



Association of *CYP1A1* gene polymorphism with breast cancer incidence

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ABSTRACT

This study was aimed to investigate the molecular detection of the *CYP1A1* gene polymorphism and its role in breast cancer enhancement. Additionally, understanding some of the environmental factors and their relationship to the occurrence of breast cancer was proposed for this study. Blood samples were collected from 50 women at ages between 21-71 years old, that suffered from breast cancer and 50 healthy women who are considered as a control group. The samples were placed in EDTA-based tubes. Then, the samples were kept frozen at -20°C for later DNA extraction and polymerase chain reaction (PCR) laboratory procedures. The results of the mutation analyses of the *CYP1A1* gene for the cancer and the healthy groups revealed that the occurrence frequencies of the homozygous wild type isoleucine/isoleucine were 52% and 26% respectively. While they were 42% and 60% respectively for the homozygous mutant Val/Val. Moreover, the heterozygous mutant Val/Ile type showed presence at 6% and 14% respectively. The statistical analyses confirmed that the incidence of the breast cancer could increase with genetic trait of the homozygous mutant Val/Val in a difference rate of 1.4 and confidence interval at 3.337-0.587. The findings of this study also declared that the women who carried the heterozygous mutant Val/Ile and their menstrual cycle started at the ages between 14-17 years old had 4 times of the risk factor of developing breast cancer. The study also revealed that there were no significant differences between the women who had breast cancer if they had early or late menopause. Finally, the study results showed that there was 3 times risk factor of developing breast cancer in uneducated women when linked to the genotypic trait Val/Ile in a difference rate of 3.2 and confidence interval of 40.057-0.265. It has been concluded that the risk factor of breast cancer was increased in the uneducated women in the presence of the Val/Ile genotype. This could be reasoned to the lifestyle of exposing to chemicals, paints, smoking, and many environmental carcinogens.

Keywords: *CYP1A1* gene polymorphism; breast cancer; Polymerase chain reaction (PCR).

INTRODUCTION

Cancer is a serious disease condition that is represented by huge and uncontrolled cell proliferation which can be locally or metastasized via blood or lymph vessels to other parts of the patient body. Developing cancer could affect all ages but mostly when getting old (Siegel et al., 2016). Mortality of cancer around the world could reach up to 3%, and the American Association of Cancer expects that 7.6 million people might die of cancer in about (Louis et al., 2016)

Breast cancer is considered as one of the malignant diseases that affect women which starts with a single cell and continues to grow up. Breast cancer is one of the common cancers and is the main reason for mortality in women around the world. The number of deaths that breast cancer did globally in 2004 was 519000 patients and was higher (25%) than that by lung cancer in

women. Mortality rate by breast cancer was 7% among other death cases by other cancers and 1% of mortalities among other reasons of death in the world (Release, 2013).

Both of the environmental and genetic factors play important roles in developing cancers (Parkinson et al., 2004). Some cancers are not caused by inherited genetic materials but could be induced by certain factors called carcinogens. These carcinogens could be ranged from exposing to X-Ray, UV, or chemical substances produced by body metabolism such as reactive oxygen species (ROS) that interferes with the DNA and leads to mutation (Jeong et al., 1999). These carcinogens might provide deletions in a particular gene that is responsible for developing a certain type of cancer, and this means that most of the cancers are associated with certain causative environmental factors such as smoking (Reynolds, 2013). Both environmental and genetic factors enhance the development of cancer, and in the recent years, studied have been focused on the interference between those two factors in understanding the etiology of a certain type of cancer (Mucci et al., 2001). The interference between environmental and genetic factors need an enzymatic-based corporation to develop cancers because

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most carcinogens require metabolic enzymes to enhance the development cancers. *CYP1A1* is one of the genes responsible for coding enzymes to enhance the metabolism of certain carcinogens such as polycyclic aromatic hydrocarbon (PAHs) which are considered as carcinogens for breast cancer in rodents. These compounds such as benzo (a) pyrene and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) are stored in the adipose tissue of the breast. The *CYP1A1* is one of the phase I genes that are responsible for coding the phase I enzymes. These enzymes metabolize these carcinogenic compounds and add to them a (-OH) group. Later, this hydroxyl group will receive an oxygen atom from the phase II enzymes to generate hydro-compound derivatives (Mezher et al., 2017). This study was aimed to investigate the molecular detection of the *CYP1A1* gene polymorphism and its role in breast cancer enhancement. Additionally, understanding some of the environmental factors and their relationship to the occurrence of breast cancer was proposed for this study.

