Glutathione S transeferase as a prediagnostic tool and tumor marker in breast cancer

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Abstract: Breast cancer is one of leading causes of death in women, polymorphism of glutathione s- transferase (GSTT1) gene is known of risk factor for some environmental related diseases. The aim of the present study was to investigate the role of polymorphism in GSTT1 gene and breast cancer in Egyptian women at Sharkia governorate, and to analyze the correlation of GSTT1gene polymorphism with some hematological parameters.GSTT1 gene polymorphism were genotyped by using multiplex polymerase chain reaction (PCR) in 200 Egyptian women,100 patients suffered from breast cancer and 100 healthy women had no history of cancer, other physiological parameters were elevated as kidney function parameters (creatinine. Urea), liver function parameters (Albumin, AST, ALT, LDH), reproductive parameters (FSH, LH, PRL and Estrogen). Breast cancer women had a significance prevalence of GSTT1 null p=(0.002) than control group, GSTT1 null genotype in age group (40-60) in cancer cases p=(0.000) comparing with age group (40-60) in control group. GSTT1 null genotype in cancer group (40-60) associated with higher significance in some physiological parameters as creatinine, FSH, PRL and Estrogen.GSTT1 gene polymorphism may play an important role in pathogens and susceptibility to breast cancer in Egyptian women.
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Keywords: Breast cancer, Glutathione-s transferase T1, DNA extraction, Polymerase chain reaction

1. Introduction:

Breast cancer is one of leading causes of cancer-related deaths in women within economically developed regions of the world. Breast cancer is considered a multifactor disorder caused by both non-genetic and genetic factors. Breast cancer is the most common malignancy in women and is highly curable if diagnosed at an early stage. Traditional prognostic factors include the age, axillary lymph node status, tumor size, tumor grade, and hormone receptor status **(Fabiolaet at., 2012).**

Biomarkers can be helpful in redefining the diseases and their therapies by shifting the emphasis of traditional practices of depending on symptoms and morphology to a more rational objective molecular basis **(Manne etal., 2015).**

The glutathione-s transferases (GSTs) gene represent major group of detoxification enzyme are the main defense against oxidative stress they comprise several isoenzymes including alpha families and are known to be induced under condition of oxidative stress (**Hayes and Pulford 2011).**

The glutathione S-transferase (GST) family of metabolizing enzymes plays an important role in the metabolism and detoxification of mutagens and carcinogens. GST genes encode a family of enzymes that have major roles in catalyzing the conjugation of glutathione to a wide variety of hydrophobic and electrophilic substrates and carcinogens such as benzpyrene and reactive oxygen species (ROS) **(Kang et al., 2011).**

An increased frequency of GST-null genotypes has been associated with several malignancies, including, lung cancer, stomach cancer**,** bladder cancer, colorectal cancer, astrocytoma, and esophageal squamous cell cancer. The influence of GST on susceptibility to cancer may be influenced by a variety of factors such as smoking, diet and gender (**Smith et al., 2014).**

The GSTT1 gene polymorphism (GSTT1) was through to play a role in the susceptibility of several disease e.g. asthma and rheumatoid arthritis it was suggested to have a role in susceptibility to breast cancer **(Faramawy et al., 2009).**

In order to determine the genetic risk factors associated with breast cancer in Sharkia government we examined the known genetic polymorphisms of GSTT1 and the physiological changes that might be attributed.

2. Subjects and methods:

Patients

200 Egyptian women attending oncology department at zagazig university hospital, 100 women suffered from breast cancer at different ages and this group was divided into three age subgroups, the other 100 women (healthy females who received a routine examinations and had no findings of cancer or any other serious medical problems).

Blood sampling

A 5 ml blood samples were drawn from all subjects after an overnight fasting and divided into two portions: 1 ml of whole blood was collected into tubes containing EDTA, for genomic DNA extraction and complete blood count (CBC). Serum was separated immediately from remaining 4 ml part of the sample and stored at -20^{o}Cuntil biochemical and hormonal analyses.

Biochemical measurements:

Aspartate Aminotransferase (AST), alanine transaminase (ALT), and Albumin concentration was measured using commercially available kit (Spinreact, Girona, Spain).Total cholesterol and triglyceride levels were measured by routine enzymatic methods (Spinreact, Girona, Spain).HDL cholesterol concentration was determined and the LDLcholesterol level was calculated using the Friedewaldformula.We determined serum creatinine concentrationsusing a Jaffe reaction method (Spinreact, Girona, Spain).

Hormonal assays

We estimated FSH, LH, prolactin and estrogen concentrations using chemiluminescence immunoassay (CLIA) assay kit provided by (Immunospec Corporation, Canoga Park, CA, USA).

DNA extraction

Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol(QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany)DNA was stored at -20^{o}C till the time of use.

