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Molecular and Biochemical Study for the Association of CYP1A1 Gene Polymorphisms with Increase the Risk of Breast Cancer

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Abstract: As a prevalence disease, breast cancer studies focus on the variations occurring with genes involved on the carcinogenesis. The CYP1A1 one of the most important cytochromes in (CYP1) family, which is exists at most in tissues (extrahepatic) and contributing in the metabolism process of a wide kinds of the xenobiotics, as well as many of endogenous substrates. Cytochrome CYP1A1 catalyzes several of reactions, but there is a unique reaction, hydroxylation of an aromatic ring at an unoccupied position producing of high reactive species that can cause carcinogenic mutations, considered to be the hallmark of CYP1A1 to initiate the carcinogenesis. so that one of the most interesting genes was CYP1A1.The current study was included 100 patients affected with breast cancer and others 100 healthy women as control group, where aimed to evaluating the role of (CYP 1A1) gene polymorphisms and the association with the risk of breast cancer among Egyptian women and its relation with biochemical investigations, hematological parameters, and tumor marker.

keywords: CYP1A1 rs4646903: c.*1189 T > C gene; breast cancer

1.Introduction

The breast cancer is most popular malignancy in Egyptians women, with percentage approximately 32 % of cancer cases in women, which breast cancer the basic reason of cancer related with mortality, the result refers to 15% of totally deaths per year worldwide. ***(1).*** There are more than 1.3 million patients affected with cancer are diagnosed every year around the world ***(2).*** The single average of risk in developing the breast cancer was (1 from 173) for ages of 40 years old and approximately (1 case in 1,500) with age of 30 years (***3***). The western women have lifetime risk diagnosed with breast cancer is around 12% (***4***). The histopathological of breast cancer classified based on the variety of the morphological features of the tumor cells **(*5*)**. There are many risk factors for breast cancer such as; Age , Body Mass Index(BMI), , Hormonal factors, menopause drugs like Hormone Replacement Therapy(HRT) , breast cancer family history, exposure to radiation, and the family history related to Breast Cancer genes 1 and 2 (BRCA1 or BRCA2) (***6)*** in addition to genetic factors, breast cancer susceptibility genes high effective genes (*BRCA1, BRCA2 and PTEN*) and low effective genes (*CYP450, GSH, UGTA*) that encode xenobiotic metabolizing enzymes in phase I and II of metabolism (***7***). Although, the family history prevalence is low relative, but it gives a strong increasing in the risk and gives a marker of risk for physicians to recognize and advice women (***8)***. Phase I enzymes (cytochrome P 450 family, CYP19A1 and CYP 1A1) are responsible for oxidizing many carcinogenic compounds, giving reactive metabolites which then are phase II enzymes detoxified it, such as Glutathione S- transferases P1 (GSTP1) (***9***). The mutagenicity and oxidative DNA damage can stimulate carcinogenesis, many presumably functional in (CYP1A1) polymorphisms were identified as a possible associated with breast carcinoma**(*10*).**

The Cytochrome (CYP1A1) gene is supposed to involving in breast carcinoma due to the important role in the metabolism of the aromatic compounds such as polycyclic aromatic hydrocarbons and in the estrogens oxidative metabolism that perhaps increase risk of cancer because the effect of oxidative stress.

The (CYP1A1) gene Polymorphisms have association with increase the activity of Aryl Hydrocarbon Hydroxylase, this lead to increase the risk of breast cancer. ***(11).*** The Cytochrome (CYP1A1) enzyme have important function in drug metabolism and the activation process of procarcinogen (***12).*** There are many genes involving in functions, synthesis, secretion and metabolism of steroid hormones, where the family of P 450 have the import role in this process. The most important gene CYP1A1, have functions to control the concentration of estrogen and androgen hormones (***13).*** These catechol estrogens are further oxidized to quinone metabolites by peroxidases or phase I metabolizing enzymes of CYP450 (CYP)1A1 and CYP1B1, respectively ***(14).*** The breast cancer tumors related with express (CYP1A1) gene. These feedbacks encourage us to evaluate the hypothesis of promotions the breast carcinoma by (CYP1A1) by studying the effects of (CYP1A1) gene ***(15).***

Aim of the Work:-

The aim of our study was to estimate the association between CYP1A1 rs4646903: c.*1189 T > C gene polymorphism and the risk factor of breast carcinoma among Egyptians women.

2.Materials and methods:-

Healthy controls and breast cancer patients*:*

This study included 100 patients. The mean of ages 46.5 ±SD ± 7.8 years. Patients were collected randomly from the Outpatients' Clinic of oncology Center, Mansoura University, Egypt. For comparison, a control sample was token accordingly 100 healthy unrelated subjects. Their mean age was 47 ± 9.9 years. An approval was obtained from the local ethical and scientific committees in addition to an informed approval that was token from all volunteers in this study. The data of patients included (age, sex, residence, parental consanguinity, family history of breast cancer, occupation, education and laboratory investigations.

