

## Relationship between Antioxidants Glutathione and Total $\alpha$ -L- Fucose as Tumor Markers in Breast Cancer Patients

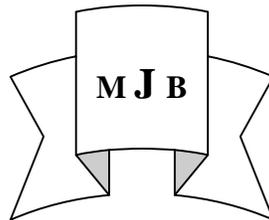
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### Abstract

Glutathione (GSH), 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(from December 2006 to December 2007) to estimate the levels of GSH and TF. The result of the study revealed that serum GSH concentration decrease in breast cancer patients, while serum TF concentration increases in the same patients.

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 ranging age from 40-49 years old. So this study recommends the use of these biomarker to early detect the disease.

### الخلاصة

تمت دراسة التغيرات في الكلوتاثيون المختزل وال - فيوكوز في مصل دم مرضى سرطان الثدي والبالغ عددهم 100 مريض وتم اخذ العينات من المرضى في مستشفى مرجان التخصصي خلال الفترة ( الأول من كانون الأول عام 2006 لغاية الأول من كانون الأول عام 2007 ) وأظهرت النتائج انخفاضاً في مستويات الكلوتاثيون المختزل مقارنة مع تراكيز الفيوكوز الذي اظهر ارتفاعاً معنوياً في نفس المرضى.

سجلت الدراسة وجود أكبر نسبة من سرطان الثدي من الدرجة الثانية.كما بينت الدراسة وجود تأثير لكل من العمر والوزن والتاريخ المرضي للعائلة بالإصابة بأورام سرطانية .

وأظهرت 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 الأجهزة الخاصة لقياسها .

### Introduction

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 [1].The breast also contains connective tissues called tascia which

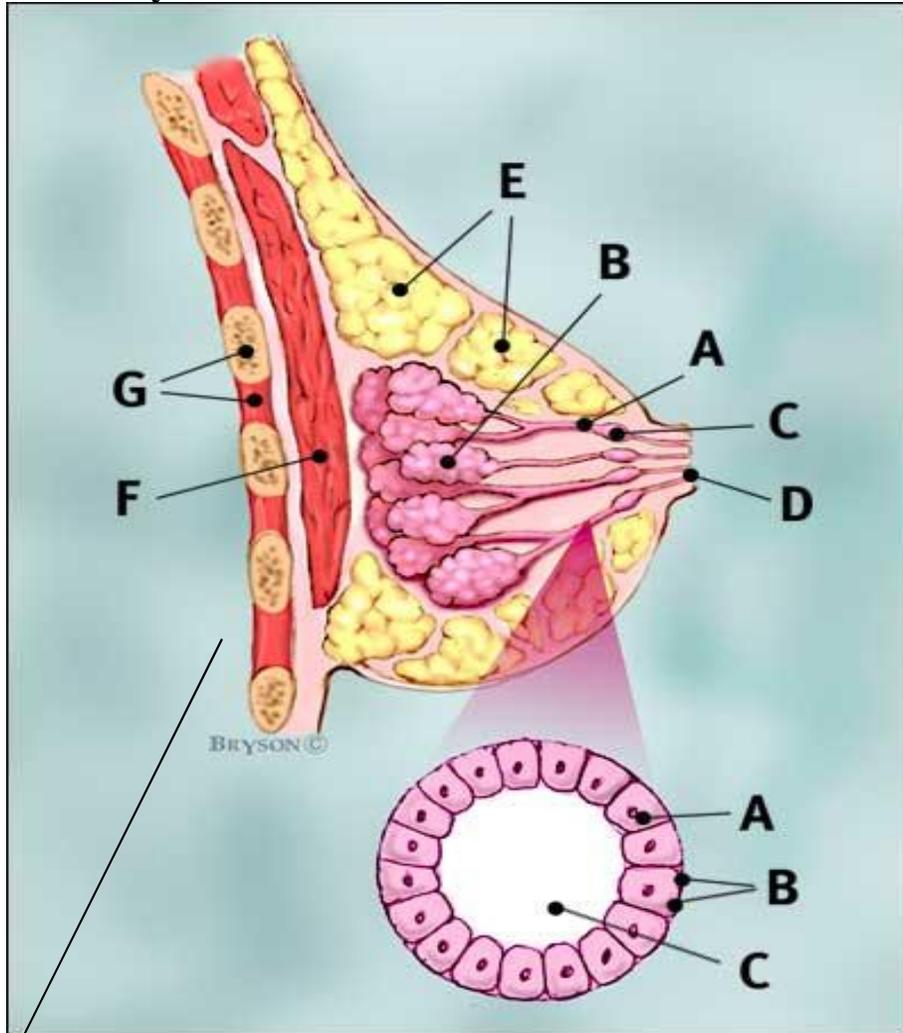
cover the milk glands and sport them. There are blood vessels, which supply the breast with nutrients and oxygen and nerves which give it sensation. [2].