Genotyping of GSTT1

The GSTT1 null genotypes were detected using a multiplexpolymerase chain reaction (PCR) method (Pemble et al., 1994). Briefly, 100 ng of DNA were amplified in 50 µl multiplex reaction mixture containing 0.90 pmol of each of the GSTT1 primers as follows: forward: 5′ GAACTCCCTGAAAAGCTAAAGC-3′; and reverse primer: 5′ GTTGGGCTCAAATATACGGTGG 3′. As an internal control, the albumin gene was also amplified with 0.2 pmol of each primer:forward:5′ GCCCTCTGCTAACAAGTCCTAC 3′; and reverse primer:5′ GCCCTAAAAAGAAAA TCGCCAATC 3′, and 1X PCR mix (Taq PCR Master Mix Kit, QIAGEN, GmbH, Hilden, Germany) containing (200 µmol/l of each d NTP, 5 µl of 10 X reaction buffer, and 1.25 U Taq Gold Polymerase, and 4 mmol/l MgCl2). Amplification was performed according to the following PCR protocol: 94°C for 5 minutes, followed by 35 cycles at 94°C for 20 seconds, 64°C for 20 seconds, and 72°C for 30 seconds. A final extension step was carried out at 72°C for 7 min. The PCR products were analyzed on 3% agarose gels after staining with ethidium bromide (Figs. 1,2). The subjects were classified as either (+), when at least one specimen of the gene was detected, or (−) when they showed a null genotype. Heterozygous individuals with GSTT1 (GSTT1+/− and GSTT1+/+) were reported to present similar enzyme activity **(Seidegard et al., 1988)** and expression levels (**Bell et al., 1993)** and were pooled together for statistical analysis.

Statistical methods:

- Data were collected, arranged and reported, summarized and then analyzed using the computer program SPSS/ version 15.0). In this study qualitative data were presented by numbers and percentage and quantitative data were presented as mean and standard deviation.
- Statistical tests for quantitative variables were performed two-tailed using student t-test for parametric data and Mann-whittney for non-parametric.
- For comparing qualitative variables, chi square (x^2) test or fisher´s exact test (if the cell value was less than 5).
- All statistical tests were considered significant at $p< 0.05$.

3. Results:

Polymorphism of GSTT1 associated with breast cancer

Among the polymorphism investigation, the GSTT1 deletion had a statistically significant link with breast cancer suggesting that the GSTT1 enzyme might play a critical role in the detoxification of quinones and protection against oxidative damage in cells.

A) GSTT1 gene polymorphism in cancer group:

The products of PCR amplification that were subjected to agarosegel electrophoresis.

The PCR product was 480 bp fragment at GSTT1.

And β-globin (control gene) was 268bp as in figure (1).

The absence of the amplified product was consistent with null genotype.

Table 1 showed the result of hematological parameters comparing the three age groups of control group with the three age group of cancer group, and it estimated that there were significance increase in some parameters in cancer group (40-60) years in creatinine, FSH,PRL, and Estrogen.

B) GSTT1 gene polymorphism in control group:

The products of PCR amplification that were subjected to agarose gel electrophoresis.

The PCR product was 480 bp fragment at GSTT1.

And b-globin (control gene) was 268 as on figure bp as in figure (2).

The absence of the amplified product was consistent with null genotype.

