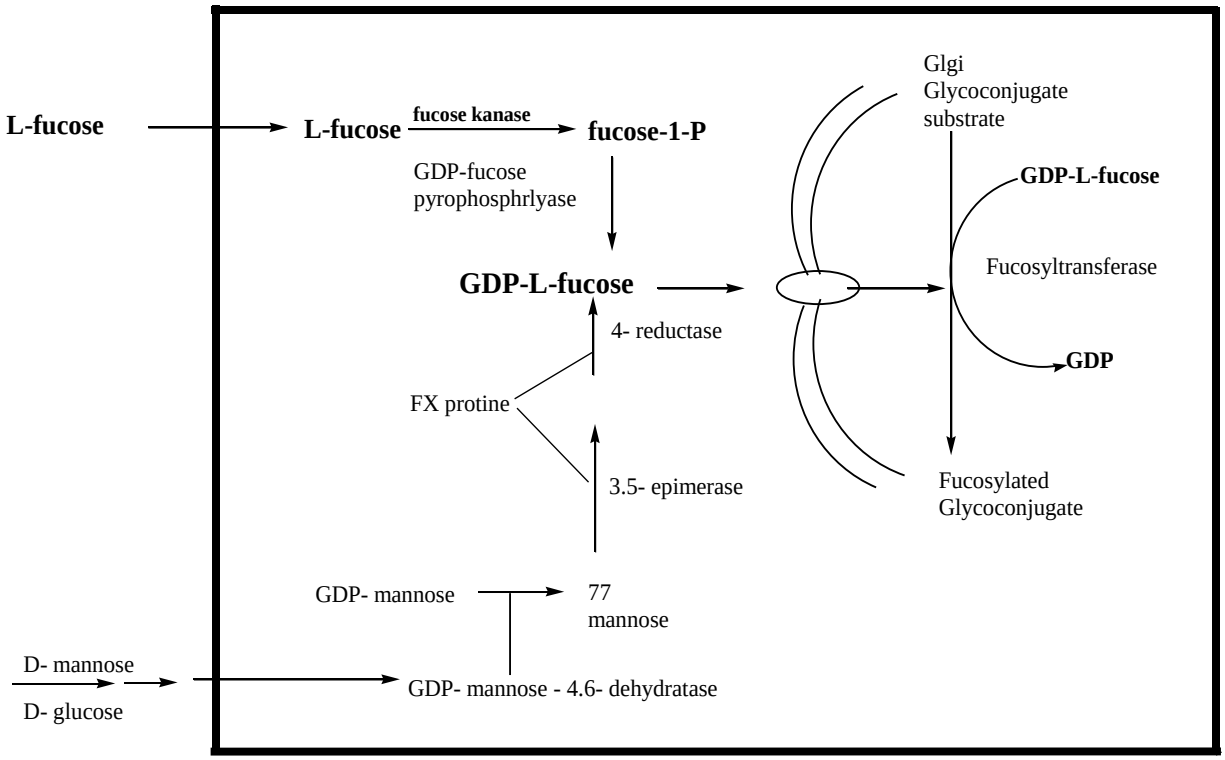


Fig (6) Fucose metabolic pathways

1.8.2 Absorption of fucose

Fucose can be readily absorbed when given orally in the diet, for example, animal studies show that the fucose is absorbed from the small intestine in vitro by a non – active diffusing trance port process (81).

Many cells also possess a specific facilitative transport for fucose (82), and fucose can inhibit the transport of some other actively transported sugars. Thus it appears that there is a potential for regulation of fucose entry into certain cells based on fucose concentration and the presence of other sugars (83).

1.8.3 Distribution

Fucose is widely distributed throughout the body in glycoprotein's and glycolipids consistent with a cell –cell communication role for fucose glyconjugates. Although endogenous fucose ,produces from other sugar precursors, it is utilized in theses possess, ingested or injected fucose is also incorporated into glycoprotein for example fucose is incorporated in vitro into photo receptor layer of the human eye retina, where it may be involved in biosynthesis of red cell glycoprotein (75).

Fucose is also incorporated into human skin epidermal cells, in vitro, where it may involved in synthesis of membranes of cells involved in maintaining skin hydration (84). Fucose is also distributed in various normal skin structures, such as glands and vascular endothelium (85).

Fucose glyoconjugates have been indentified in various other this use as well. For example, fucose glycoprteins are found in animal, and human brain cell (86, 87).They are present in synaptic junction areas where never cell meet, implying a role in synaptic membrane involvement in nerve impulse transmission. Human testes germ cells are rich in fucose glycoconjugates, which are altered during germ cell differentiation (88).Fucose glycoconjugates have been loclized to the proximal tubles of the human kidney, implying divers functional roles for these complex carbohydrate in this important organ(89) . Fucose is also distributed in macrophages which are critically important cell in the immune system (90). Additionally fucose is found in glycoproteins and glycolipid red blood cell antigens which are involved in determining type (75).

Fucose distribution is altered in certain disease states , for example, there is a higher fucose content in the serum glycoproteins of cancer patient (75,82,83), however the level of fucose are increased in serum of cell breast cancer patients (90).

1.8.4 Excretion

Fucose is excreted in urine at a rate of a approximately 17 micrograms /minute (91). Fucose-containing oligosqcchrides, probably from breast milk, have been identified in infant feces (92). Concentrations of fucose –containing glycopeptides in human urine have been found to increase markedly during the latter stages of pregnancy and during lactation, consistent with a role in late- stage preganancy (93).

1.8.5 Biological Activity

Fucose appears to be an important immune modulator, which is active in inflammatory disease. For example, fucose suppressed the skin reaction of allergic (but not irritants) contact dermatitis induced by dinitrochlorobenzene in guania pigs (94).Fucose also stimulated rabbit macrophage migration, suggesting that it might be part of amacrophage cellular releptor site migration enhancement factor, an essential of the immune system (75,95) . Moreover, fucose inhibits macrophage-chemotactic and neutrophil – chemotatic fucose(75) , which are also important in the immune system cascade (96).

Fucose appears to inhibit cancer growth and metastasis. For example, fucose inhibit rat mammary tumor cell growth in in vitro .Different concentrations of fucose uniformly produced a suppression in the growth rate and a change in the morphology of cell , grown in tissues culture (97). Fucose also in inhibit mouse tumor cell – induced platelet aggregation , a process important in cancer cell metastasis (98) .A natural derivative of fucose (2-deoxy-L- fucose) inhibited leukemia an mammary tumor cell growth in cell culture systems in vitro(99). Injection of 600mg in rats with alchemically -induced mammary tumor resulted in suppressed tumor growth, and serum and tumor

levels of fucose were increase in the treated animals (96,100).

Fucose also has therapeutic implications treating or preventing respitory tract infections (97).Fucose also reduced collagen production in cultured cereal micro vessel endothelial cell obtained from animals ingesting a diet composed of 20% fucose (98).

1.9 The correlation between pentose phosphate pathway and glutathione, -L-fucose as tumor biomarker

Tumor biomarker are either intracellular proteins or cell surface glycoproteins and glycolipids released into the circulation and detected by immuno assays.

The pentose phosphate pathway handles (5 - 10) % of metabolized glucose in normal red cell in the process generating 2 mol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for each 1 mol of glucose metabolized, NADPH is an essential cofactor for enzyme glutathione reductive, which maintains glutathione in the reduced state necessary for the detoxification of toxic oxygen products such as super oxide anion (O_2^-) , hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) as shown in fig (7).