Sampling:

Samples were collected by taking 3 ml of venous blood from each patients and controls via EDTA tubes for DNA extraction and hematological analysis. Another sample of 5 ml venous blood was token for biochemical investigations. DNA extracted then it analyzed by PCR, followed by gel electrophoresis for detection CYP1A1 gene polymorphisms.

Detection of genotypes of CYP1A1

DNA extraction **(Genejet ™ Genomic DNA Purification Kit #0721**) :

The DNA isolated from whole blood according to the instructions of the manufacturer of kit purchased by (**Fermentas , K0721 , USA**).

Procedure:

Lysis Solution 400 µl and 200 µl of whole blood were added to Eppendorf contained of 10 µl of (proteinase K) and mixed gently by vertexing in order to obtain a uniform suspension, then the sample was incubated at 56 Celsius degree, then vortexing gently, using shaking water bath for 0 minutes followed by addition of 200 µl of ethanol (96-100%) then mixed by vortex.

Then transferring the prepared lysate to purification column (gene jet genomic DNA) , then centrifugated for one minute at speed (6,000 × g). After discarding the sample tube contains solution of flow-through, the Column was place for (2 ml) sample tube.

The Wash buffer I with volume 500 µl was added, then centrifuged for one minute at (8000×g), then discarding and place back the purification into the sample tube. Again (500 µl) of wash buffer II added to the column, Centrifuged for 3 minutes at (12,000 × g).

The sample tube contains the solution that was discarded, the Column was transferred to a (1.5 ml) sterile micro centrifuge tube then by adding elution buffer (200 µl) slipping to the bottom of the column, elution of (genomic DNA) takes place. Incubate for 2 minutes at the temperature of room 25C, then centrifuge for 1 minute at (8000 × g) . The last step is , discarded purification column and the purified DNA used directly or stored at (-20 Celsius degree) .

Detection of extracted DNA:

DNA samples were analyzed by horizontal agarose electrophoresis through 1.0% agarose slab gel. The gel containing DNA samples was submerged in 1× Tris-Phosphate-EDTA buffer

(TPE) and electrophoresed at 200 volts for 20 minutes. Then visualized the DNA by staining with ethidium bromide, and photographed the gel by digital camera.

Polymerase chain reaction (PCR):

Reagents and materials for PCR:

-Master Mix, Ladder DNA marker (100 bp) (fermentas) and Agarose Powder (Laboratories Conda, Madrid, Spain).

Ethidium bromide 0.5%: Distilled water 100 ml added to solution of Ethidium bromide (1.25 mg/ml) : ethidium bromide 0.25 gm and stirred by magnetic stirrer for several minutes to ensure completely dissolve for the dye. The tube wrapped with aluminum foil and stored in the refrigerator at 4˚C.

Tris-Phosphate-EDTA buffer 10x (TPE 10x): Tris base powder (43 g), EDTA powder (18.5 g) and phosphoric acid (40 g) were dissolved in distilled water and the volume was completed to 1 liter. The solution was stored in the refrigerator at 4˚C.

Polymerase Chain Reaction Technique of CYP1A1 Gene Polymorphism Conducted According to (Adem Altunkol, 2018):

The (CYP1A1) (rs4646903) c *1189 T > C), in 3'-untranslated region (3'- UTR) polymorphic site was inspected by the PCR (RFLP technique). The primers of CYP1A1: c.*1189T > C used to detect the alleles and genotypes status of the gene.

Forward and reverse

Forward 5'-cagtgaagaggtgtagccgc-3'

Reverse 5'-taggagtcttgtctcatgcc-3'

The DNA amplified in 25 µl reaction volume contained 13 µl of PCR master mix, 4 µl of each primer (BioBasic Inc, Ontario, Canada), 4 µl of genomic DNA. For the CYP1A1: polymorphic site c.*1189T > C, program of PCR applied at 94 Celsius degree for 3 minutes for the initial denaturation process, then 35 cycles with 94 Celsius degree for 45 sec, 59 Celsius degree for 45 sec, and 72 Celsius degree for 45 second for elongation step, and then 72 Celsius degree for 5 minutes for the later extension step.

Ten microliters of the yield of PCR in a 30 microliter volume for CYP1A1: c.*1189T > C separately digested with (1.5 Units of MspI (HpaII) (Fermentas, St. Leon-Rot, Germany), respectively, at 37celsius degree for 2Hrs . The digested of PCR products were electrophoresed with 2 % agarose gel and visualized using ethidium bromide under ultraviolet illumination, the digested (CYP1A1: c.*1189 T allele produced two fragments of (209bp and 133), and the C allele 342 bp produced fragment.