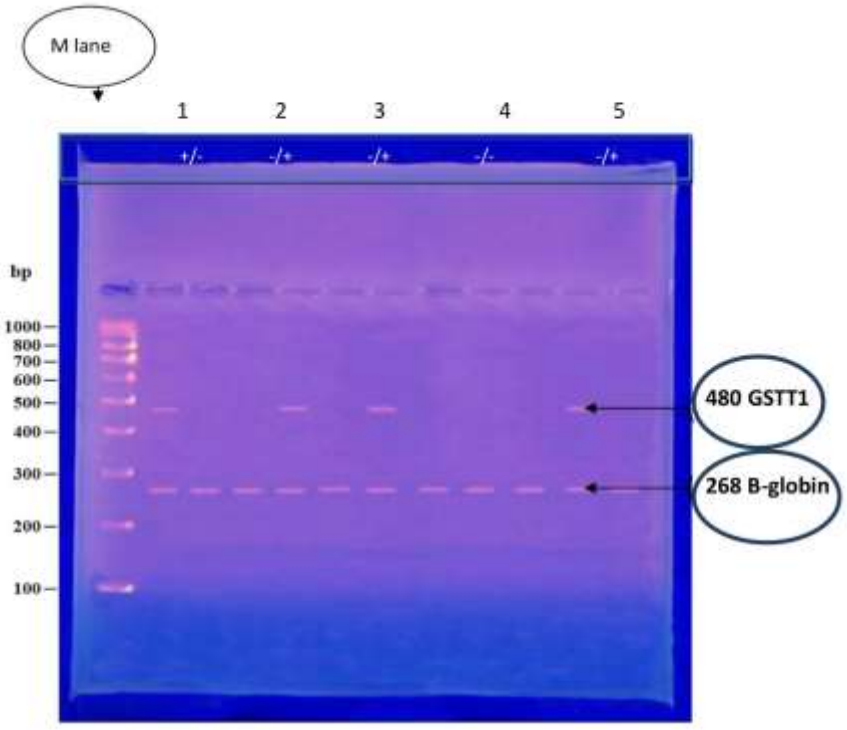


Figure (1): an agarose gel electrophoresis show PCR prodcuts of GSTT1 gene in cancer group patients (showing 480 bp DNA fragment amplification GSTT1).

- Lane M: PCR DNA marker (100bp DNA ladder purchased from Sigmacompny-St. Shokri, Cairo).
- Lane 1,2,3,4,5 shows PCR products of GSTT 1 gene (480pb) in cancer group.

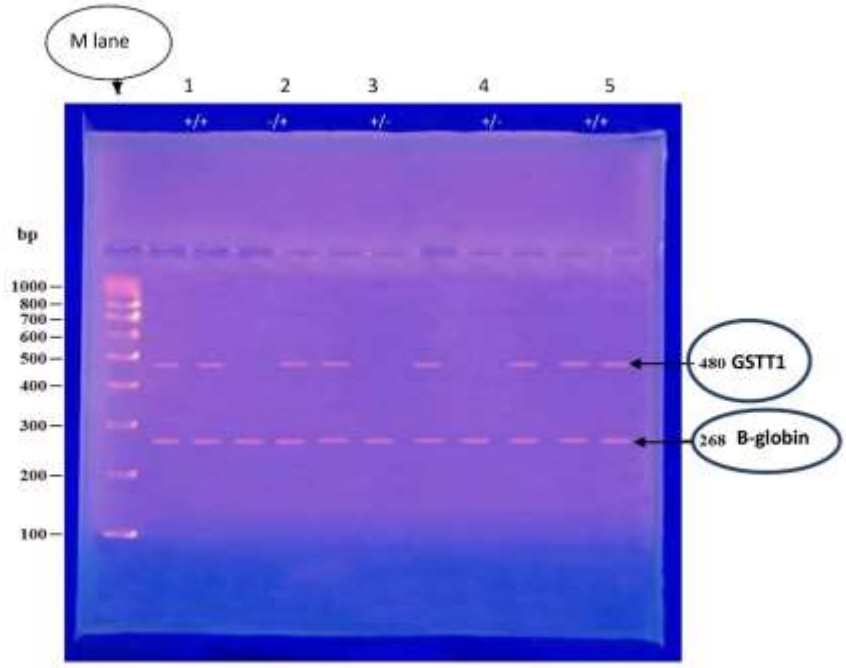


Figure (2): an agarose gel electrophoresis show PCR prodcuts of GSTT1 gene in control group patients.

- Lane M: PCR DNA marker (100bp DNA ladder purchased from Sigmacompny-St. Shokri, Cairo).
- Lane 1,2,3,4,5 shows PCR products of GSTT 1 gene (480pb) in control group.