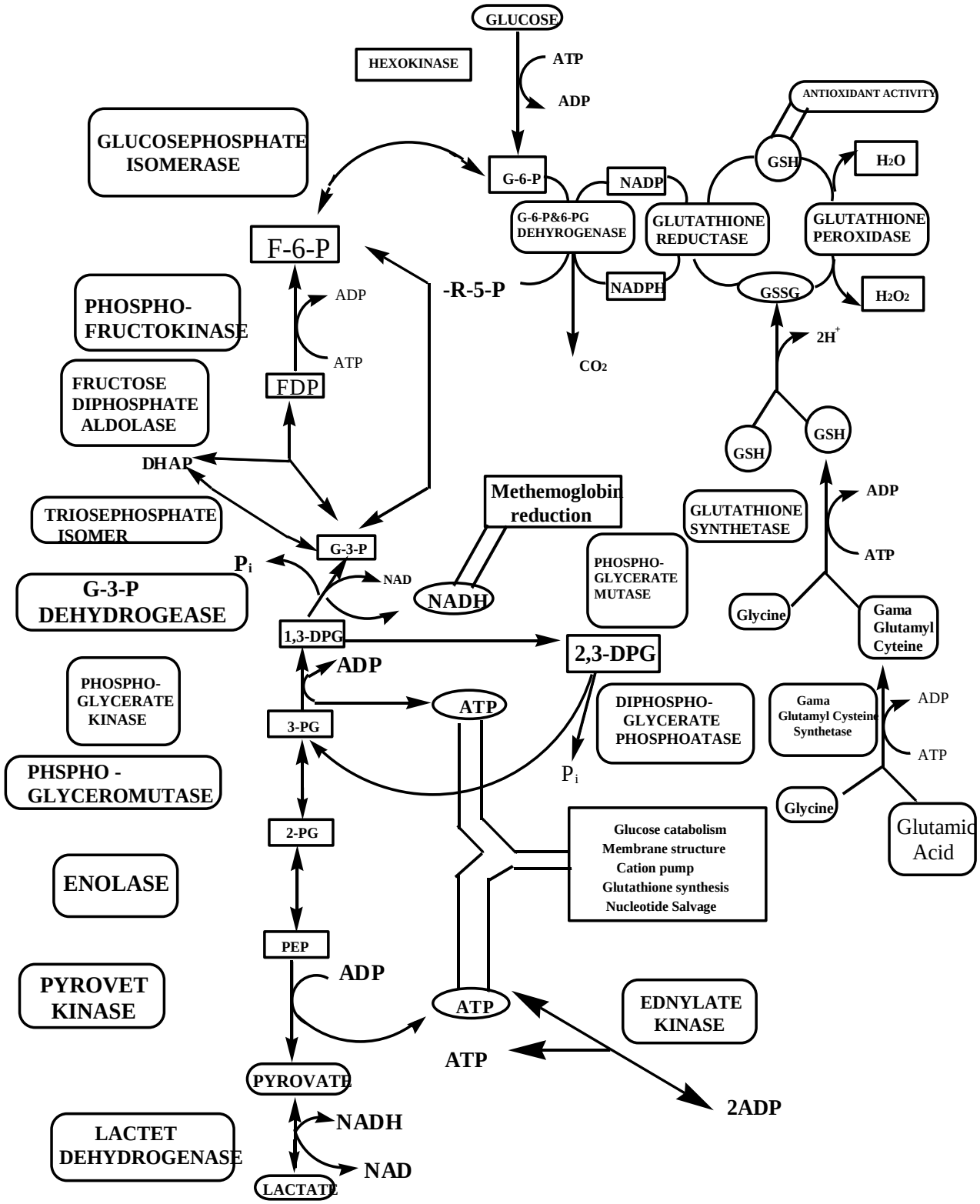


Fig (7) the pentose phosphate pathway and glutathione red blood cell (100).

The enzymatic free radical formation resulting in consuming more (NADPH) in a, reaction being catalyzed by NADPH oxidase enzyme and the cytochrome reducatase lead to increase level of toxic oxygen

Products especially , superoxide (O_2^-) moreover , reduction of NADPH concentration, as a cofactor the glutathione enzyme which also reduced casing finally a noticeable reduction of the reduced glutathione (GSH) , and in the absence of reduced glutathione toxic oxygen products (oxyradicals) can damage red cell lipids, proteins and results in hemolysis and anaemia.

However , normal red cell are continually subjected to the oxy radiacls as a result of intracellular hemeoxidation beign strictly and obviously concerned with non-enzymatic free radical formation in which the oxidation of Fe^{+2} to Fe^{+3} that will rises in the intracellular levels of free radical superoxide and hydroxyl group, resulting in an imbalance between pro-oxidant and anti- oxidant levels, so the fact of reduced glutathione level (GSH) inside the cells opposed by rising levels of free radical that if are not inactivated their chemical reactivity can damage all the cellular macromolecules including proteins, carbohydrate, lipids and nucleic acid . as shown in fig (7) (100) .

1.10 Aim of study

1- To detect the reference of serum total fucose (TF) and serum reduced glutathione and serum glutathione –S- transferase as valuable biomarkers in transformation particularly of breast cancer.

2- To investigate and measure the values of the total fucose (TF) in serum of patients with breast cancer and correlate with reduced glutathione and glutathione –S- transferase activity as possible useful biological tumor markers in the early diagnoses of breast cancer .

3- To present the effect of age, weight, family history, occupation, and area of living.

Materials and Methods

2. Materials and Methods

2.1 Materials

2.1.1 Chemical Materials

All common laboratory chemicals were obtained from the Firms, Fluka, Hopkins and Williams, Sigma Chemicals, Merck.

All chemicals were used as supplied without farther purification.

Table (1): The chemical compounds

Chemicals	Purity %	Supplied Company
1.glutathione	99.5	Sigma chemicals company, U.S.A
5,5-dithiobis (2- nitrobenzoicacid)DTNB	99.5	Sigma Chemicals company ,U.S.A
Trichloroacetic acid (TCA)	99	Hopkins and Williams
Ethylene Diaminetetracetic acid dihydrate (EDTA). 2 H2O	99.5	Fulka company.
Tris (hydroxyl methylene) aminomethane	99.5	Merck
Methanol	99.8	Fluka company.
Alpha L- Fucose (standard)	99.8	Fluka company.
Cystien Hydrochloride	99	Merck
Concentrated H2SO4	99.5	Merck

2.1.2 Instrumental Analysis and Equipment

Table (2):The equipments of laboratory

Instrument	Supplied Company
pH meter microprocessor pH meter HI 9321	HANNA instruments HI-9321, (Portugal)
Sensitive balance	Sartorius AG GOTTINGEN BL 2105 (Germany)
Vortex mixer (Electronic)	VIOBROFIX JANKE and IKA-labrotechnik (Germany)
Water bath	Schutzart DIN 400050-IP 20 Memmert Gmbh , Schwabach FRG (Germany)
Magnetic stirrer with Hot plate	J1assco (India)
Spectrophotometer Type 21 (Digital ultraviolet and visible)	Spectronic (21) MILTON ROY COMPANY, Bouch and Lamp (USA)
ELISA Reader and washer	Bikaman Keouldeir (USA)
Centrifuge	Griffin and George BS 4402- D(UK)
Centrifuge tube	AFMA (Jordan)
Plane tube	AFMA – Dispo (Jordan)
Micro pipette 10-50 ml	SLAMED (Germany)
Micro pipette 100-200 ml	SLAMED (Germany)
Micro pipette 100-1000 ml	SLAMED (Germany)
Disposable syringe	Bulim medical (South Korea)
Incubator (isotemp)	Fisher scientific company model 5370, CAT.11-690 -538D , (USA)

2.1.3 Patients and control groups

One hundred patients female only with diagnosed breast cancer in deferent stages as well as one hundred healthy female on control which they are volunteers.

The patients were visitors for Marjan Teaching Hospital in Hilla City

2.1.4 Collection of Blood and Serum preparation

Blood samples were obtained from patients and control group.

The vein on the front of elbow and forearm is all most employed .

The tourniquet was put around the arm and applied pressure on the vein then sterile the area of vein with iodine disinfectant then drawn 5 ml of blood from vein by sterile syringe then removed the tourniquet and syringe and closed the vein opening by tight pressure of cotton with iodine till there was no bleeding .

2.2 Methods

2.2.1 Determination of serum reduce glutathione

All analytical methods such as, photometric enzymatic flourometric, and HPLC methods that used to determine tissue homogenate, erythrocytes, and serum glutathione (GSH) depend on the action of the sulfhydryl groups (102-104).

Principle

5,5 dithiobis (2- nitrobenzoic acid) (DTNB) is a disulfide chromogen that readily reduced by sulfhydryl Group of GSH to an intensely yellow compound . The absorbance of the reduced chromogen is measured at 412 nm and directly proportional to the GSH concentration.