Sampling

DNA extracted and the CYP gene was amplified by PCR, followed by analysis in agarose gel electrophoresis for detection of gene polymorphisms for CYP1A1.

Statistical analysis

The genotype and allele frequency of the genes were tested for both patient and control group using chi-square test, Fisher exact test, quantitative (numerical) parameters analyzed by t-student test Odds ratio (OR), confidence intervals (CI) and P-values were calculated using unconditional logistic regression and adjusted estimate the association between genotypes or some other clinicopathological data and the risk of breast cancer. In this research.

3.Results and Discussion:

Clinical Parameters for breast cancer patients compared with control group. The result in **Table 1.** showed that there are no significant differences between the breast cancer patients and control group regarding the age and age groups, which the p value > 0,05. The frequencies of tumor staging, Stage 1 (23 %), Stage 2 (64%), Stage 3 (9%), and Stage. 4 (4%). For the frequencies of tumor grade, the obtained results were Grade 1, (22%), Grade 2 (52%), Grade 3 (26%). Regarding the family history, results of this study showed that the represent of Positive breast cancer (36%), and Negative breast cancer (64 %).

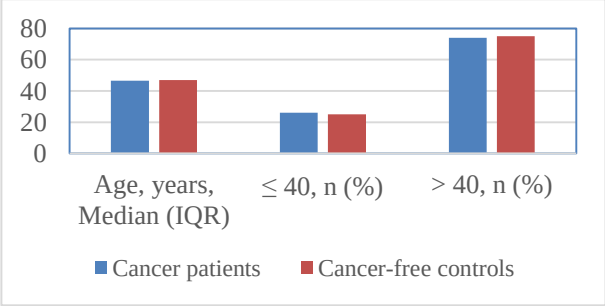


Fig 1. Clinical parameters (Age and age

group) for breast cancer patients compared with control group.

Table 1. Clinical parameters (Age and age group) for breast cancer patients compared with control group

Variable	Cancer patients (n=100)	control (n=100)	P-value
Age, years, Median (IQR)	46.5 (40.0-55.7)	47.0 (40.2-53.7)	0.531
Age groups			
≤ 40, n (%)	26 (26.0)	25 (25.0)	1.0
> 40, n (%)	74 (74.0)	75 (75.0)	
Tumor staging			
Stage 1, n (%)	23 (23.0)	---	NA
Stage 2, n (%)	64 (64.0)	---	
Stage 3, n (%)	9 (9.0)	---	
Stage 4, n (%)	4 (4.0)	---	
Tumor grade			
Grade 1, n (%)	22 (22.0)	---	NA
Grade 2, n (%)	52 (52.0)	---	
Grade 3, n (%)	26 (26.0)	---	
Family history			
Positive, n (%)	٣٦ (36.0)	---	NA
Negative, n (%)	64 (64.0)	---	

The Genotypic and Allelic frequencies for the (CYP1A1 (rs4646903)) Variants.

Table 2. The genotypic and allelic frequencies of CYP1A1 (rs4646903;g.3801T>C) gene polymorphisms for breast cancer patients compared with control group.

Genetic polymorphism	Cancer patients	Cancer-free controls	OR (95% CI)	P value
Genotypic frequencies	n (%) 100	n (%) 100		
TT	51 (51.0)	60 (60.0)	1.0	
TC	42 (42.0)	37 (37.0)	1.33 (0.75-2.38)	0.38
CC	7 (7.0)	3 (3.0)	2.74 (0.67-11.17)	0.19
HWE	$\chi2$=0.174, p=0.68	$\chi2$=0.924, p=0.34		
Allelic frequencies	n (%) 200	n (%) 200		
T	144 (72.0)	157 (78.5)	1.0	
C	56(28.0)	43 (21.5)	1.42 (0.90-2.24)	0.16

The genotypic frequencies of CYP1A1 (rs4646903 ; g.3801T>C) gene polymorphism showed in this study **(Table 2.)** that the genotype frequency (TT) in cancer patients (51%) was less than control group (60 %), while genotype (TC) in breast cancer patients (42%) was higher in the control group (37%), regarding to (CC)genotype in breast cancer patients (7%) was higher than control group (3%).

Hardy-Weinberg equilibrium (HWE) showed that there is no significant difference between the allelic frequency of (T) allele in the breast cancer patients (n=144, 72%) compared with the frequency of (T) allele in control group

(n=157, 78.5%). For the allelic frequency of (C) allele, the (HWE) showed that there is no significant difference between breast cancer patients (n=56, 28%) compared with control group (n=43, 21.5%) where the P value =0.16. The results obtained from **(16)** study suggested that supposedly (CYP1A1) polymorphisms individually didn't plays significant function in the breast cancer risk in Indians women. CYP1A1 rs4646903 T > C polymorphism have several studies due to their probably participation in increasing the risk carcinomas such as, lungs cancer, leukemia and breast cancer **(17).**