Table(1): Demographic data and lab. finding of the two studied group

	Cases (n=100)	**Control (n=100)**	**P**
Age group "years" (%)			
(20 – 40)	15 (15%)	28 (28%)	
(40 – 60)	60(60%)	40(40%)	
(60 – 70)	25 (25%)	32(32%)	
Leucocyte*:*			
(20 – 40) Mean ± SD	13.94 ± 1.72	14.39 ± 1.67	0.314
(40 -60)Mean ± SD	11.96 ± 1.98*	13.82 ± 1.75	0.162
(60 – 70)Mean ± SD	8.94 ± 1.87*	13.40 ± 1.92	0.622
Albumin		cc	
(20 – 40) Mean ± SD	7.22 ± 3.68	8.84 ± 4.85	
(40 -60)Mean ± SD	6.51 ±4.23	7.14 ± 4.07	0.311
(60 – 70)Mean ± SD	8.35 ± 5.01	5.9 ± 3.48	0.739
AST		44.85	0.22
(20 – 40) Mean ± SD	14.7 ± 5.59	13.71 ± 4.55	
(40 -60)Mean ± SD	16.51 ±4.23	17.14 ± 4.07	0.83
(60 – 70)Mean ± SD	19.57 ± 12.16	18.36 ± 4.76	0.8
ALT			0.53
(20 – 40) Mean ± SD	24.5±14.92	29.29±5.35	
(40 -60)Mean ± SD	28.94±10.23	26.65±12.51	0.085
(60 – 70)Mean ± SD	25.8±10.15	23.77±9.03	0.635
LDH			
(20 – 40) Mean ± SD	203.36 ± 33.39	194.6 ± 30.03	0.06
(40 -60)Mean ± SD	207.36 ±58.4	169.29 ± 26.86	0.09
(60 – 70)Mean ± SD	191.4 ± 29.09	185.25 ± 25.12	0.08
Creatinine			
(20 – 40) Mean ± SD	1.47 ± 0.73	1.11 ± 0.93	0.350
(40 -60)Mean ± SD	8.44 ±5.9*	1.47 ±1.03	0.03*
(60 – 70)Mean ± SD	2.98 ± 2.26	2.88 ± 2.1	0.938
Urea			
(20 – 40) Mean ± SD	31.23 ± 9.4	29.45 ± 9.3	0.18
(40 -60)Mean ± SD	35.18 ±8.4	33.15 ±6.86	0.06
(60 – 70)Mean ± SD	28.4 ± 9.09	25.25 ± 5.12	0.42
FSH			
(20 – 40) Mean ± SD	30.47 ± 10.5	26.6 ± 9.18	0.438
(40 -60)Mean ± SD	43.36 ±21.95**	20.83 ±5.6	0.005*
(60 – 70)Mean ± SD	34.40 ± 23.23	27.7 ± 9.76	0.413
LH			
(20 – 40) Mean ± SD	30.47 ± 10.5	11.97 ± 6.49	0.43
(40 -60)Mean ± SD	43.36 ±21.95**	16.18 ±6.86	0.1
(60 – 70)Mean ± SD	34.40 ± 23.23	5.35 ± 5.12	0.680
PRL*:*			
(20 – 40) Mean ± SD	23 ± 7.69	18.7 ± 15.5	0.176
(40 -60)Mean ± SD	16.11 ± 5.65*	8.35 ± 4.55	0.004*
(60 – 70)Mean ± SD	9.6 ± 6.16	8.4 ± 5.3	0.36
E2			
(20 – 40) Mean ± SD	153.21±23.49**	134.6 ± 23.45	0.001**
(40 -60)Mean ± SD	237.6 ±48.5**	209.45 ± 26.86	0.001**
(60 – 70)Mean ± SD	30.4± 9.11	28.7 ± 5.12	0.11
		44.85	

Table (2): Distribution of GSTT1 genotypes in breast cancer patients and control group in different age groups.

	Control N=100	**Cases N=100**	**OR (C.I)**	χ^2	**P**
Genotyping:					
Present (normal)	56 (56%)	36 (36%)	2.26 (1.23 – 4.16)	8.65	**0.004***
Null	44 (44%)	64 (64%)			

- O.R. = ODDS ratio
- CI= Confidence interval * Significantly differently
- χ^2= Chi-square
- The prevalence of GSTT1 genotype is significant higher in cases when compared to controls as shown in the above to
- Chi-square revealed significant difference regarding the distribution of GSTT1 gene between cases and control
- (χ^2= 8.65, P< 0.05) with 2.26 times increase risk(O.R.: 2.26, 95%, CI: 1.43 = 5.3).
- (χ^2= 8.65, P< 0.05) with 2.26 times increase risk(O.R.: 2.26, 95%, CI: 1.43 = 5.3).

Table (3): Distribution of genotypes in different ages groups:

Group	**Control n=100**		**Cancer n=100**		**OR (CI)**	χ^2	**P**
(20-40) years	**Null**	**Present**	**Null**	**Present**	8.25(1.23-4.16)	9.46	0.002*
	7	21	11	4			
(40-60) Years	**Control n=40**		**Cancer n=60**		2.71(11.3-70.7)	21.6	<0.001*
	Null	**Present**	**Null**	**Present**			
	29	11	36	24			
(60-70) years	**Control n=32**		**Cancer n=25**		1.41(0.38-2.25)	1.2	0.27
	Null	Present	Null	Present			
	8	24	17	8			

This table (Table 3) showed that there were statistical significance differences between cancer groups and control groups in both age groups (20-40) and (60-70) but no statistical difference in the age group (60-70).

Table 4 showed that there were significance increase in some hematological parameters and gene polymorphism as in creatinine, FSH, PRL and Estrogen.

Association of some physiological parameters.

Effect on kidney function tests:

On serum creatininethere was a significant increase in Creatinine level in the second age group (40- 60) comparing cancer group with control groups.

Effect on leukocytelevel:

No significant changes were observed on leukocytic level in the three age groups of cancer group comparing with control groups.

Effect on liver function tests:

According to serum GOT and GPT (AST&ALT),LDH and Albumin levels there were no significant difference observed on comparing the three age groups of cancer group comparing with control groups.