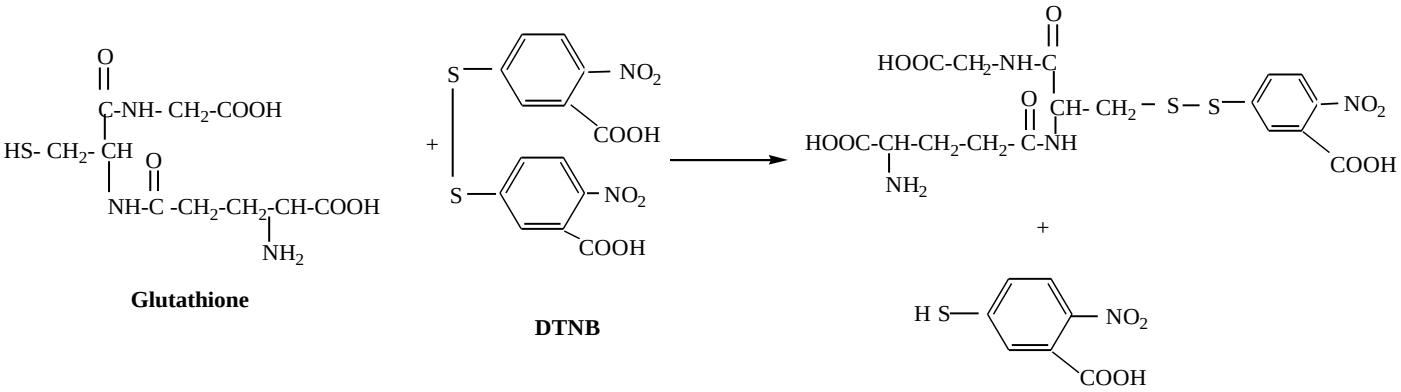


Fig (8) Reaction between GSH and DTNB

Preparation of Reagents

1. precipitating Solution (Trichloroacetic acid (TCA) 50 %)

A weight of 50 gm of TCA is dissolved in final volume of 100 ml of DDW.

2. Ethylenediamine tetra acetic acid – di Soduim (EDTA Na_2) (0.2M)

A weight of 7.445 gm of EDTA Na_2 is dissolved in final volume of 50 ml DDW or 3.7224 gm of EDTA Na_2 dissolved in a final volume of 25 ml DDW.

3. Tris EDTA buffer (0.2M) pH 8.9

A weight of 0.4845 gm of Tris is dissolved in 8 ml of DDW.

1ml of (0.2M) EDTA Na_2 Solution are added and bring to a final volume of 10 ml with DDW.

The PH was adjusted to 8.9 by the additional 1 M of HCL. (This Solution is stable for at least 10 days)

4. DTNB reagent (0.01M)

A weight of 0.037 gm of DTNB is dissolved in absolute methanol, and brings to a final volume of 10 ml.

(This Solution is stable for at least 13 weeks at 4C°)

5. GSH Standards Solution (0.001M)

Stock standards solution (0.001M) is prepared by dissolved 0.00768 gm of GSH in a final volume of 25 ml of (0.02 M) EDTA solution , dilution are made in EDTA Solution to 5,10,15,20,30,40,50 µm

(This working standard solution should be prepared daily)

Procedure

Serum GSH was determined by using a modified procedure using Elmans reagent (DTNB), which is summarized as follows:

Duplicates of each standard and sample test tube are prepared then pipette into test tubes.

Table (3):Procedure of GSH part one

Reagents	Sample µL	Reagent blank µL	Standard µL
Serum	100		
Standard			100
DDW	800	900	800
TCA	100	100	100

Tubes are mixed in vortex mixer, intermittently for 10-15 min , and centrifuged for 15 min at 3000xg , then pipetted into test tubes.

Table (4):procedure of GSH part two

Reagents	Sample µL	Reagent blank µL	Standard µL
Supernatant	400	400	400
Tris- EDTA buffer	800	800	800
DTNB reagent	20	20	20

Tubes are mixed in vortex mixer, the spectrophotometer is adjusted with reagent blank to read zero absorbance (A) at 412 nm and the absorbance of standard and sample is read within 3 minutes of the addition of DTNB reagent.

Calculation of serum GSH :

The concentration of serum GSH is obtained from the calibration curve in µM. As shown in fig (9).

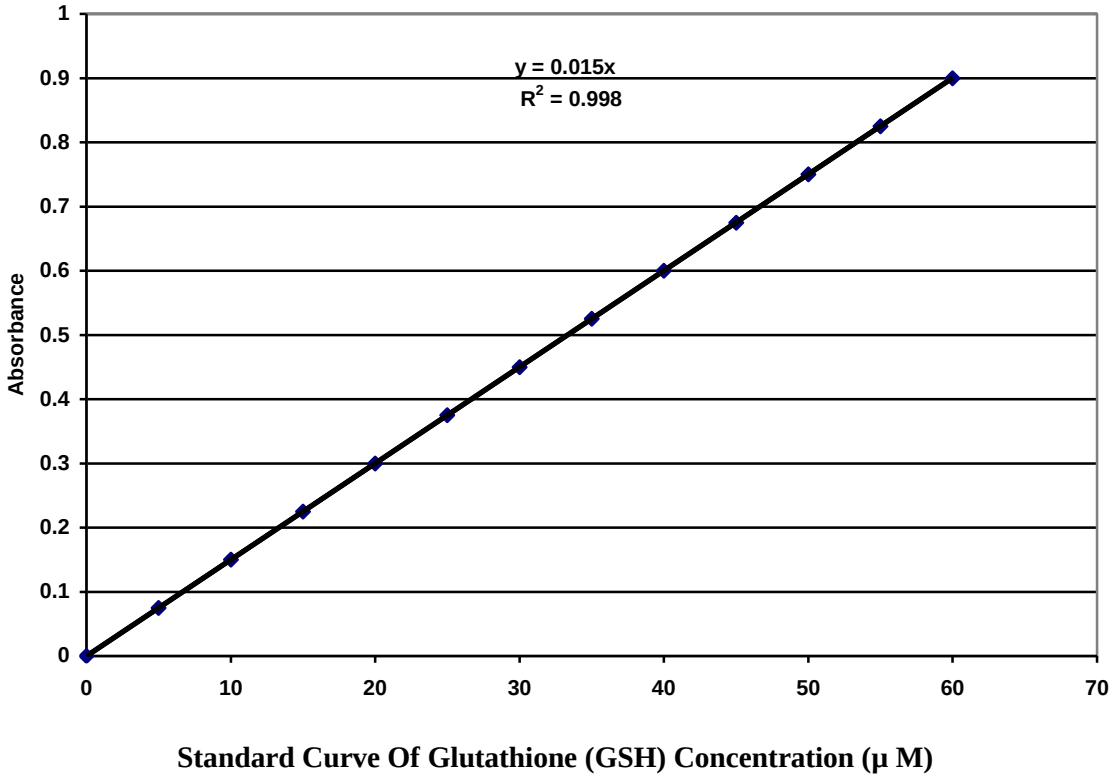


Fig (9) Standard Curve of Glutathione (GSH) Concentration (µ M)

2.2.2Enzymatic assay of glutathione –S- transferase

Principle (106)

GSH + CDNB $\xrightarrow{\text{GST}}$ G-CDNB conjugate + Hcl

Conditions: T= 25C° pH= 6.25, A340nm, Light path= 1cm

Method: Continuous spectrophotometer Rate Determination

Reagents

1- Glutathione solution, (0.092 of reduced standard glutathione is dissolved in (10 ml) distilled water.
2- 1-chloro 2,4- Dinitro benzene (CDNB) solution, this is formed by dissolving (0.455 gm) of CDNB in (100 ml) ethanol.
3- Phosphate buffer is prepared by dissolving (0.436 gm) of dipotassium hydrogen ortho- phosphate, and (3.060gm) of potassium dihydrogen ortho phosphate in (250ml) distilled water, pH was set to (6.25).

Procedure

Table (5):Procedure of GST

Reagents	Sample	Blank
Phosphate buffer	2.7 ml	2.7 ml
Serum	100 µl	-
Distilled water	-	100 µl
CDNB Solution	100 µl	100 µl
After 3 minutes add GSH Solution	100 µl	100 µl

The absorbance is measured after 10 minutes at a wave length (340 nm).