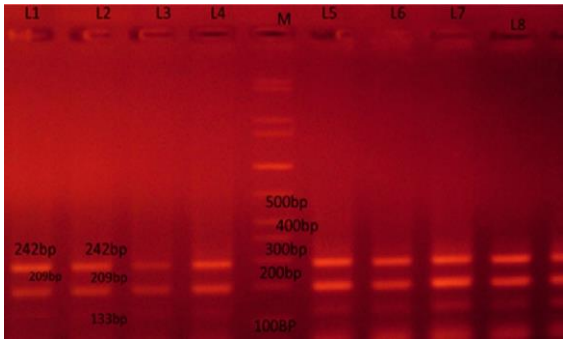


Fig (2) The photomicrograph shows heterozygote mutation TC of CYP1A1 (rs4646903;g.3801T>C) gene polymorphism 1n(lanes 1-9)bands appeared at 133,209 for T allele and 342 for C allele.

M represent DNA ladder 100bp (ferments).

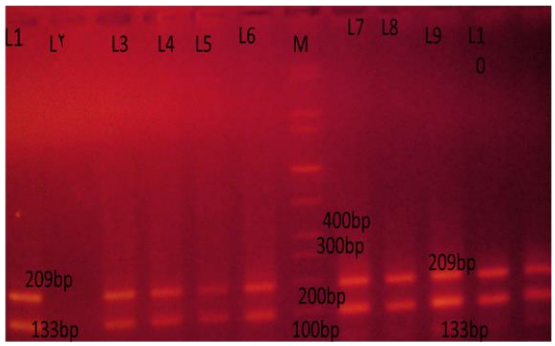


Fig (3) The photomicrograph shows homozygote mutation TT genotype of CYP1A1 (rs4646903;g.3801T>C) gene polymorphism 1n lanes 1, 3-11 bands appeared at 133,209 for T allele). Lane 2 showed negative control without DNA. M represent DNA ladder 100bp (ferments).

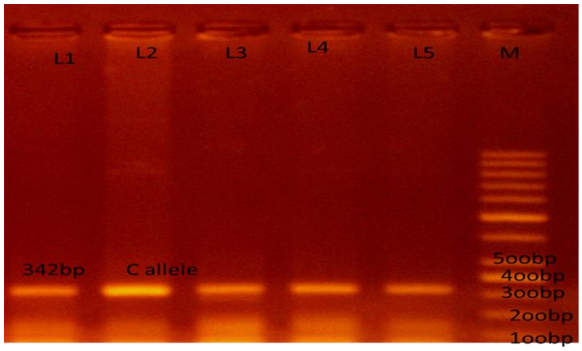


Fig (4) The photomicrograph shows homozygote mutation CC genotyping of CYP1A1 (rs4646903;g.3801T>C) gene polymorphism 1n lanes (1 – 5) bands appeared at 342 bp for C allele. M, represent DNA ladder 100bp (ferments).

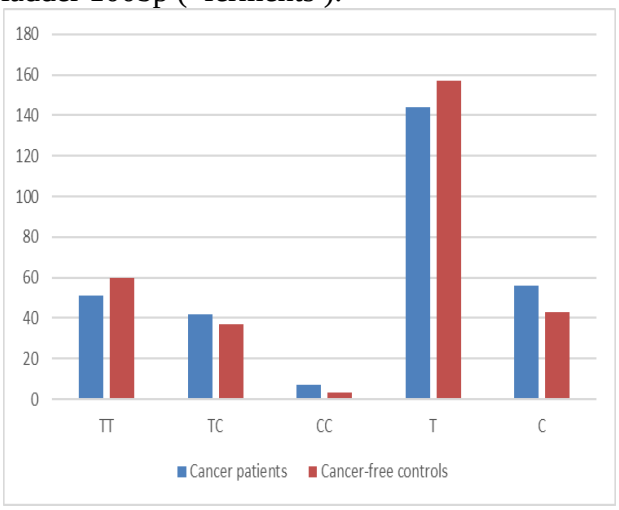


Figure 5. The genotypic and allelic frequencies of CYP 1A1 (rs4646903) for the cancer patients compared with cancer-free control

Genetic Association models of CYP1A1 (rs4646903; g.3801T>C) Variants and the Risk of Breast Cancer risks.

The obtained results as showed in **Table 3.** indicate that the codominant model (TT) of CYP1A1 gene had had no significant association to the risk of breast cancer (OR (95% CI) = 1 and P value =0.19). For the codominant model (TC) of CYP1A1 gene; the results showed that there is no association with the risk of breast cancer (OR = 1.37, 95% CI = (0.76-2.45). For the codominant (CC) of CYP 1A1 gene, our obtained results showed that there is no association with the risk of breast cancer (OR = 3.21, 95% CI = (0.77- 13.42).