Under beneath each breast is of two large flat mostly the pectoral's

muscles, which help to move the arms and under these muscles, ribs [3] .

Figure 1.

**Breast Anatomy**



**Breast profile**

- A- Ducts
- B- Lobules
- C- Dilated section of duct to hold milk
- D- Nipple
- E- Fa
- F- Pectoralis major muscle
- G- Chest wall/rib cag

**Enlargement**

- A- Normal duct cells
- B- Basement membrane
- C- Lumen (center of duct)

**Figure 1** The breast anatomy

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 [4,5].

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). [6]

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. [7,8]

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. [9,10]

Reduced Glutathione ( GSH ) or commonly Glutathione , It is a tripeptide composed of the amino acids glutamic acid , cysteine 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[11].

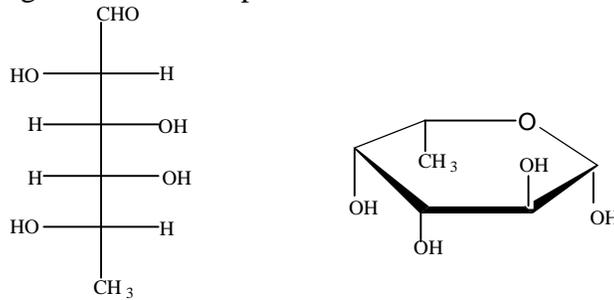
Glutathione is involved in detoxification. It binds to toxins such as heavy metals, solvents and pesticides. Glutathione transform them to the form can be excreted in urine or bile. Glutathione is also an important anti- oxidant; dietary Glutathione take from fruits raw vegetable has been associated with protection against some forms of cancer [12,16].

The science of glycobiology is exploding as various sugars and complex carbohydrates being recognized for their importance as more than just energy sources.. One of those sugars, fucose is found in wide variety of natural substances from [17]many sources and occurs in abundance in glycoproteins and glycolipds in animals and humans .Fucose – congaing 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 [18]

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 [19,20]. 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. Fucose metabolism is abnormal in cystic fibrosis, and cancer and during episode of shingles which is caused by herpes virus. Studies suggest the sugar is active against other herpes

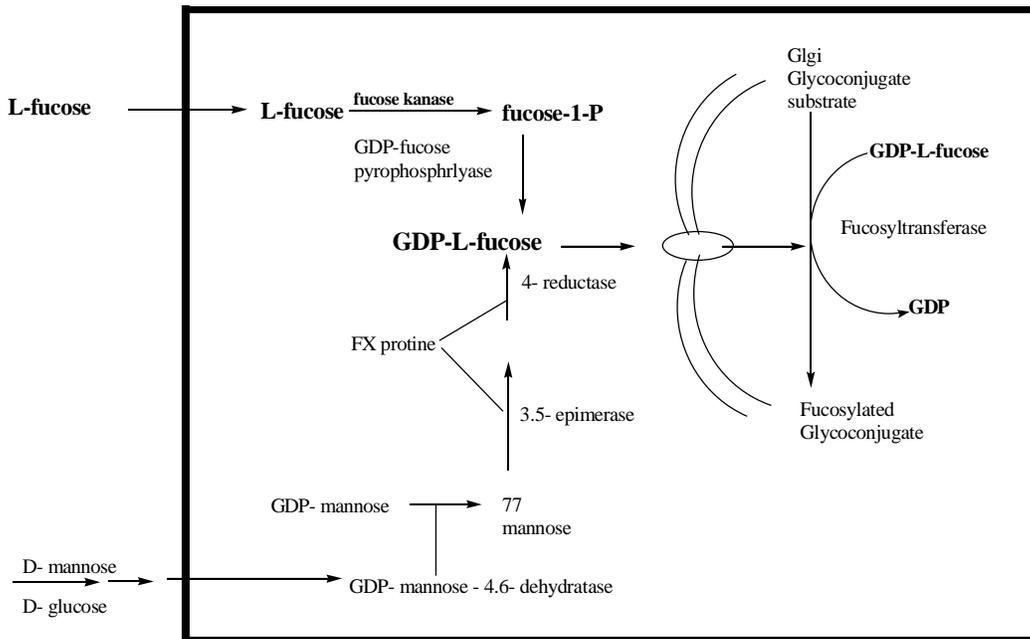
viruses including herpes I are cytomegalovirus. The saccharide also guards against respiratory tract infections and inhibits allergic reaction, [21].