Calculations

GST (U/L) = (ΔA / t * V_t *1000) / (ε * V_s)

= (ΔA /10 * 3 *1000) / (9.6 *0.1)

ΔA= Change in absorbance.

t= time in minute

V_t = total volume in milliliters

ε = extinction coefficient

V_s = Volume of Sample

2.2.3 Determination of total fucose (TF)

Principle (107)

This methods depends on a direct reaction of concentrated sulfuric acid with serum components the reactants combine with cysteine, and the colour product measured at (396 and 430 nm) . The differences in absorbance were directly proportional to alpha-L – fucose content of the solutions.

Reagents

Reagent (1) six parts of concentrated H_2SO_4 + one part of distilled water. (6 ml H_2SO_4 + 1ml DDW).

Reagent (2) . 3% cysteine hydrochloride solution was prepared weekly and stored in the refrigerator.

3% i.e . 3 gm in 100 ml DDW.

To avoid loss of chemicals , 0.3 gm were prepared and dissolved in 10 ml of DDW., since its weekly prepared.

Reagent (3) Standard alpha- -L- fucose. 10 mic gm of methyl pentose to be dissolved in 1000 ml DDW to avoid loss of chemicals.

Reagent (4) Distilled water

Procedure

1. Volume of (4.5 ml) of chilled solution of reagent (1) was add slowly with contrast shaking in ice bath to (0.1) ml or 100 mic. L ice cold serum.
2. The tubes were transferred to a water bath at room temperature for few minutes exactly 10 min, then tubes transferred to vigorously boiling water bath for exactly 3 min. Lastly the tubes placed in water bath at room temperature for 15 minutes.
3. Volume of (0.1ml) or 100 mic L of reagent (2) was added to each tube then was mixed after two hours the absorbance was measured at 396 nm and at 430 nm.
4. The same procedure was applied for standard fucose solution.

Calculations

$$\text{Total fucose (mg/dl)} = \frac{A_T\,396 - A_T\,430}{A_S\;396 - A_S\,430} * 12$$

Where :

A = absorbance.

A_T = the absorbance of the test with cysteine.

A_S = the absorbance of the standard with cysteine

12 = dilution factor.

Results and discussion

Glutathione concentration, total fucose (mg/dl) and Glutathione – s – transferase U /L in serum blood of breast cancer patients were determined as follows:

From 240 samples being collected very hard; 26 samples had been lost because of bad electricity, and14 other samples were spoiled during analysis, and only 200samples yielded sufficient readings and the following results were obtained:

3.1 GSH concentration in serum:

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

Table (6): 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	0.333	±0.286	$p<0.05$
Control	100	2.35	±0.755	

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 $p < 0.05$.

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 (30).

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 (30).

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 (18).

GSH play 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 (30) .

3.2 GST activity in serum :

Glutathione – s – transfers enzyme had shown an increase in patients with breast cancer in comparison with control.

Table (7): The mean, of GST in contrast patients with control.

Group	No. of samples	Mean GST U/L	S D U/L	significance
Patients	100	2.406	±0.26	$p<0.05$
Control	100	0.32	±0.27	

According to t- test, there was significance difference between the mean activity of serum blood of patient and mean GST in serum blood of control $p < 0.05$.

GST catalyze the formation of the thioeither conjugated between glutathione and xenobiotics which the body of patients contain many free radicals and medicals substances such as chemotherapy compounds, all that causes increasing GST activity (54) .

That's mean the conversion to GSH concentration is high and enzyme activity is small.

3.3 Total fucose concentration in serum :

The mean of TF in serum blood of patient had shown an increase in comparison to the total fucose concentration in serum blood of control.

Table (8): The mean, of fucose concentration in contrast patients with control.

Group	No. of samples	Mean in mg/dl	S D mg/dl	significance
Patients	100	35.98	±1.276	$p<0.05$
Control	100	20.35	±1.897	

According to t- test, two samples mean, there were significance difference between the mean of TF in serum of patient and mean TF in serum of control which $p < 0.05$.

Fucose is widely distributed through out the body in glycoprotein and glycolipds dependable with cell-cell communication, which suggested that fucose play a role in the inhibition of growth of this mammary tumors which fucose metabolism is abnormal in cystic fibrosis, diabetes and cancer (81).

There is a study that proved there were high increase in TF concentration in patient of cancers specially patients with breast cancer (70).

3.4 GSH concentration in serum of patients before and after 48 hours

The mean of reduced glutathione had shown a decrease after 48hr in contrast with the mean of GSH before 48hr.

Table (9): The difference in mean GSH concentration in serum blood of patients before and after 48 hrs.

Group	No. of samples	Mean in mg/dl	S D mg/dl	significance
GSH before 48 hr	100	0.333	±0.286	p<0.05
GSH after 48 hr	100	0.0169	±0.0165	

According to t-test there were a significance difference between the mean of GSH before and after 48hr ,which GSH reacts in time scale measured in minutes with itself to form Oxidized glutathione (GSSG) ,as well as with cysteine to form CSSG and with cystein residues of proteins in the plasma as well as serum blood; $P < 0.05$ (26) .

3.5 GST activity before and after 48 hours for patients

GST enzyme activity had shown a decrease before and after 48 hr in serum blood of patients with breast cancer.

Table (10): The mean activity of GST before and after 48 hrs in serum blood of patients.

Group	No. of samples	Mean in mg/dl	S D U/L	significance
before 48 hr	100	2.406	±0.2316	$p<0.05$
after 48 hr	100	0.8774	±0.2196	

According to t- test, there was significant difference between the activities of GST before and after 48 hr, which $p < 0.05$.

The enzyme activity increases or decreases with increasing or decreasing of substrate concentration , So according to the previous table there was decreasing in GSH concentration after 48 hr.

3.6 Total fucose concentration in serum before and after 48 hours:

The mean of total fucosse in serum blood of patient had show a very small decrease before and after 48 hr.

Table (11): The mean, of fucose concentration in serum blood of patient before and after 48 hr.

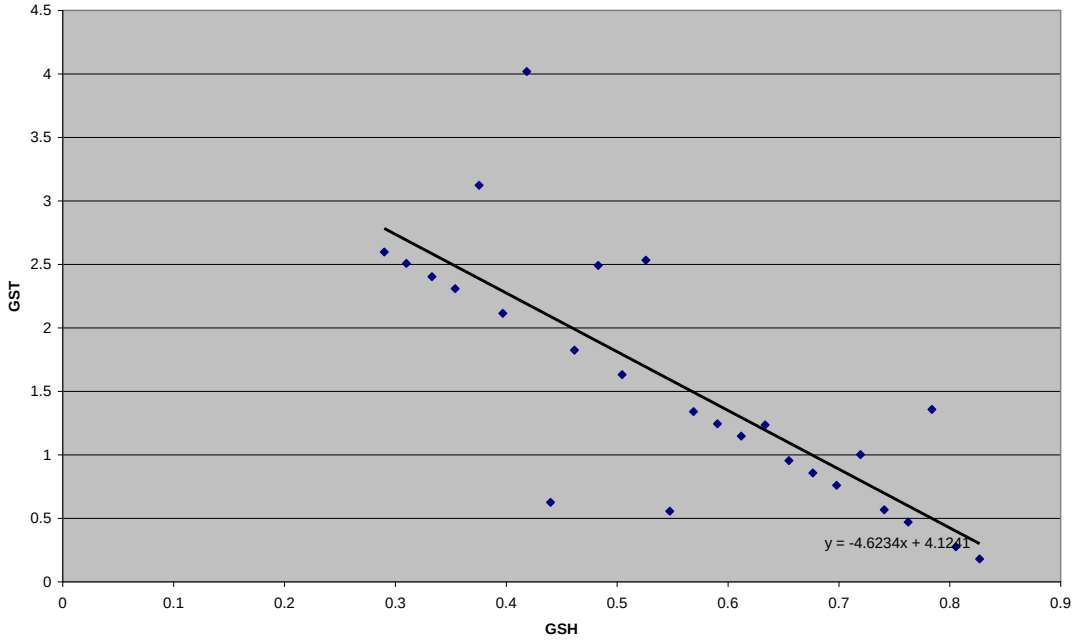
Group	No. of samples	Mean in mg/dl	S D mg/dl	significance
before 48 hr	100	35.98	±1.276	p>0.05
after 48 hr	100	35.91	±3.86	

According to t-teat of two samples means , that referred there were no significant difference was found before and after 48 hr for serum blood of patients which p >0.05 . Carbohydrate can be attached by glycosidic bonds with carbohydrate and non carbohydrate structures, including purines and pyrimidines and other compounds.

