Cytochrome P450 (CYP1A2) Genetic Polymorphism as a Genetic Marker for Treatment Outcome in Early Breast Cancer Patients

Abstract

Background: Cytochrome P450(CYP1A2) has a crucial role in drugmetabolism, and some neurotoxins. Polymorphisms of Cytochrome P450(CYP1A2)have a role in cancer activity through affecting aromatic hydrocarbon metabolism.This work was aimed at studyingCYP1A2polymorphism as a genetic predictive marker of treatment out come in breast cancer (BC) female cases.

Methods: This case control research included 70 cases. Patients' group (50 cases) were categorized into two groups: Group I A (n=20): failed BCcases for hormonal therapy during treatment period, group I B (n=30): after 5 years of hormonal treatment which subdivided into two groups: [treatment failure (recurrence or metastasis) and patients with successful outcome] and group II (n=20): normal female subjects.

Results: The occurrence of treatment events was significant in patients carryingPolymorphism of Cytochrome P450(CYP1A2)(CYP1A2 rs762551) C/A. Also,relapse was earlier in patients with CYP1A2 carrying C allele and earlier among CC compared to C/A and wild allele. There was significantly higher in positive family history than in negative ones, with about 85.0% of positive family patients carrying C/A polymorphism and 77.8% in CC polymorphism.The incidence of BC events was studied using Kaplan-Meier scale to study event free survival in relation to CYP1A2 rs 762551 in postmenopausal BC patients treated with AI along five years treatment.

Conclusions:CYP1A2genetic polymorphism is associated with early treatment events amongfemale cases developingBC treated by aromatase inhibitors.

Keywords: Cytochrome P450, Genetic Polymorphism, Marker, Breast Cancerز

Introduction:

Breast cancer (BC) is a prevalent cancer type associated with mortality as well as morbidity. It affects over 230,000 new cases every year in the United States [1].

The tumorexpresses hormonal receptors so hormonal therapy is considered in BC treatment [2].

Enzymes associated with Estrogen metabolism and synthesis include Cytochrome P450(CYP19A1) and other enzymes like CYP1A1, CYP1A2, COMT as well as CYP3A4 [3].

Multiple enzymes involved in the estrogen metabolism pathway are involved in the aromatase inhibitor (AI) metabolism pathway [4]. Also, AIs interfere with the metabolism of such enzymes anastrozole interferes with CYP1A2 and CYP2C9 also exemestane interferes with CYP1A1 and 2. So, several studies for the correlation between polymorphism of these enzymes and disease-free survival have been postulated as polymorphism may be behind AI resistance [5].

CYP1A2exhibits a crucial role in drug metabolism and some neurotoxins. It also has aromatic amine activity and hence it has a role in cancer activity through the binding of aromatic hydrocarbon to Ah receptors[6].The two genes CYP1A1 as well as CYP1A2 possess a similar promotor and are regulated by aryl hydrocarbon receptors. CYP study shows interindividual variability polymorphism [7].

Polymorphisms in these genes associated with estrogen and AIs metabolism can be a cause of primary aromatase inhibitor resistance as estrogen has a role in BC events [8].

This research aimed to study the polymorphism of CYP1A2as a genetic predictive marker of treatment outcome in BC female cases.

Patientsand Methods:

Our team designed case control researchincluding50postmenopausal BCcasesdiagnosed as early BC with poor hormonal therapy (aromatase inhibitor therapy) outcome either local or

distant metastasis in Tanta University Hospitals in Clinical Pathology Department for 2 years from February 2020 and February 2022.

The research was done after it got approved by the Ethical Committeeat Tanta University Hospitals, Tanta, Egypt.All participants were asked to sign an informed consent.

Patients having a history of another cancer, patients receiving any other type of anticancer therapy and premenopausal BC patients were excluded from our research.

All participantswere categorized into two groups:Group I A (n=20): failed BCcases for hormonal therapy during treatment period, group I B (n=30): after 5 years of hormonal treatment which subdivided into two groups: [Patients with treatment failure either recurrence or metastasis and patients with successful outcome] and group II (n=20): normal female subjects.