For the dominate model (TT) of CYP1A1 gene the results showed there is no association with the risk of breast cancer (OR (95% CI) = 1, P value =0.17). Regarding the model (TC+CC) of CYP1A1 gene the results showed there is no association with increase breast cancer risk factor (OR= 1.49, 95 % CI= (0.85-2.62).

The results of this study indicate that the Recessive model (TT+TC) of CYP1A1 gene had no association to the risk of breast cancer (OR (95% CI) = 1, P value = 0.13). For the recessive model (CC) of CYP1A1 gene, our obtained results indicate that there is no association with increase the risk of breast cancer (OR = 2.80, 95% CI = (0.69-11.41).

This study showed that the overdominant model (TT+CC) of CYP1A1 gene had had no association to the risk of breast cancer (OR 95% CI) = 1, P value =0.45). Regarding the overdominant (TC) of CYP1A1 gene, our obtained results indicate that there is no association with the risk of breast cancer (OR= 1.24, 95% CI = (0.70-2.20).

The biggest share of the studies regarding (CYP 1A1) gene polymorphism and the related with breast cancer were applied specially in developed countries **(18).** Results from **(19)** study suggest that (rs4646903) in the (CYP 1A1 gene) probably a genetic marker for breast cancer prediction in Chinese women. Also, **(20)** deduce that (CYP 1A1 polymorphism) is a portability risk factor for the postmenopausal Chinese women in Taiwan.

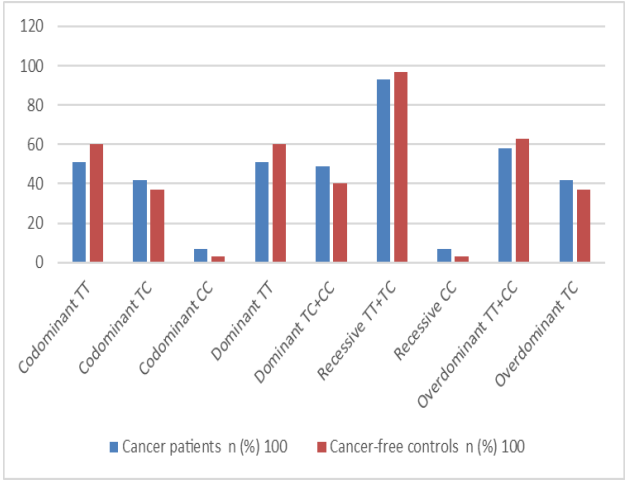


Figure 6. Genetic association models of

CYP1A1 (rs4646903) variants and the risk of breast cancer.

Table 3. The Genetic association models of CYP1A1 variants and the risk of breast cancer.

Model	Genotypes	Cancer patients n (%) 100	Cancer-free controls n (%) 100	*OR (95% CI)*	*P value*
Codominant	TT	51 (51.0)	60 (60.0)	1.0	0.19
	TC	42 (42.0)	37 (37.0)	1.37 (0.76-2.45)	
	CC	7 (7.0)	3 (3.0)	3.21 (0.77-13.42)	
Dominant	TT	51 (51.0)	60 (60.0)	1.0	0.17
	TC+CC	49 (49.0)	40 (40.0)	1.49 (0.85-2.62)	
Recessive	TT+TC	93 (93.0)	97 (97.0)	1.0	0.13
	CC	7 (7.0)	3 (3.0)	2.80 (0.69-11.41)	
Over-dominant	TT+CC	58 (58.0)	63 (63.0)	1.0	0.45
	TC	42 (42.0)	37 (37.0)	1.24 (0.70-2.20)	
Log-additive	---	---	---	1.52 (0.94-2.46)	0.09

Genotypic Frequencies of CYP1A1 (rs4646903) Variants Stratified by the Clinical, Biochemical, Hematological Parameters, and Tumor Markers Among Breast Cancer Patients.

Clinical parameters:

The data in **Table 4.** showed that there is high significant association between genotype frequencies of CYP1A1 (rs4646903) and the parameters (age and age group) for all cases with breast cancer patients, where the P value for age parameter = 0.028 and P value for age group = 0.001.

In other hand, the results showed that there is no significant association between genotypic frequencies of CYP1A1 (rs4646903) and the clinical parameters (tumor staging (S1 +S2/S3 + S4), tumor grade (G1 + G2/G3) and family history) for all cases with breast cancer patients which the P value > 0.05.

Table 4. The clinical parameters for genotypic frequencies of CYP1A1 in breast cancer patients.