Effect on reproductive parameters:

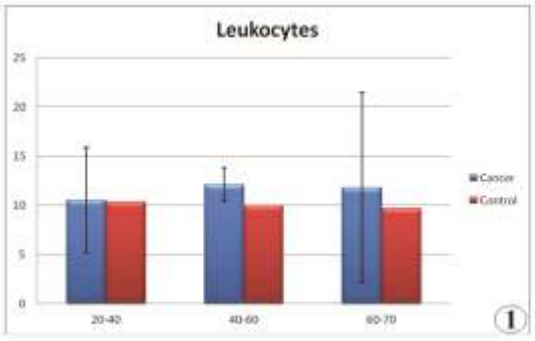
There was a highly significant increase in prolactin and FSH levels in the second age group (40-60) of cancer group comparing with control group.

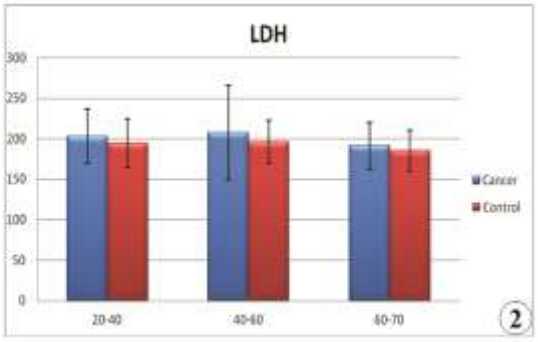
Meanwhile non significant observed on LH level in the three age groups of cancer groups and control group.

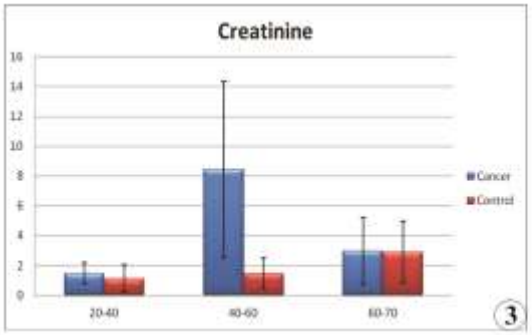
As well as no significance were observed on other risk factors as body mass index when comparing the three age groups of cancer group and control groups.

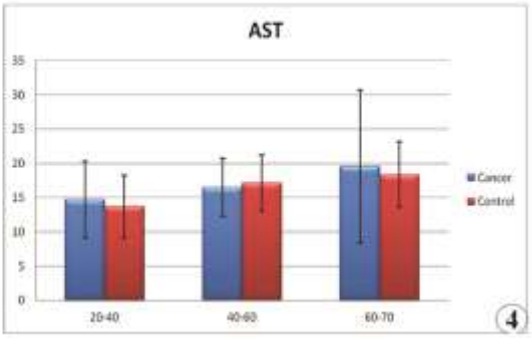
Table (4): Relation between lab. Finding and gene polymorphism in cancer group

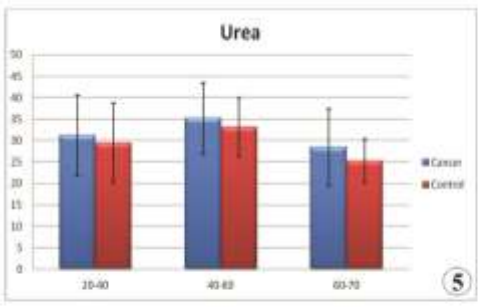
	NULL	PRESENT	P
Leucocyte*:*			
Mean ± SD	34.43 ± 16.71	37.60 ± 20.80	0.41
Albumin			
Mean ± SD	22.08 ± 12.92	25.71±16.5	0.22
AST			
Mean ± SD	16.93 ± 21.98	21.73 ± 26.78	0.33
ALT			
Mean ± SD	26.27 ± 35.3	31.27 ± 42.71	0.54
LDH			
Mean ± SD	207.58 ± 120.88	201.49±113.12	0.8
Creatinine			
Mean ± SD	12.89 ± 8.89	6.71± 3.39	0.0001**
Urea			
Mean ± SD	31.60± 26.89	36.28± 30.98	0.43
FSH			
Mean ± SD	108.23 ± 55.68	158.44± 90.69	0.0009**
LH			
Mean ± SD	16.67± 25.02	12.41±21.20	0.39
PRL			
Mean ± SD	48.71±19.15	81.60 ± 35.21	0.0001**
Estrogen			
Mean ± SD	140.4 ±27.03	181.3± 41.2	0.0001**