MATERIALS AND METHODS

Sample collection

Blood samples were collected from 50 women at ages between 21-71 years old that suffered from breast cancer and 50 healthy women who are considered as a control group. Each blood sample of (2-3) ml was placed in an EDTA-based tube. The samples were collected in Al-Hussein Teaching Hospital, Al-Diwaniyah, Iraq. Then, the samples were kept frozen at -20°C for later DNA extraction and polymerase chain reaction (PCR) laboratory procedures. Questionnaire information was performed on the sampled women that included age, living address, marital status, type of nursing, educational level, and family history of cancer.

DNA extraction

The DNA was extracted using PK/SDS method that was mentioned by Miller et al. (1989).

Polymerase chain reaction (PCR)

The materials used for the PCR were Go Taq Green Master mix, sterilized distilled water, mineral oil, Forward (F) and Reverse (R) primers, and 5µl of DNA. The PCR technique that was utilized to amplify a pre-selected piece of the *CYP1A1* gene was mentioned by Wang and Liehr, (1995). The primers that were used in this study were F: CAGTCAACAGGTGTAGC and R: GAGGCAGGTG-GATCACTTGAGCTC. The kit that was used for this study was Go Taq Green Master mix (Promega, USA). The manufacturer's protocol was followed to prepare the PCR mastermix. The mastermix solution contained 12.5µl Go Taq Green Master Mix, 1µl from each primer, 5µl DNA, and 5.5µl D.W.

The PCR mastermix was then placed in a thermocycler to perform the PCR reaction. The conditions used were 1 cycle of initial denaturation at 94°C for 4 min, 30 cycle of denaturation at 94°C for 1 min followed by annealing

at 65°C for 45 Sec. and extension at 70°C for 1 min, and 1 cycle of final extension at 70°C for 1 min. The PCR products were tested using 2% agarose gel electrophoresis. The gel was finally visualized under a UV imager to check for the presence of a band at 295bp of the *CYP1A1* gene. The PCR products were digested using Msp1 restriction enzyme for 3 hours at 37°C. This was done by adding 0.5µl of the enzyme to 7µl of the PCR product, 0.2µl BSA buffer, 2µl of digestion buffer PX, and 10.3µl of D.W.

Statistical Analysis

The result data were analyzed using Chi-Square χ^2 . Fisher Exact Test was also used to measure the frequency of the occurrence of the genotypic polymorphisms of the *CYP1A1* gene and their relationship to the development of breast cancer. SPSS10 was utilized to analyze those data.

RESULTS

Figure 1 shows the bands of the DNA that was extracted from the blood samples for the patient and the control groups. Figure 2 represents the image of the 2% agarose gel electrophoresis of the *CYP1A1* gene-PCR products. Where L is the ladder at 100bp, and 2-6 are the PCR products. The product of the gene is at 295 bp. Figure 3: The image of the PCR products of the *CYP1A1* gene after digestion with Msp1 restriction enzyme. L is the ladder at 100 bp. Lanes 1 and 3 are Val/Val genotype. Lane 2 is Ile/Ile genotype. Lane 4 is Ile/Val genotype.