**Figure 2** The structure of alpha -L- fucose

Fucose metabolism is important for formation glycoproteins and glycolipids. Endogenous fucose is produced in the sugar-nucleotide form (GDP- Fucose) from GDP-mannose via a dehydratase and an epimerase-reductase enzyme. Exogenous (i.e.dietary) fucose is

converted to fucose-1-phosphate by fucokinase and then to GDP- fucose by a pyrophosphatase enzyme. Inhibitors of fucokinase lower fucose incorporation into glycoproteins [22]. **Figure 3**



**Figure 3** Fucose metabolic pathways

A deficiency in fucosidase will lead to disease termed fucosidosis due to accumulation of fucose-containing mucopolysaccharide and glycolipids. Fucose metabolism also appears to be altered in various other diseases. [23]

**Materials and Method**

**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 hospital in hilla city. Blood sample which withdrawn from control and patients (5 ml –vein) 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 obtain from the Firms, Fluka, Hopkins and Williams, Sigma Chemicals, Merck. and used as supplied without farther purification.

**Methods**

**Determination of serum reduce glutathione**

All analytical methods such as, photometric enzymatic fluorometric, and HPLC methods used to determine tissue homogenate, erythrocytes, and serum glutathione (GSH) depend on the action of the sulfhydryl groups [24].

**Determination of total fucose (TF)**

**Principle**

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 . [25]

**Results**

The mean of reduced glutathione in blood serum had shown a decrease in its patient with breast cancer in comparison to that of control group.(Table 1)

**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, p<0.05
Control	100	2.3451	±0.755122	

According to t-test of two sample means, there was a significant 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 [26].

Also GSH is required to carry out an immune response since it is needed by the lymphocytes to multiply in order to develop a strong immune response for killing cancer cell [27].

GSH directly reduces the radicals that are critical to anti tumor activity

and 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 [26] .

**Total Serum fucose concentration .**

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 2)

**Table 2** 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	35.98221	±1.2759	P<0.01, p<0.05
Control	100	20.35458	±1.8967	

According to t- test, two samples mean, there were significance difference between the mean of TF in serum blood of patient and mean TF serum blood of control  $p < 0.01$ ,  $p < 0.05$ .Fucose is widely distributed through out the body in glycoprotiens 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 cystien fibrosis, diabetes and cancer , however there is a study that proved there were high increase in TF concentration with patient of cancers specially patients with breast cancer [28].

**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 48 hr.(Table 3).

**Table 3** 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	0.333064	±0.2857	P<0.01, p<0.05
GSH after 48 hr	100	0.0169	±0.0165	

According to T- test there was a significant 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 GSSG, and with cystein residues of proteins in the plasma as well as serum blood;  $P < 0.01$ ,  $P < 0.05$  [29] .

**Total serum fucose concentration before and after 48 hours**

very small decrease before and after 48 hr.(Table 4)

The mean of total fucose in serum blood of patient had show a

**Table 4** 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	35.98221	±3.7988	P>0.01, p>0.05
after 48 hr	100	35.90878	±3.8596	

According to t-test of two samples means , that referred there were no significant difference was found before and after 48 hr for serum blood of patients  $p > 0.01$  ,  $p > 0.05$  . Carbohydrate can be attached by glycosidic bonds to 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 [18].