Parameter		BC patients			
CYP1A1 (rs4646903)		**TT (n= 51)**	**TC (n= 42)**	**CC (n= 7)**	**P value**
Age , years	Media n (I QR)	51 (44-59.0)	44.5 (40.7-52.5)	38.0 (35.0-40.0)	0.028
Age groups (≤40/>40)	n (%)/n (%)	10 (19.6) /41 (80.4)	10 (23.8) /32 (76.2)	6 (85.7) /1 (14.3)	0.001
Tumor staging (S1+S2/S3+S4)	n (%)/n (%)	45 (88.2) /6 (11.8)	36 (85.7) /6 (14.3)	6 (85.7) /1 (14.3)	0.932
Tumor grade (G1+G2/G3)	n (%)/n (%)	37 (72.5) /14 (27.5)	33 (78.6) /9 (21.4)	4 (57.1) /3 (42.9)	0.462
Family history (Positive/Negative)	n (%)/n (%)	20 (39.2) /31 (60.8)	15 (35.7) /27 (64.3)	1 (14.3) /6 (85.7)	0.435

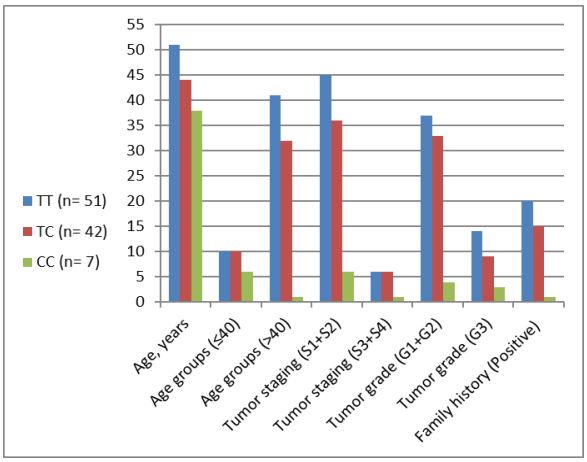


Figure 7. The clinical parameters (Age, Age group, Tumor Stage, Tumor Grade and family history) for Genotypic frequencies of CYP1A1 (rs4646903) in breast Cancer patients

Biochemical investigations

The data in **Figure 8.** showed that there is no significant association between the genotypic frequencies of CYP1A1 (rs4646903) and the biochemical parameters (ALT, AST, Albumin, Total bilirubin, ALP, Creatinine, Uric Acid) for all cases with breast cancer patients (P value >0.05).

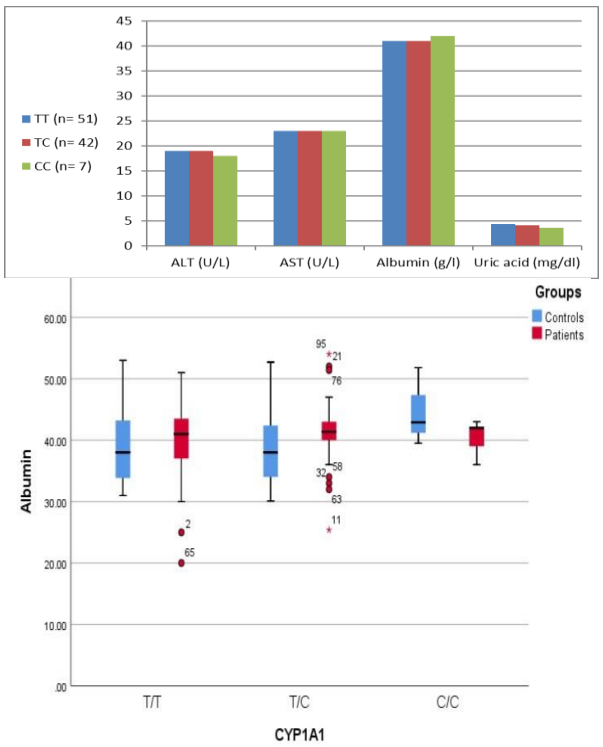
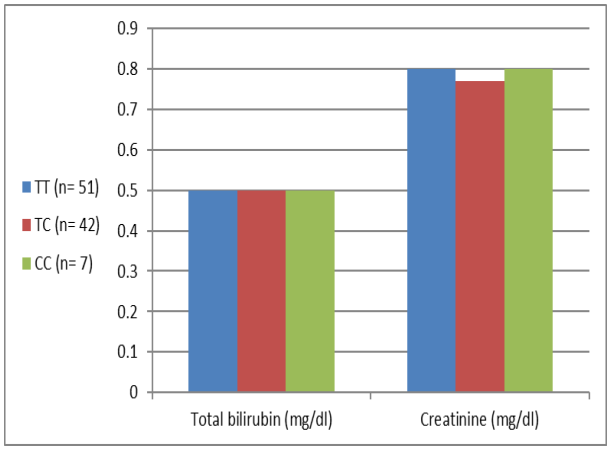


Figure 8. The biochemical parameters (ALT, AST, Albumin and Uric Acid) for genotypic frequencies of CYP1A1 (rs4646903) gene in cancer patients.