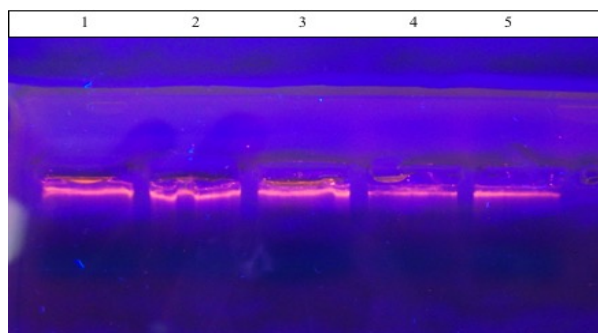


Figure 1: Image of the electrophoresis using 0.8% Agarose gel which targeted the DNA that was extracted from the blood samples of the patient and the control groups. Where 1-4 are the patient group and 5 is the control group

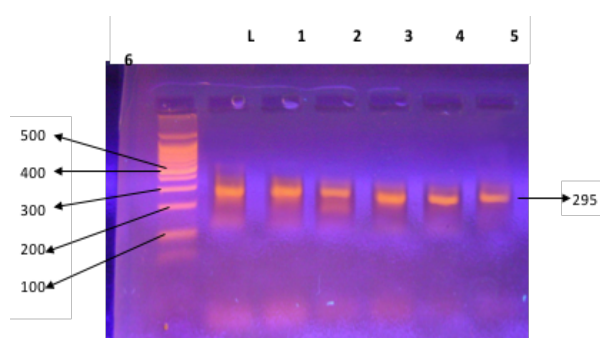


Figure 2: Image of the 2% agarose-gel electrophoresis of the *CYP1A1* gene-PCR products. Where L is the ladder at 100bp, and 2-6 are the PCR products.

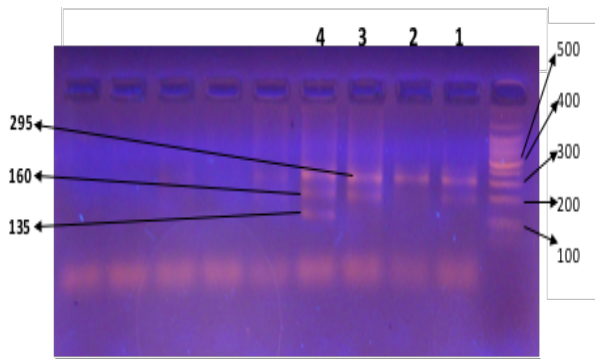


Figure 3: The image of the PCR products of the *CYP1A1* gene after digestion with *MspI* restriction enzyme. L is the ladder at 100 bp. Lanes 1 and 3 are Val/Val genotype. Lane 2 is Ile/Ile genotype. Lane 4 is Ile/Val genotype.

Distribution of the *CYP1A1* gene polymorphisms in both groups

The results of the current study revealed that there was a link between the mutant homozygous Val/Val and the mutant heterozygous Ile/Val with the incidence rate of breast cancer. The study found that Val/Val showed high association with breast cancer at (OR=1.4), while the genotype Ile/Val was the highest (OR=4.66) when linked to the occurrence of breast cancer, Table 1.

Table 1: The frequent occurrence of the *CYP1A1* gene polymorphisms in the patient and control groups

Genotype	Control	Patients	OR*	95% CI**	p value***
Ile/Ile	13 (26%)	26 (52%)	1	-----	-----
Val/Val	30 (60%)	21 (42%)	1.4	3.337-0.587	0.446
Val/Ile	7 (14%)	3 (6%)	4.66	21.072 – 1.034	0.035
Val/Val+Val/Ile	37 (74%)	24 (48%)	3.08	7.149-1.33	0.0076

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 2: The frequent occurrence of the *CYP1A1* gene polymorphisms in the patient group according to living in urban or rural regions

Genotype	Urban regions	Rural regions	OR*	95% CI**	p value***
Ile/Ile	19 (38%)	7 (14%)	1	--	--
Val/Val	17 (34%)	4 (8%)	0.63	2.569 –0.159	0.526
Val/Ile	2 (4%)	1 (2%)	0.22	2.075– 0.025	0.16

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 3: The frequent occurrence of the *CYP1A1* gene polymorphisms in the patient group according to the marital status