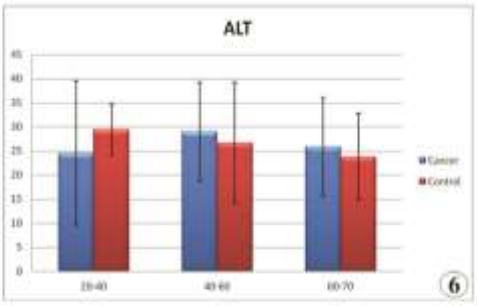


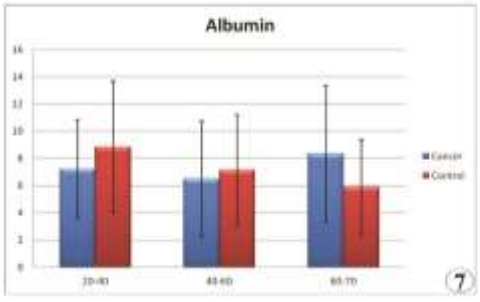


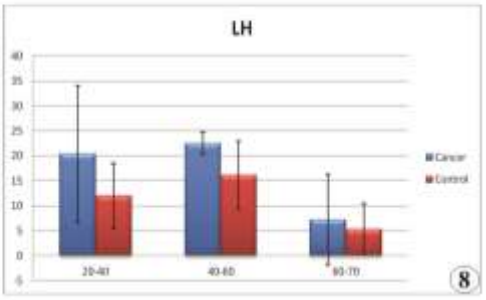


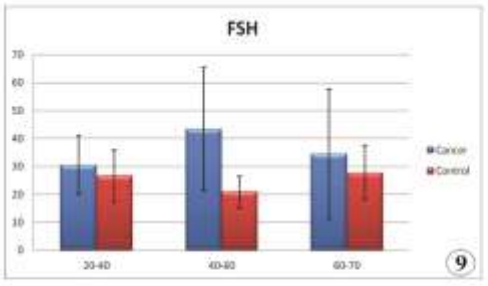


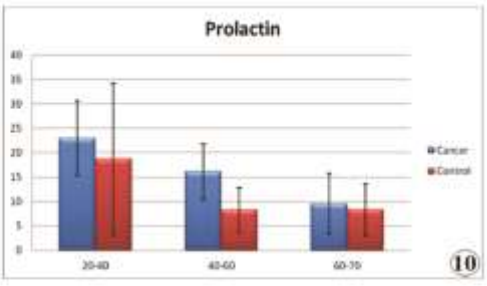












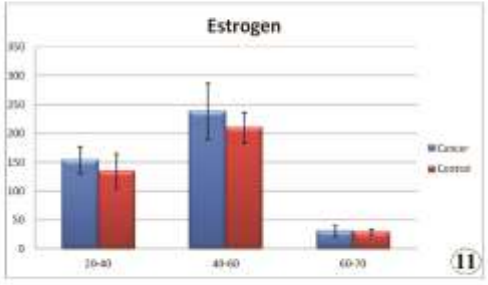


Figure (1): Leukocytes (10 9/L) level of the three age groups between cancer groups and control groups
Figure (2): LDH level between cancer group and control group in different age groups.
Figure (3): Creatininelevel(mg/dl) in cancer and control groups in different age groups.
Figure (4):AST(U/L) level between cancer group and control group in different age groups
Figure(5):Urea level between cancer group and control group in different age groups.
Figure (6):ALT(U/L) level of cancer group and control the three age groups
Figure (7): Albumin level (gm/ml) of the three age groups in cancer groups and control groups
Figure (8): LH (MIU/dl) level between cancer group and control group in different age groups.
Figure (9): FSH (MIU/dl) level in the three groups in cancer group and control group
Figure (10): PRL(mg/ml) level in cancer group and control group in different age groups
Figure (11): E2 level between cancer group and control group in different age groups.

4. Discussion

Worldwide breast cancer comprises 10.4% of all cancer incidence among women making itthe second most common type of non-skin cancer (after lung cancer) and the fifth most common cause of cancer death in 2009 breast cancer caused 519,000 deaths worldwide (7% of cancer deaths, almost 1 % of all deaths) **(yang et al., 2015).**

The estimated annual incidence of breast cancer worldwide is about one million (**Jemal et al., 2009),** In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (**Omar et al., 2013**).

Most known risk factors for breast cancer can be linked to hazardous effects of hormonal exposures **(Fisher et al, 1998**), although other risk factors such as female (1% male), aging, relative (mother or sister), menstrual history (early onset or late menopause), child birth after the age of 30, exogenous estrogen, radiation exposure and obesity are also revelant in some populations **(Annezet al.,2011).** Approximately 15% of all breast cancer cases can be attributed to familial and genetic influences (**Saslow et al., 2012).**

The etiology of breast cancer is still poorly understood with known breast cancer risk factors explaining only a small proportion of cases **(Dumitrescu RGCotarlo, 2005)**.