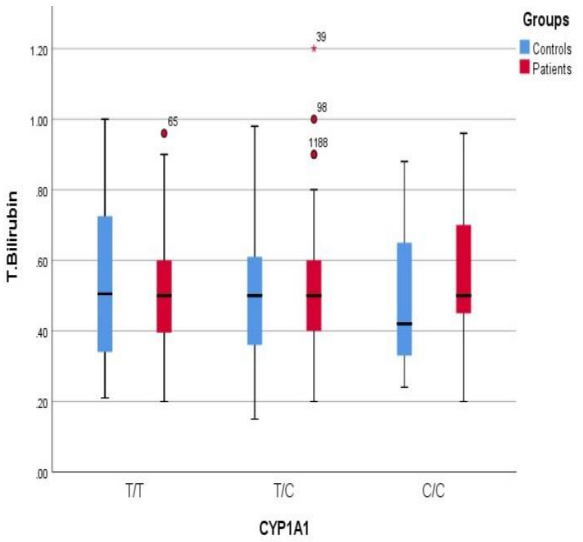


Figure 9. The biochemical parameters (Total Bilirubin and Creatinine) for genotypic frequencies of CYP1A1 (rs4646903) gene in Cancer patients

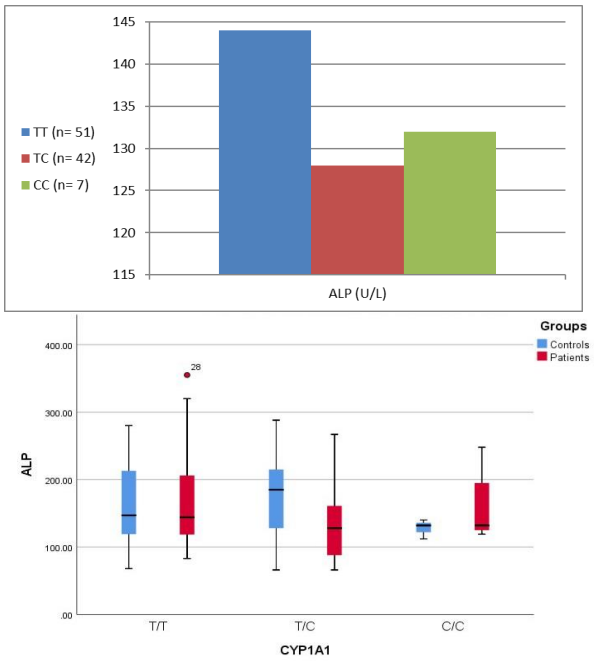


Figure 10 The Biochemical parameters (ALP) for Genotypic frequencies of CYP1A1 (rs4646903) gene in Cancer patients.

Hematological parameters

The data in the **Figure 11.** Showed that there is no significant association between the genotypic frequencies of CYP1A1 (rs4646903 and the hematological parameters (WBCs, Lymphocytes, RBCs, Hemoglobin, Platelets) for all cases with breast cancer patients, which the P value > 0.05.

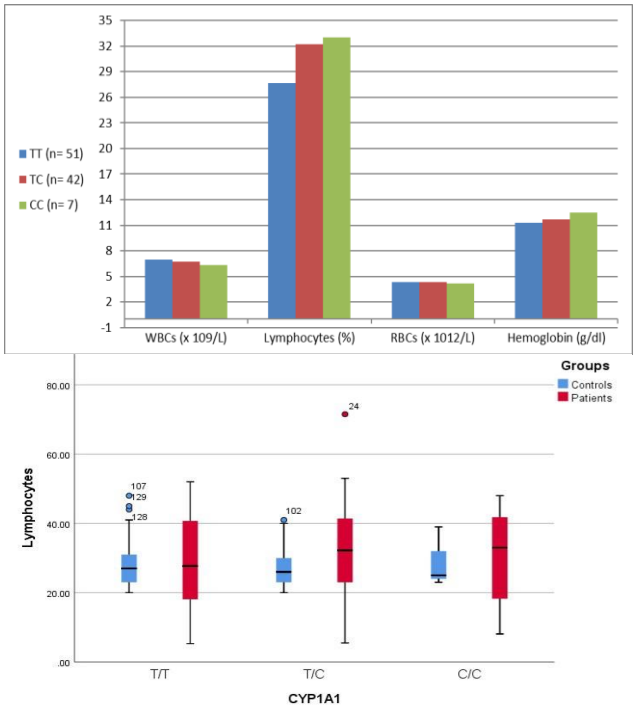


Figure 11. The hematological parameters (WBCs, Lymphocytes, RBCs and Hemoglobin) for the genotypic frequencies of CYP1A1 (rs4646903) gene polymorphisms in Cancer patients