Genotype	Married	Unmarried	OR*	95% CI**	p value***
Ile/Ile	24 (48%)	2 (4%)	1	--	--
Val/Val	20 (40%)	1 (2%)	0.6	7.113-0.051	0.68
Val/Ile	2 (4%)	1 (2%)	6	98.72-0.365	0.167

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 4: The frequent occurrence of the *CYP1A1* gene polymorphisms in the patient group according to the age of getting married

Marriage age	Val/Val	Val/Ile	OR*	95% CI**	p value***
10 – 20	13 (54.16%)	2 (8.33%)	1	--	--
21 – 30	4 (16.66%)	1 (4.16%)	1.62	22.981-0.115	0.717
31 – 40	3 (12.5%)	1 (4.16%)	2.166	32.528-0.144	0.569

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the living regions

The study findings showed that there were no significant differences between the *CYP1A1* gene polymorphisms, Val/Val (OR=0.63) or Ile/Val (OR=0.22), and the living in urban or rural regions when linked to the incidence rate of developing breast cancer, table 2.

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the marital status

The current study results demonstrated that there was a significant link between the presence of the mutant heterozygous Ile/Val in unmarried women and the incidence rate of developing breast cancer. Table 3 shows that the occurrence of the Ile/Val in these women increases the significant rates of developing breast cancer at 6 times more than usual. The genotype Val/Val didn't show any significant effect on this incidence rate.

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the time point of getting married

The findings of this study revealed that there was a significant relationship between the presence of the genotypic polymorphisms of the *CYP1A1* gene and the age of getting married on increasing the incidence rate of

breast cancer. Indeed, the incidence rate of developing breast cancer increases at (OR=1.62) of age range between 21-30 years old and doubled-increases (OR=2.166) at ages between 31-40 years old, table 4.

Distribution of the CYP1A1 gene polymorphisms in the patient group according to the baby nursing type

The results showed that there were no significant differences of developing breast cancer in the women who do breast- or artificial feeding when having the mutant homozygous genotype (OR=0.28) or having the mutant

heterozygous genotype (OR=1.33) of the CYP1A1 gene, table 5.

Distribution of the CYP1A1 gene polymorphisms in the patient group according to the starting age of menstrual cycle

The results of the present study showed that there was a four-time increase (OR=4.2) in the incidence rate of breast cancer when having the genotype Ile/Val in the women who had started their menstrual cycle at the ages between 14-17 years old. However, there were no

Table 5: The frequent occurrence of the CYP1A1 gene polymorphisms in the patient group according to the baby nursing type

Genotype	Breastfeeding	Artificial feeding	OR*	95% CI**	p value***
Ile/Ile	16 (34.78%)	6 (13.04%)	1	--	--
Val/Val	19 (41.30%)	2 (4.334%)	0.28	20.155–0.63	0.134
Val/Ile	2 (4.34%)	1 (2.17%)	1.33	9.78–0.057	0.82
Ile/Ile	16 (34.78%)	6 (13.04%)	1	--	--

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 6: The frequent occurrence of the CYP1A1 gene polymorphisms in the patient group according to the starting age of the menstrual cycle

Genotype	Menstrual-cycle starting age (years old)		OR*	95% CI**	p value***
	10-13	14-17			
Ile/Ile	19 (38.77%)	9 (18.36%)	1	--	--
Val/Val	13 (26.53%)	5 (10.2%)	0.81	2.984–0.221	0.75
Val/Ile	1 (2.04%)	2 (4.08%)	4.2	52.90–0.337	0.234

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 7: The frequent occurrence of the CYP1A1 gene polymorphisms in the patient group according to the menopause starting age

Genotype	Menstrual-cycle starting age (years old)		OR*	95% CI**	p value***
	36-45	46-55			
Ile/Ile	8 (27.58%)	6 (20.68%)	1	--	--
Val/Val	8 (27.58%)	6 (20.68%)	1	4.468 –0.224	1
Val/Ile	0 (0%)	1 (3.44%)	--	--	--

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 8: The frequent occurrence of the CYP1A1 gene polymorphisms in the patient group according to having or not having a family history of breast cancer