Genetic polymorphism is the simultaneous occurrence in the same locality of two or more discontinuous forms in such proportions **(Morinage et al., 2013).**

Genetic polymorphism is a difference in DNA sequence among individuals groups or populations sources include sequence repeats insertions, deletion and recombination **(Milikan et al., 2008).**

Genetic polymorphism may be the result of chance process of may have been induced by external agents (such as viruses or radiations) **(Miller et al., 2012).**

If a difference or a change in DNA sequence among individuals has been shown to be associated with disease it will usually be called genetic mutation rather than polymorphism **(Woodhouse et al., 2012).**

The glutathione-S transferase (GST) family of metabolizing enzymes that play on important role in the metabolism and detoxification of mutagens, carcinogens and anti-cancer drug.

Homozygous deletion of GSTT1 are present in a large proportion of individuals as a genetic polymorphism this causes absence of specific enzymayic activity **(Sprenger et al.,2011**).

Previous reports on GST null genotype as a risk factor for breast cancer gene homogenous results.

GSTT1 is involved in the metabolism (mainly inactivation, but activation is possible) of a wide range of carcinogens that are ubiquitous in the environment, also it is active in endogenous mutagenic processes and homozygous deletion of this gene result in a lack of enzyme activity **(Garcia-closas et al.,2009).**

Our obtained results seems to be conceivable with that obtained by **Zheng et al (2010)** as they reported among women with GSTT1- null genotype there was a sign 50% increase in breast cancer risk for all women combined(OR = 1.5, 95 %,CI 1.2-3.0)**.**

On contrary to our result **Garcia-Closas et al (2005)** who mentioned thatGSTT1 null genotypes were not associated with an increased risk of breast cancer (OR = 1.05 [95% CI = 0.80-1.37] for GSTM1 null; OR = 0. 86 [95% CI = 0.61-1.21] for GSTT1 null). On the contrary, a suggestion of a decreased risk of breast cancer associated with the GSTT1 null genotype was observed among premenopausal women. When considered together, no combination of the GSTM1 and GSTT1 genotypes was associated with an increased risk of breast cancer. The relationship between GSTM1 and GSTT1 gene deletions and breast cancer risk was not substantially modified by cigarette smoking.

Also another meta-analysis suggests that GSTM1 and GSTT1 polymorphism may not be associated with increased risk of breast cancer (for GSTM1: OR = 0.99; 95% CI, 0.86 - 1.4; for GSTT1: OR = 0.96; 95% CI, 0.80 - 1.14, respectively**. Marce-Amara et al.,2013.**

An Egyptian study has shown an increased risk of breast cancer in adult women associated with the GSTT1 null genotype, a significantly increased incidence of GSTT1 null genotype was found in group of patients compared to controls (34% versus 15%, P= 0.03, OR= 2.98, 95% CI 1.6= 7.6) **(Helal et al., 2013).**

A Chinese study showed that the genetic variability in GSTM1, Pi and T1 genotype may be associated with an increases susceptibility to breast cancer **(Feng et al., 2009).**

While another Chinese study showed that the GSTT1 null genotype is a low penetrate risk factor for developing breast cancer and the null genotype has been proven to have a complete absence of GSTT1 enzyme activity thus may increase the risk of breast cancer **(Chen et al., 2011).**

A Greece study showed at the overall analysis the null GSTT1 genotype was associated with elevated breast cancer risk (OR = 1.114, 95%, CI: 1.035-1.199) (P= 0.04) **(Theodoros et al., 2010)**.

Another study from Taiwan showed that among the polymorphisms investigated only the GSTT1 deletion had a statically significant link with breast cancer, suggesting that the GSTT1 might play a critical role in the detoxification of quinines and protection against oxidative damage in cells **(Chang et al., 2006).**

Contradicting our results a study from United States found that GSTM1, GSTT1, and GSTP1 genotypes don't play a strong role in susceptibility to breast cancer **(Nelon et al., 2009).**

While similar to our study in United States a case-control study proved that no evidence for an increased risk of premenopausal or postmenopausal breast cancer and GSTM1 and GSTT1 genotype **(Mansterrate et al., 2011).**

In agreement with our study **(Parkin et al., 2012)** found no significant between age groups of control group and age groups of cancer group in Leukocytic level.

In our study we found no relation between breast cancer risk and body mass index (BMI) this disagrees with **Wolmark et al., (2012)**that proved that high body mass index (BMI) associated with increased risk for breast cancer among postmenopausal women.

Another study showed that no significant effect on the incidence of breast cancer and the body mass index (BMI) **(Cheraghi et al., 2012)** which agreed with our study.