Fucose can be attached with GDP, and the bonds between carbon atoms of fucose is covalent bond (72).

3.7 The correlation among GSH, GST and fucose in patients with breast cancer

3.7.1 The correlation between GSH and GST:



Fig(10) The correlation between GSH and GST

The correlation is significant at the level 0.01 (2 – tailed), which significance value (p-value) <0.01 .The relation between GSH concentration and GST activity is inverse, which a decrease in GSH conc. is related with an increase in GST activity.

Plotting GSH concentration against GST activity in breast cancer patients finds a significant negative correlation with correlation coefficient(r) value (-0.732).

3.7.2 The correlation between fucose and GST :

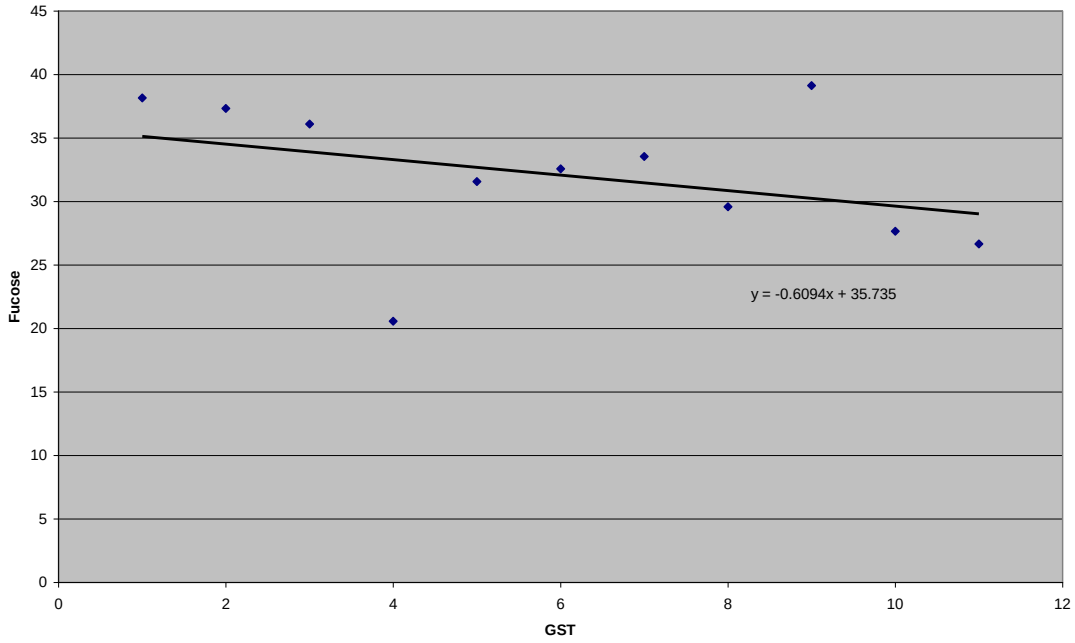


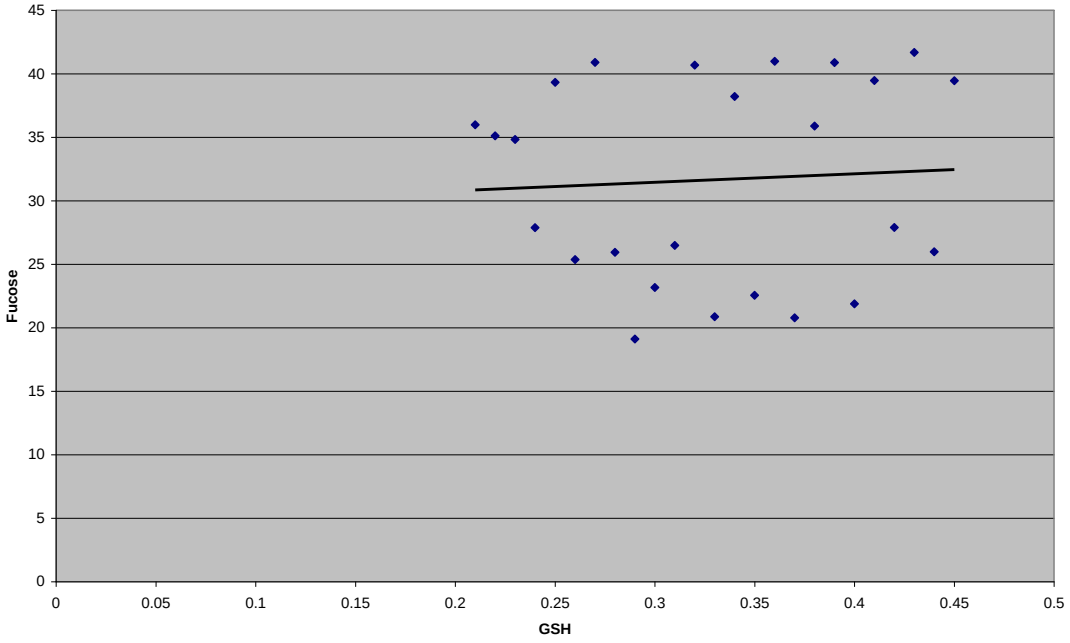
Fig (11) The correlation between fucose and GST

The correlation is significant at 0.05 level(2-tailed) ,which means the relation between GST and fucose concentration is inverse, which significance value (p-value)<0.05,which a decrease in fucose concentration is related with an increase in GST activity.

Plotting fucose concentration against GST activity breast cancer patients

finds a significant negative correlation with correlation coefficient(r) value (-0.22).

3.7.3 The correlation between en fucose and GSH:



Fig(12) The correlation between GSH and fucose

The correlation is not significant between (GSH) and (fucose) at level 0.05, 0.01, which significance value (p-value)<0.05 and (p-value)<0.01 . There is no relation between GSH and fucose concentration.

Plotting GSH concentration against fucose concentration finds linear (zero) correlation with correlation coefficient(r) value (0.09).

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

Table (12): 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 the percentage of patient with breast cancer who was taken contraceptive drug is more than the patients who were not taken contraceptive drugs.

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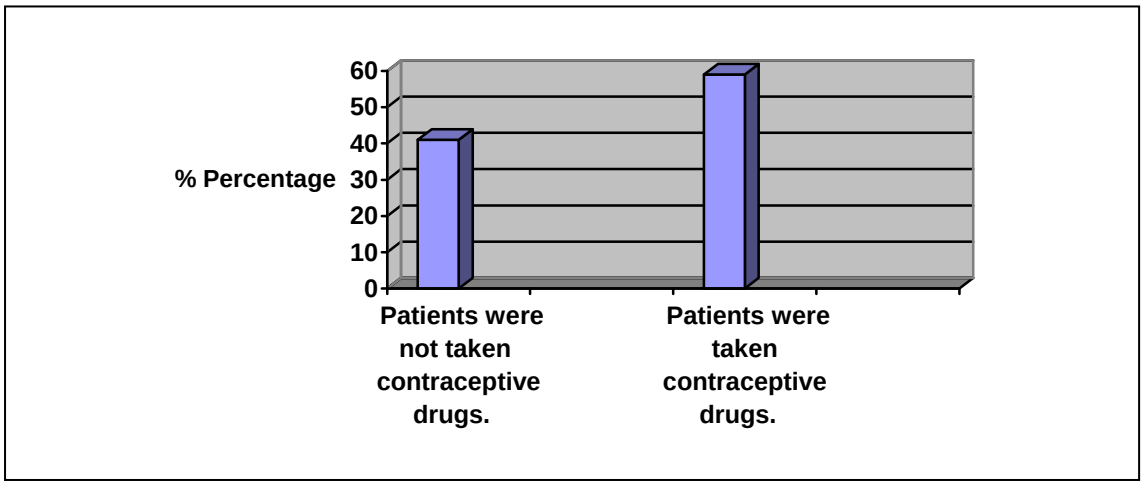

Fig (13): The percentage of Patients were taken and not taken contraceptive drugs.