Genotype	Family history of breast cancer		OR*	95% CI**	p value***
	Absent	Present			
Ile/Ile	22 (44%)	4 (8%)	1	--	Ile/Ile
Val/Val	19 (38%)	2 (4%)	0.57	3.52–0.095	Val/Val
Val/Ile	2 (4%)	1 (2%)	0.45	4.57–0.046	Val/Ile

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

Table 9: The frequent occurrence of the CYP1A1 gene polymorphisms in the patient group according to the educational levels

Genotype	Educated	Uneducated	OR*	95% CI**	p value***
Ile/Ile	10 (20%)	16 (32%)	1	--	--
Val/Val	8 (%16)	13 (26%)	0.984	3.215–0.302	0.9846
Val/Ile	2 (%4)	1 (2%)	3.2	40.057–0.265	0.3476

*OR: Odd Ratio; **95%CI: 95% Confidence Interval; ***p<0.05

significant (OR=0.81) differences when having the genotype Val/Val on this incidence rate, table 6.

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the menopause starting age

The study findings did not indicate any significant relationship between having these mutant traits, Val/Val or Ile/Val, and the age of menopause on the incidence rate of breast cancer in women who had menopause at ages between 36-45 or 46-55 years old, table 7.

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the family history of breast cancer

No significant differences were noticed when having any of the mutant traits in the women who had or did not have a family history of breast cancer on increasing the incidence rate of the breast cancer, table 8.

Distribution of the *CYP1A1* gene polymorphisms in the patient group according to the educational level of women

The findings of the current study have realized that there was no significant (OR=0.984) relationship between having the genotype trait Val/Val and the educational levels of the patient women on increasing the incidence rate of this cancer. On the other hand, there was a significant (OR=3.2) relationship between having the genotype trait Ile/Val and the educational levels of the patient women on increasing the incidence rate of this cancer, table 9.

DISCUSSION

There are many risk factors that enhance the development of breast cancer such as age, family history of breast cancer, the early-age start of the menstrual cycle (as early as 12 years old), the lateness of menopause, and first birth after 30 years of old (Russell et al., 2013). The breast cancer results from many genetic or environmental factors and some of these environmental factors are the polycyclic aromatic hydrocarbon (PAHs). These compounds are deposited in the breast tissues which are metabolized later to generate active carcinogenic substances (Gorlewska-Roberts et al., 2002). There are many metabolic enzymes that are responsible for stimulating or inhibiting carcinogenic chemical compounds such as endogenous sexual hormones (Dunning et al., 1999). Therefore, there are individual variations in the ability of these enzymes to metabolize these carcinogens, and this might lead to breast cancer occurrence variation (Shimada and Fujii-Kuriyama, 2004). Genetic polymorphism is an important factor in breast cancer development (Huang et al., 1999). In the current study, the frequent occurrence of the genotypic polymorphisms of the *CYP1A1* gene in the patient and the control groups were 52% and 26% for the Ile/Ile, 42% and 60% for the Val/Val, and 6% and 14% for the Val/Ile, table 1. The results also revealed that there was no relationship between the Val/Val and the risk of developing

breast cancer (OR=1.4, 95% CI= 3.337-0.587), However, this risk increases 5 times (OR=4.66, 95%CI = 21.072-1.034) when having Val/Ile genotype. These results partially agree with (16 and 17) who found that there were no significant links between having these mutant traits and the risk of breast cancer development. On the other hand, Taioli et al. (1999) have realized that there were significant links between having these genetic polymorphisms, *CYP1A1* M1, and *CYP1A1* M3, and increasing the risk of breast cancer development. The *CYP1A1* gene polymorphism is important in the formation of Catechol estrogen (CE) form the hydroxylation of estrogen. *CYP1A1* gene codes for an enzyme that leads to this hydroxylation (Zhu and Conney, 1998). This gene also plays a crucial role in distributing the substances that are produced from the metabolism of estrogen (Taioli et al., 1995). Exposing breast epithelial cells to CE might lead to DNA damages and mutations (Eubanks, 1997).