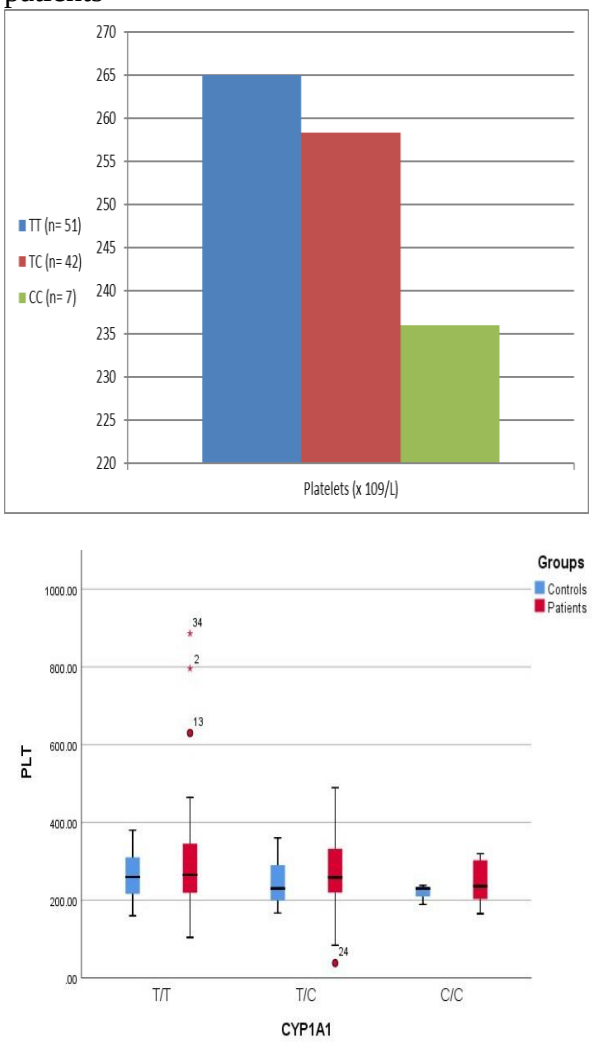


Figure 12. The hematological parameter (Platelets) for genotypic frequencies of CYP1A1 (rs4646903) gene polymorphisms in Cancer patients

Tumor markers and Hormone receptor status

The results in **figure 13.** Showed that the human epidermal growth factor receptor HER2/neu) had had statistically significant association with the genotype distribution of CYP1A1 (rs4646903) gene polymorphisms, which the P value = 0.037.

Regarding the tumor markers, the results showed there is no significant association between the genotype frequencies of CYP1A1 (rs4646903) gene polymorphisms and the Cancer antigen 15-3 (CA 15-3) nor the carcinoembryonic antigen (CEA), which p>0.05. Also, there is no significant association related to Estrogen/progesterone (positive/ Negative), which p>0.05.

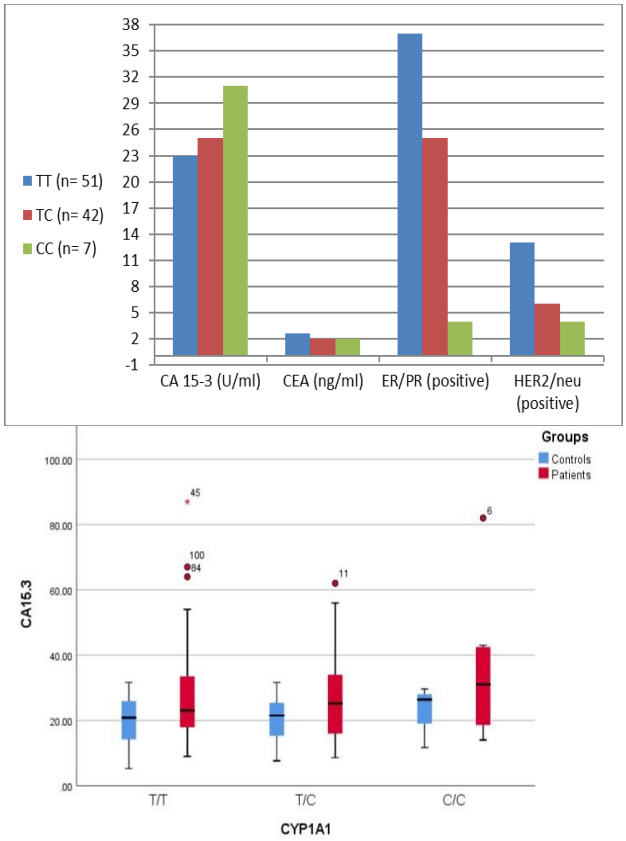


Figure 13. The Tumor marker parameters (CA 15-3, CEA, ER/PR AND HER2) for Genotypic frequencies of CYP1A1 (rs4646903) gene among Cancer patients.

Conclusion

The polymorphisms of CYP1A1 (rs4646903)gene probably contribute in breast

cancer. However, this study showed that there is no significant association between CYP 1A1 (rs4646903) gene polymorphism and the risk of breast cancer among Egyptian women**4.**

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