Table (13): The mean of GSH 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	2.35	±0.755	
41%	0.54	±0.276	p>0.05
59%	0.1664	±0.159	p<0.05

According to t-test, there were no significance different between were not taken contraceptive drugs and healthy control which P. Value more than 0.05.

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

Table (14): The mean GST activity in both were taken and were not taken contraceptive drugs.

Group	Mean of GST activity	SD U/L	significance
Control	0.32	±0.27	
41%	2.277	±0.868	$P < 0.05$
59%	2.49166	±0.88	$P < 0.05$

At level 0.05 there was a significant difference which is $P < 0.05$ between patients were not taken contraceptive drugs and healthy control.

There was a significant difference between patients were taken contraceptive drugs and healthy control which $P < 0.05$.

Table (15): The mean of fucose in the Patients were taken contraceptive drugs and were not taking contraceptive drugs.

Group	Mean of fucose mg /dl	SD mg /dl	significance
Control	20.355	±1.897	
41%	35.87	±1.186	$P < 0.05$
59%	36.05	±1.338	$P < 0.05$

According to t-test, there was significant difference between the mean concentration of fucose to healthy control and patients were not taken contraceptive drugs, which $P < 0.05$.

Also, there was significant difference between patients were taken contraceptive drugs and healthy control, which $P < 0.05$.

A recent study suggests a link between high doses combined oral contraceptive that was 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 still available, women should use two – dose pills instead, especially if they have family history of breast cancer (8).

3.9 Stage and age effect on the breast cancer patients

Table (16): The stage and the No. of cases with individual stage:

Stage	No. of cases
I	15
II	71
III	9
IV	5

According to study, stage II is the most common stage with breast cancer patients which there were 71 cases.

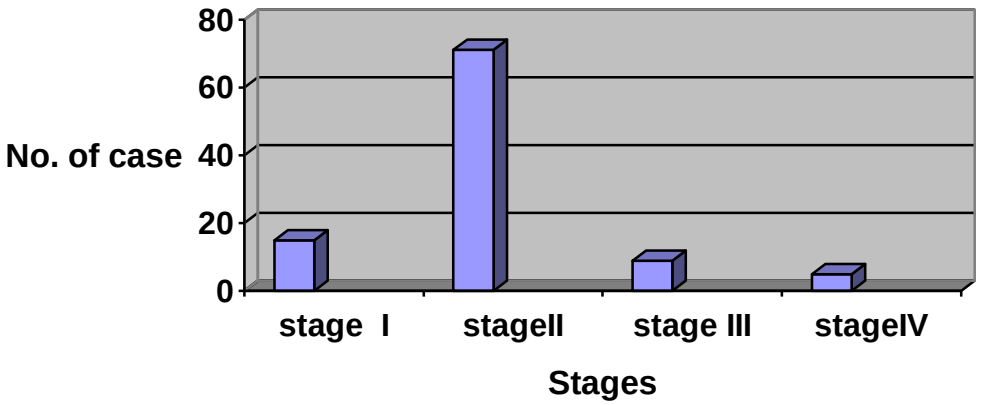

Fig (14): The stage and the no. of cases

3.10 Age effect with breast cancer

According to the previous section the most common stage with breast cancer patients is stage, II.

From these result we can known the most common age.

Table (17): The most common age with breast cancer.

Ages	No. of cases
30-39	7
40-49	27
50-59	23
60-69	12
70-80	2

We see the most common age is 40-49. This could be due to the diagnosis of disease at our country.

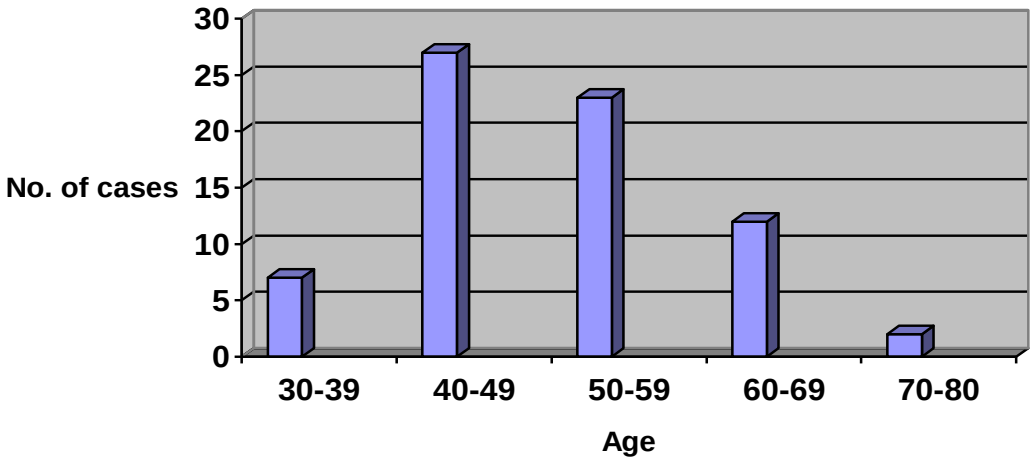

Fig (15): The most common age with breast cancer

3.11 The correlation among fucose , GSH and GST at age 55 years old with breast cancer patients :

We took 55 years only because there were more cases at that age in stage II only so, we wants to know the correlation among GSH, GST and fucose with breast cancer patients There were 12 cases at age 55 years old at stag

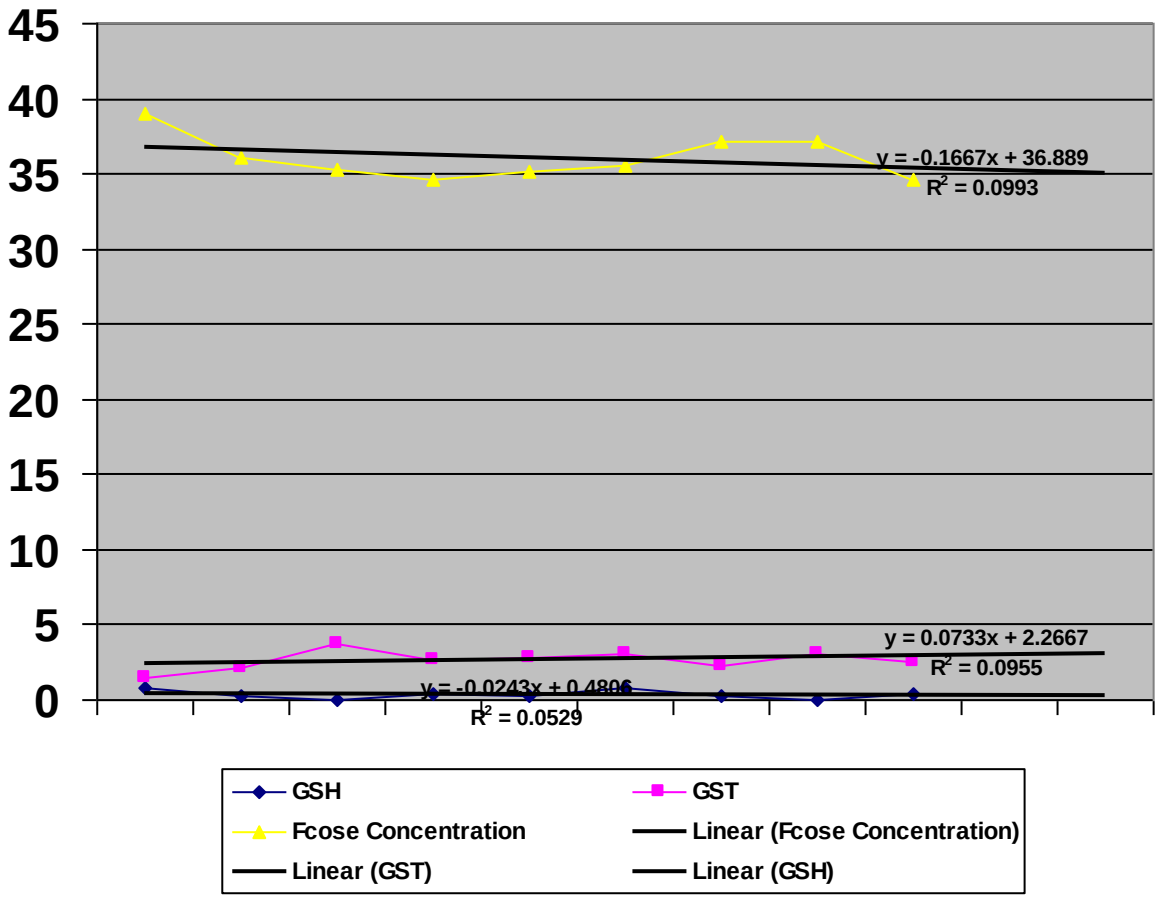

Fig (16):

Shows The correlation among fucose , GSH and GST at age 55 years old with breast cancer patients

The significance (p-value) is more than 0.1 there is no correlation among GSH ,GST and fucose at age 55years old .