**The correlation between fucose and GSH**

**Table 5** The correlation between GSH and fucose

	Mean	S D	R. correlation
(GSH ) mg/dl	0.333064	±0.2857	0.09
Fucose mg/dl	35.98221	±1.2759	

The correlation is not significant between (GSH) and ( fucose ) at level 0.05, 0.01 which  $R > 0.05$ ,  $R > 0.01$ ,

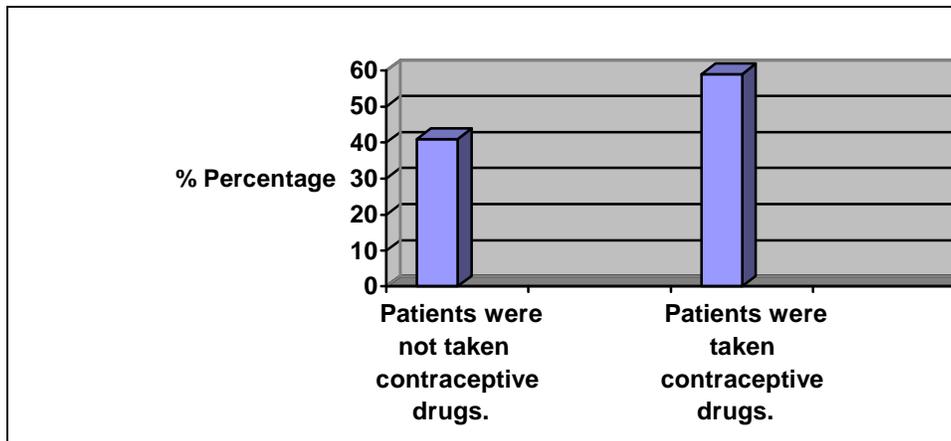
**Contraceptive drugs effect on GSH, and fucose level in serum blood:**

**Table 6** 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 are more than the patients who have not taken contraceptive drugs.



**Figure 4** The percentage of Patients were take and not taken contraceptive drugs.

**Table 7** 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	2.3451	±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 not taking contraceptive drugs and healthy control which P. Value more than 0.05 and 0.01.

The comparison between patients who have taken contraceptive drugs and healthy control shows there were significant difference between them which is P < 0.01, P < 0.05.

**Table 8** The mean of fucose in the Patients were taking contraceptive drugs and were not taking contraceptive drugs.

Group	Mean of fucose mg /dl	SD mg /dl	significance
Control	20.35458	±1.8967	
41%	35.87	±1.18558	P < 0.05, P < 0.01
59%	36.05	±1.3376	P < 0.05 , P < 0.01

According to t-test, there was a significant difference between the mean concentration of fucose to healthy control and patients were not

taken contraceptive drugs, which P < 0.05, P < 0.01.

Also, there was a significant difference between patients who

have taken contraceptive drugs and healthy control, which is  $P < 0.05$ ,  $P < 0.01$ .

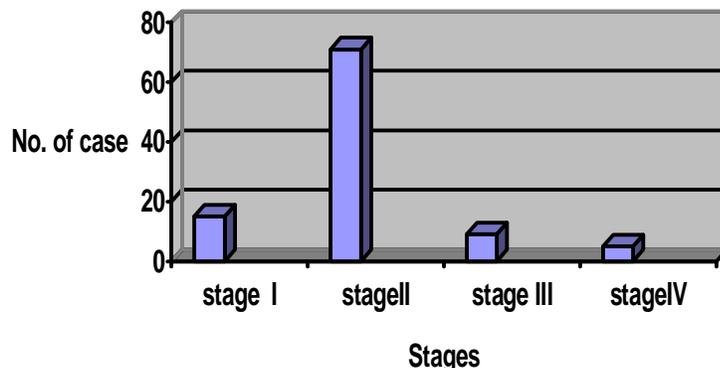
A recent study suggests a link between high dose combined oral contraceptive that were discontinued in most countries years ago and increase risk of breast cancer among women with a strong family history

**Stage and age effect on the breast cancer patients**

Table ( 9) states the stage and number of cases of breast cancer and according to the study, stage II is the most common stage with breast cancer patients where there were 71 cases.