According to the current study results, there were no significant links between having these mutant traits and the living regions, urban or rural, on the risk factor of developing breast cancer, table 2. There are many chemical and physical mutagens and carcinogens in the environment that could affect living beings especially humans either directly or by taking a long time to show up their detrimental effects (Young, 2002). As a result, for Iraq to be polluted with chemical and radioactive substances, breast cancer has been increased, and this might indicate that urban and rural regions are exposed equally to these contaminants or suggest interfering of genetic and environmental factors in increasing the risk of breast cancer (Cheng et al., 2005). These environmental factors affect the DNA and lead to certain mutations which can be increased via the synergistic effects of both acquired and inherited mutations. These mutations could drive the cells into uncontrolled mitosis and lead to the formation of cancer.

The current study revealed that there was a significant effect on increasing the risk factor of breast cancer when having the heterozygous Val/Ile in the unmarried women more than that in the married women. While, there was no such effect in the case of the Val/Val, table 3. Exposure of woman body to female sex hormones such as estrogen might increase this risk factor. Estrogen enhances the mitosis of the breast cells and thus increases the chances of developing breast cancer. The *CYP1A1* gene plays an important role in estrogen metabolism. The products of this metabolism could lead to the formation of DNA adducts and increase the risk of breast cancer (Eubanks, 1997). Therefore, *CYP1A1* gene polymorphism might decrease this risk via this mechanism (Den Dunnen and Antonarakis, 2000). The current study findings demonstrated that the risk of breast cancer was increased in patients who got married at ages between 21-30 years old when having Val/Ile and Val/Val traits. While, this risk was doubled-increased in patients who got married at ages between 31-40 years

old when having these traits, table 4. The reasons behind these increases could be as a result to delayed-first pregnancy or absence of pregnancy, and this means increasing the exposure to the female sex hormones such as progesterone and estrogen (Pike et al., 1993). Table 5 shows that there was no increased risk of breast cancer in the cases of breast or artificial feedings when having Val/Val, but this risk was increased when having Val/Ile. This could be reasoned to the fact of the links between the receptors of progesterone and estrogen with cancer formation for mothers who breastfeed their babies. Table 6 reveals that there was an elevated risk of breast cancer when having the Val/Ile trait in the women who had started their menstrual cycle between the ages of 14-17 years old, while there was no such effect in the case of having the Val/Val trait in the women who had started their cycles in any time. This could be as a result of the same wild type-metabolic activities that Val/Val has (Al, 2016). Moreover, Huang et al. (1999) have found that the risk of breast cancer was increased 8 times in the women who had started their cycles in the early age when linked to the presence of the Val/Ile genotype. Table 7 revealed that there were no active relationships between the risk of breast cancer and the age of menopause when having any mutant traits of the studied gene. This agrees with (Cheng et al., 2005; Huang et al., 1999) who have also found that there were no significant links between the age of menopause and these mutant polymorphisms on the risk of breast cancer. However, Huang et al. (1999) have detected a significant and strong link after menopause when having these mutants. The risk of breast cancer increases when there is continuing accumulation of the estrogen. Starting menstrual cycle in early age, lateness menopause and delayed-first pregnancy or absence of pregnancy increases this risk (Ban and Godellas, 2014). Genes that code for estrogen metabolism increases the risk of breast cancer. Estrogen binds to its receptors on breast tissues and leads to more mitosis activation (Stingl, 2011). Table (8) showed no significant differences between the patients who had a family history of breast cancer and those who did not have such history when linked to the presence of the mutant traits, and this agrees with (Cheng et al., 2005). The breast cancer could be developed by the synergistic effect of an inherited gene and the effects of the environmental factors (Kelsey et al., 1997).

CONCLUSION

In the current study, the risk factor of breast cancer was increased in the uneducated women in the presence of the Val/Ile genotype. This could be reasoned to the lifestyle of exposing to chemicals, paints, smoking, and many environmental carcinogens.

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