3.12 Obesity effect on the patient with breast cancer

Table (18): The percentage of obesity to the patients with breast cancer.

Group	Percentage
Obese	63%
Non obese	37%

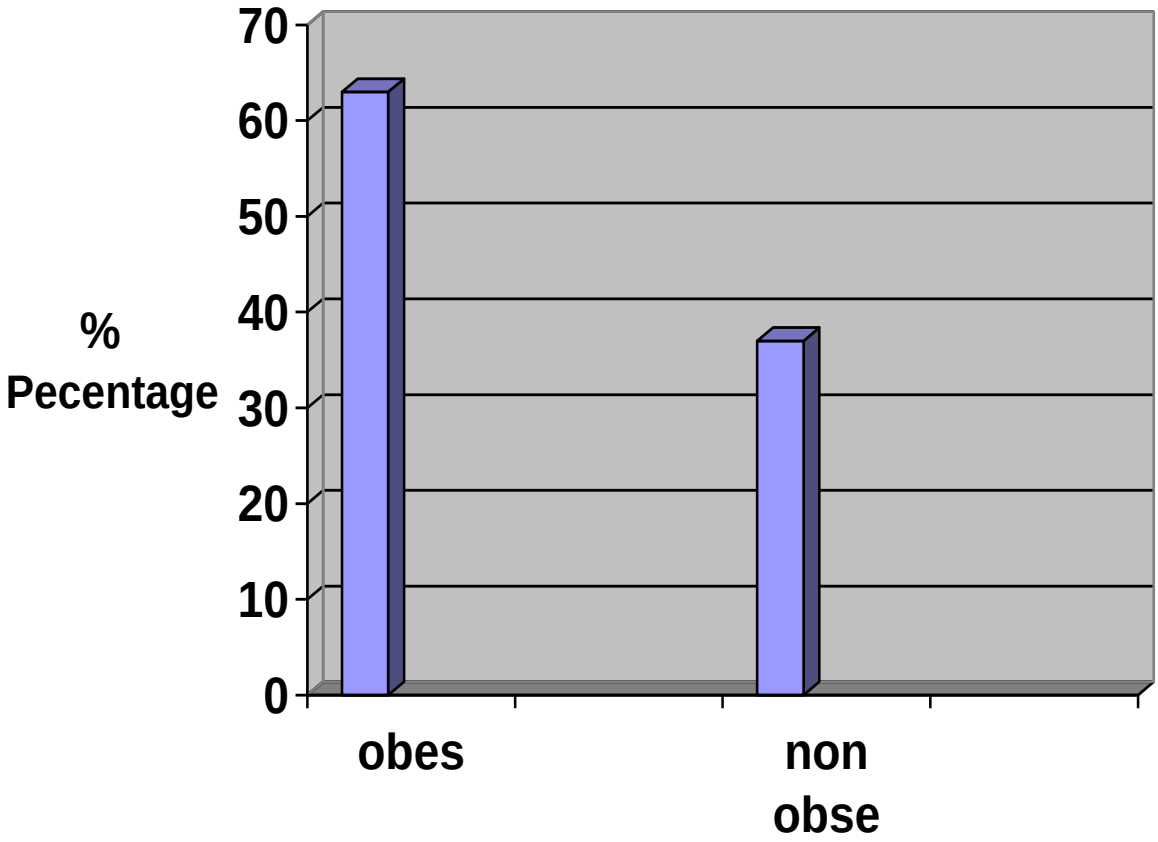

Fig (17): The percentage of obese and non obese patients.

Our study reveled that 63% breast cancer patients were obese while only 37% of them were non obese or with normal body weight there were many research suggested that the increase in breast cancer risk faced by obese postmenopausal women may largely be due to higher levels of estrogens circulating in there bodies. The study showed that the average concentration of estrogens in obese women was between 50% and 219% higher than in thin women, and risk of breast cancer increased by with each increase in the weight (height-weight).

3.13 Residence area effect in breast cancer patients

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

Table (19): Shows the percentage of breast cancer according to their residence.

Group	percentage
Urban	48%
Rural	52%

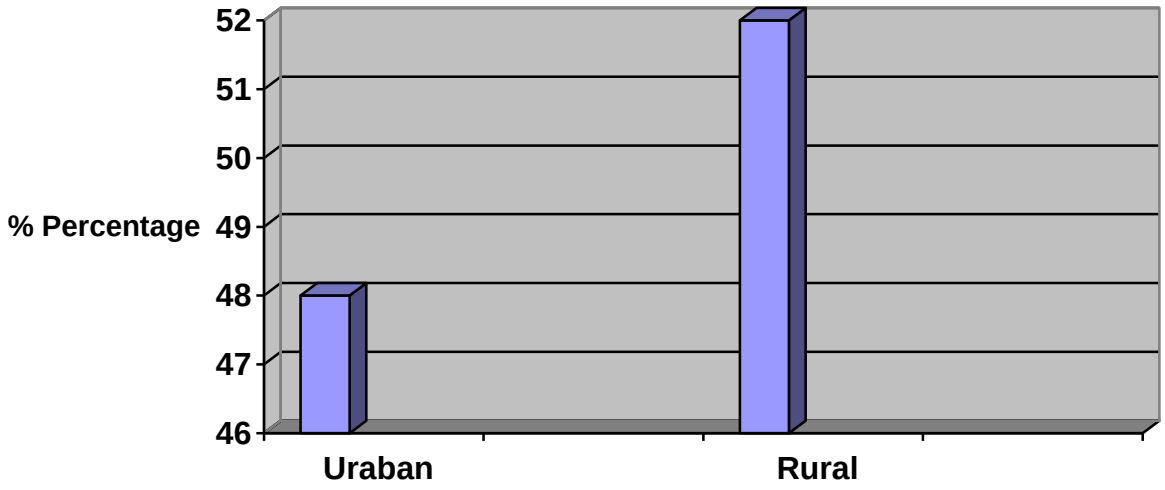

Fig (18): The percentage of urban and rural patients.

The cancerous agents a problem arise in Iraq which the use of 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 induce 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, since those people their work and life style made them under the bad effect of the use of organ phosphorus agents (insecticides and pesticides) to protect their farms from the harmful insects and pest. These agents are so harmful as they considered as a cancer inducer especially due to the wrong way of applying these agents the plants, grasses and trees, although self, home and environment contamination .

Not to mention the harmful effect of 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 has grown by more than 600% (the white death- leukemia 0, the desert dust carries death, studies indicate the more than 40% of the population around Basra will get cancer. Since unfortunately most the leukemia and cancer victims aren't soldiers. They are civilians.

While for those resident in urban areas these results explained by the notorious effect of crowding in association with air pollution from the exhaust oil refineries, factories and car engines.