Obesity becomes an increasingly serious health problem (**Carmelli et al., 2013),**extensive genetic studies have been launched to search for genes underlying the BMI variation (**Verla-Tebit et al., 2012).**

On the other hand, many investigators are contradictory to the current results. (***Chacko J. Dorgan's, 2012;*** **Dorgan et al., 2012; Chacko et al., 2010)** found that, body weight is associated positively with the serum concentration of estradiol in postmenopausal women and with an increased risk of breast cancer and therefore could be considered to be a confounding variable.

Effect on reproductive hormones:

Our result showed high statistical difference in Estrogen level between age group (20-40), and group (40-60) in cancer groups comparing the same groups in the control groups.

This result was full agreed with another study that found a positive association between endogenous estrogens and breast cancer risk in postmenopausal women (**Jones et al., 2011).**

Our present results were compatible with (**Anitaet al., 2010)** that reported Plasma E2 levels are significantly associated with gene expression of ER-positive breast cancers.

Also agreed with **Yasuo et al. (2005)** that suggested postmenopausal Women in the high tertile of E1 levels had a significantly ($P < 0.01$) increased risk of breast cancer as compared with women in the low tertile [odds ratio (OR), 4.14; 95% confidence interval (CI), 1.44–11.87).

In the current study there was high statistically significant differences in the serum level of Prolactin of breast cancer group (40-60) compared with (40-60) of control group.

The current results disagreed with the findings of previous studies of **(Wang et al., 2003)** who proved that no significant relation between risk of breast cancer and prolactin in either pre or postmenopausal women.

This seems to be conceivable to **Susan et al.,** (**2009**) who proved that the median prolactin level in case patients was significantly higher than that in control group (9.0 versus 7.9ng/ml, P= 0.01).

In our present study we found high significance difference due to increased level of FSH between cancer age group (40-60) and control group (40-60).

Our result coincides with **Jennifer et al.(2010)** whoobserved significant correlations between the serum concentrations of FSH and breast cancer risk. The genotype predicted GSTT1 activity influenced the levels of both FSH and Estrogen levels suggesting anti-estrogenic effect on the pituitary. This may explain the observed positive association between a better prognosis and FSH levels.

This was contradicting to previous findings that studied that elevated serum estrogen levels and increased urinary excretion rates of E2 have been found in breast cancer cases, compared with controls Estrogen production in the ovary is under the control of the pituitary hormones FSH and LH**.** FSH regulatesaromatase activity whereas LH is responsible for the actual production of androgens in the ovarian thecacells, thus providing the substrate for aromization to estrogens in the granulose cells **(Burstein andSchapira2012).**

Meanwhile our study showed no statistical significance difference between the three age groups of cancer and control groups in LH level.

This did not final agree with the study that said women with high level of LH have somewhat higher serum levels of estradiol, testosterone, and sex hormone binding globulin than women have low level, indicating alterations in the bioactivity of LH (**Alder et al., 2013).**Because LH is an important regulator of steroidogenesis, the variant of LH may affect the levels of endogenous sex hormones and the subsequent risk of hormone-dependent cancers (**Arslan et al., 2002).**

FSH and LH levels in patients with benign and malignant tumors of breast were significantly different

(**Rzepkaet al., 2004).**Elevated FSH levels unlike clinical menstrual status did not have a significant correlation with breast cancer risk.

Meanwhile our findings agreed with some investigators who reported that there were no significant differences in FSH and LH serum levels between breast cancer patients and control group (**Paganini-Hill et al., 2013).**

Effect on liver function parameters:

Our results demonstrated that liver parameters AST, ALT, LDL and Albumin showed no statistical difference between age groups of cancer group and control groups.

This was contradicting to another study that showed a significance elevation in AST, ALT, and LDL in cancer group comparing with control group **(Fatiha et al., 2011).**

Meanwhile this agreed with **(Faizalet al., 2013)** who proved that no statistical difference in Albumin level between cancer groups comparing with control groups.

Effect on kidney function parameters:

Our results illustrated that there is slight significance elevation difference in serum creatinine betweencancer age group (40-60) when compared with control age groups, this were in accordance with**Hong et al., (2013)** that full agree with our study and proved that creatinine has significance at p=0.005 associated with breast cancer and subtypes of breast cancer.

Also the current study showed no statistical significance difference between cancer groups and control groups in serum urea.

This agreed with**Prakruti Dash et al**.(**2001)** who assumed thatMalignant breast disease cases documented a prominent rise in serum urea with no significant alteration in serum creatinine as compared with control.

Conclusion:

We conclude that the GSTT1 null genotype is a significant risk factor for breast cancer (BC) and it can be used as a tumor marker and pre diagnostic tool for early diagnosis of the disease.

GSTT1 gene polymorphism associated with changes in some physiological parameters as creatinine, estrogen, prolactin and FSH.

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