**Table 9** The stage and the No. of cases with individual stage:

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



**Figure 5** The stage and the no. of cases

**Age effect with breast cancer**

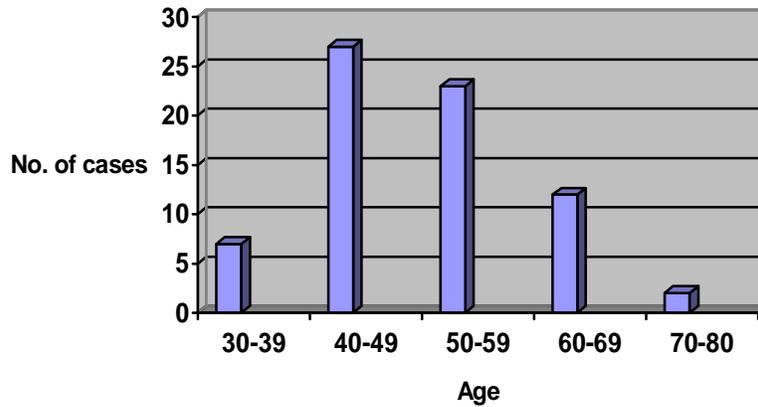
According to the previous section the most common stage with breast cancer patients is stage II, so it is

important to know the age effect for such incidence to have an idea of most common age with breast cancer.(table 10 )

**Table 10** 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.

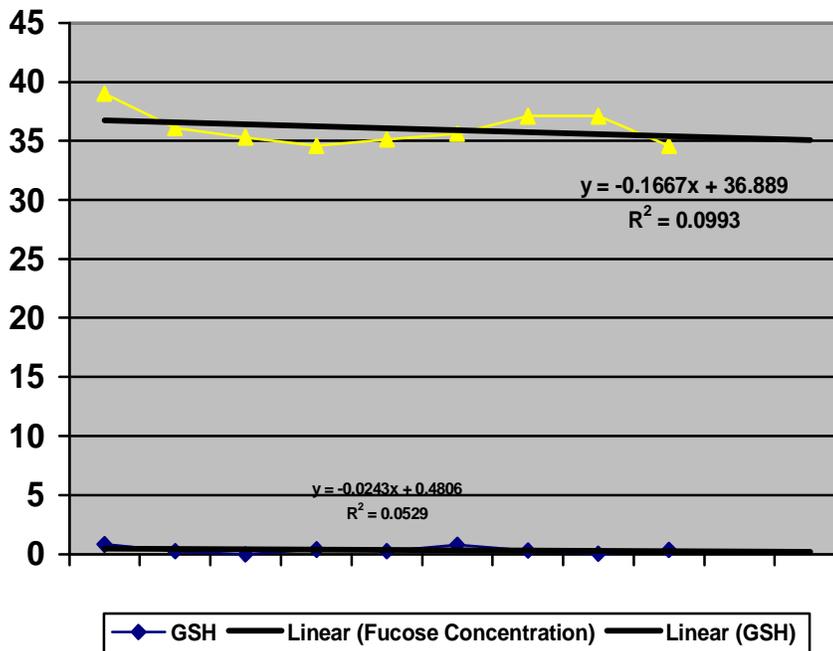


**Figure 6** The most common age with breast cancer

**The correlation among fucose , and GSH 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, it is important to know the correlation among GSH, GST and fucose with breast cancer patients and it was found that there were 12 cases at age 55 years old.



**Figure 13** Shows The correlation of fucose and GSH at age 55 years old with breast cancer patients

R is more than 0.1 there is no correlation among GSH ,GST and fucose at age 55 years old .

### **Conclusions**

1. The breast cancer affects the TF levels by increasing its level in contrast with control level.
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 patient did not take contraception drugs in contrast with patients who took contraception drugs.
3. Decreasing the level of TF control serum in contrast with patients of breast cancer, while there was proportionally decreasing in TF with patient who did not take contraception.
4. Stage and age were affected on the breast cancer patient.

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