3.14 The work of the women:

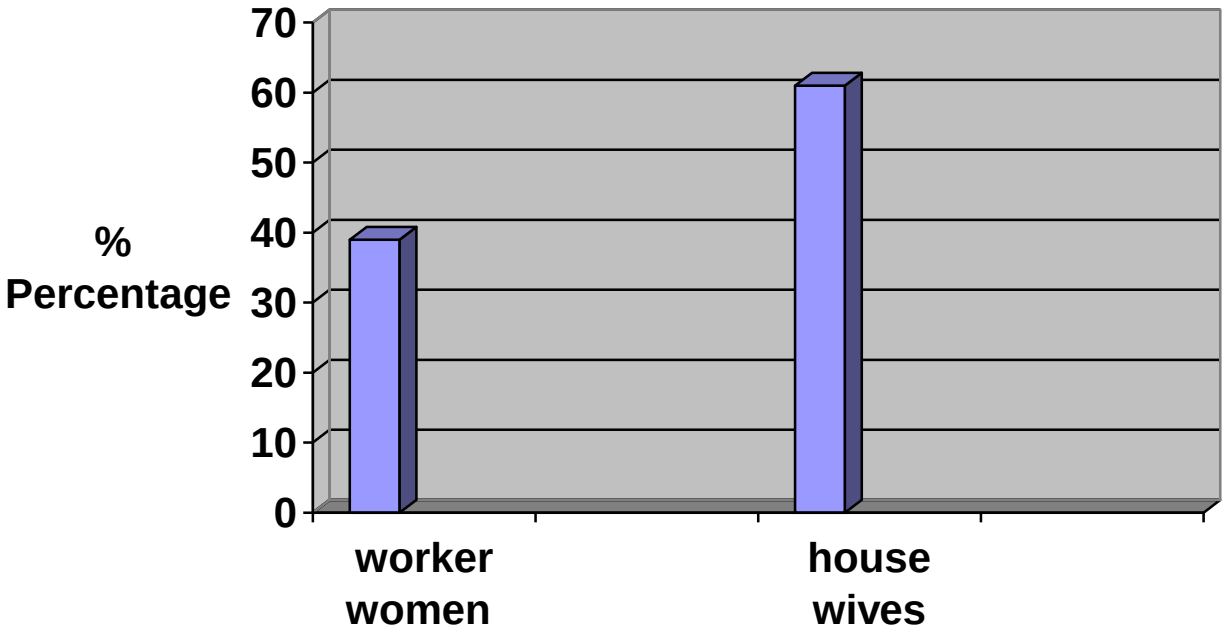

Fig (19): The percentage of worker women and house wives patients

Most housewife are farmers so we can say most those women are worker women but their living in rural area so they are exposed to the same conditions such as insecticides and pesticides

3.15 Family history of breast cancer patients:

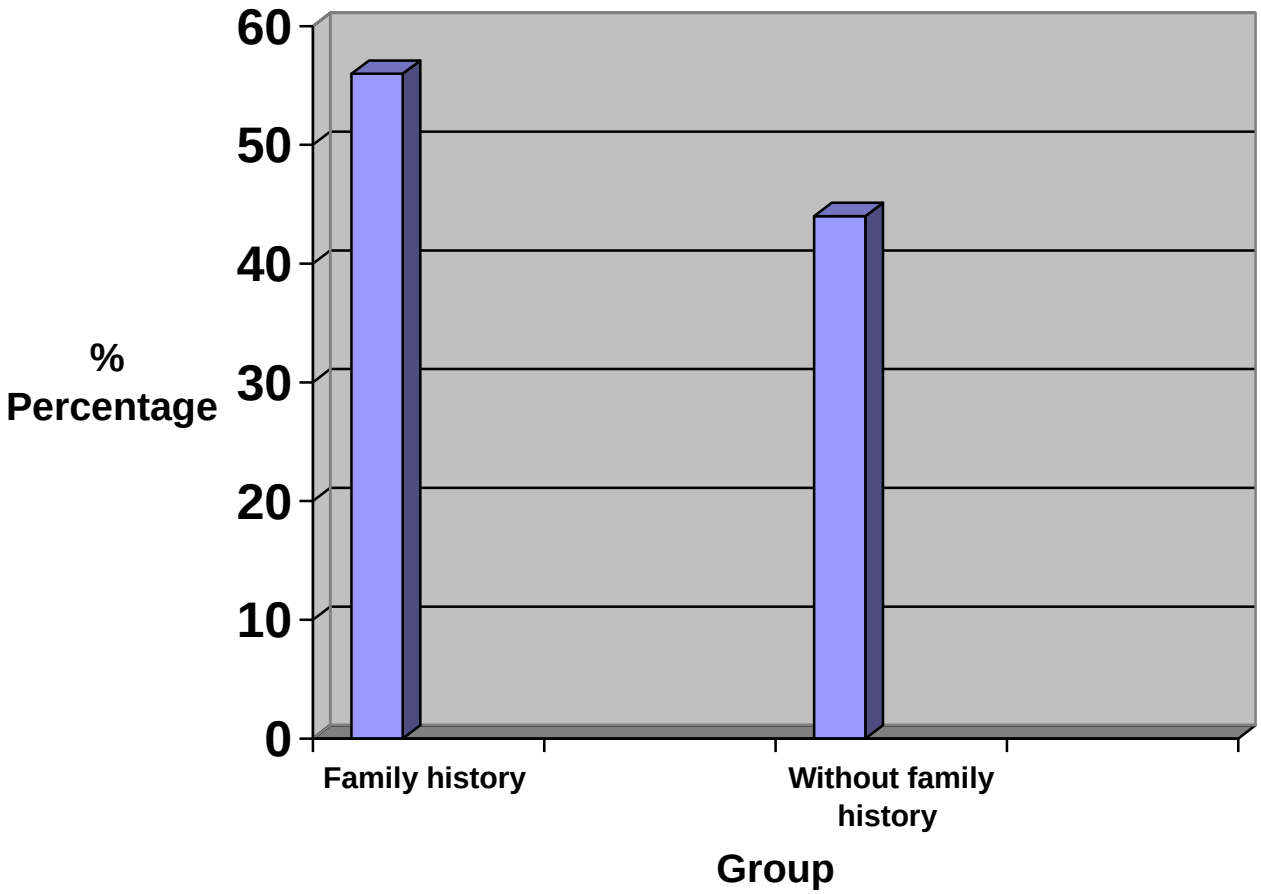

Fig (20): The percentage of patients with and without family history of breast cancer.

3.16 Exposure to chemical effect on the patients with breast cancer

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

Table (20): The percentage of exposure to chemicals in patients with breast cancer.

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

Conclusion

Conclusions

1- The breast cancer affected the GSH levels by decreasing its level while GST increased proportionally.
2- The breast cancer affected the TF levels by increasing its level in contrast with control level.
3- Increasing the level of GSH in control serum in contrast with patients of breast cancer, while there was proportionally increasing in GSH level in patient were not taken contraceptive drugs in contrast with patients who were taken contraceptive drugs.
4- Decreasing the level of TF control serum in contrast with patients of breast cancer, while there was proportionally decreasing in TF with patients who were not taken contraceptive drugs.
5- Family history affected on the levels of breast cancer.
6- Obesity affected on the levels of breast cancer.

7-stage and age were affected on the breast cancer patient.

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تمت دراسة التغيرات في الكلوتاثيون المختزل وانزيم الكلوتاثيون – اس – ترانسفريز وال – فيوكوز 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)

سجلت الدراسة وجود اكبر نسبه من سرطان الثدي من الدرجة الثانية , بينما هناك15 حاله فقط من سرطان الثدي من الدرجه الاولى وخمس حالات فقط من سرطان الثدي من الدرجه الرابعة

بينت الدراسة وجود تاثير لكل من العمر والوزن ومكان المعيشة وطبيعة عمل المراة والتاريخ المرضي للعائلة بالإصابة باورام سرطانية .

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 التغيرات كدالات بايوكيميائية للاستدلال المبكر على وجود المرض وتوفير الاجهزة الخاصة لقياسها .

بسم الله الرحمن الرحيم

قالوا سُبحانكَ لا عِلمَ لنا الاّ ما

علّمتنا انكَ أنتَ العليمُ الحكيمُ

صدق الله العظيم
سورة البقرة : الآية (32)

العلاقة بين مضادات الاكسده الكلوتاثيون والكلوتاثيون – اس – ترانسفيريز وسكر الفيوكوز كدالات ورمية لمرضى سرطان الثدي

رسالة

مقدمة الى كلية الطب جامعة بابل كجزء من متطلبات نيل شهادة الماجستير
في الكيمياء الحياتية السريرية
من قبل

نادية حسن كاظم

بكالوريوس – علوم كيمياء (جامعة الكوفه)

ميلادية 2008