Our team took a comprehensive medical history, then physical assessment was conducted followed by lab testing [Complete blood count (CBC), liver and kidney functions test and genetic marker (CYP1A2 rs762551) by real timepolymerase chain reaction(PCR)] and laboratory investigations [bilateral sonomamogram, chest X ray or computed tomography (CT) and abdominopelvic ultra-sound or GT].

Samples preservation and extraction:

The samples were likely identified and stored at a temperature of -20 degrees Celsius until the time of the experiment. The extraction process included utilizing thermal JET whole blood genomic DNA purification. The kit includes Proteinase K, lysis Solution, wash Buffer WB I, wash Buffer II, as well as elution Buffer. A volume of 20 µL of Proteinase K was introduced to 200 µL of whole blood and thoroughly combined by vortexing. Next, 400 µL of Lysis Solution was vigorously agitated utilizing a vortex to ensure a homogeneous suspension. The sample was incubated at a temperature of 56 ℃ for a duration of 10 minutes, with intermittent use of a vortex. 200 microlitres of pure ethanol were added and stirred

utilizing a pipette. The mixture was transferred to the spin column and then centrifuged for 1 minute at 8,000 rpm . The collecting tube retaining the fluid that passed through was discarded. Subsequently, the column was transferred to a fresh 2 mL collection tube (provided). We introduced 500 microlitres of Wash Buffer WB I, which had additional ethanol. Centrifugation was then performed at about 10,000 rpm for a duration of 1 minute. The flow-through was discarded and placed the column back into the collection tube. Next, 500 µL of Wash Buffer II (including ethanol) was added to the column. The mixture was then centrifuged at maximum speed (≥14,000 rpm) for 3 minutes. Genomic DNA was eluted by adding 200 µL of Elution Buffer to the centre of the column membrane. The incubation was allowed for a duration of 2 minutes at the room temperature, followed by centrifugation for a duration of 1 minute at approximately 10,000 rpm. The elution was stored at a temperature of -20 degrees Celsius until the master mix was prepared.

Detection of polymorphism:

- CYP1a2 rs 762551 with context sequence (Vic/ Fam)

  TGCTCAAAGGGTGAGCTCTGGC(C/A)CAGGACGACGATGGTGATGGAGCT

  TA was detected using TaqMan SNP genotyping assay provided by thermo fisher.
- The reaction requires:
  - TaqMan master mix
  - SNP genotyping assay
- Master mix protocol:

Ensuring all safety precautions as PPE and working area guides.

The reaction mix was prepared as:

a-10-micron TaqMan master mix for each sample.

b-0.5-micron assay mix for each sample.

c-6.5 microns DNase free water for each sample.

d-3 microns of each elute was used.

- Amplification was done using rotor gene PCR.

Statistical analysis

Our team analyzed data statistically with analysis SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were showcased through mean and standard deviation (SD) and a comparison was conducted among the three groups utilizing ANOVA (F) test with post hoc test (Tukey). Qualitative variables were illustrated through frequency and percentage and analysis was conducted utilizing the Chi-square test. A two tailed P value of below 0.05 was deemed statistically significant.

Results:

Ageand smoking exhibitedinsignificant variance between patients and control groups. The average age of menarche of both groups was a significant decrease in patient groups 1A and 1B compared with control group. The average age of menopause was a significant increase in patients' groups than control group. History of HC and family history of BC were significant different between both groups. **Table 1**

The prevalence of CYP1A2 polymorphism was significantly different between patients and control group and also between subgroups of 1B in comparison with control group ($P<0.05$).

The expression of homozygous polymorphism was more in patients of group 1A than in patients of group 1B. The genetic polymorphism either homozygous or heterozygous is more prevalent in patients with relapse than patients without relapse. **Table 2**

There was no significant relation between genetic polymorphism of CYP1A2 marker and smoking. The age was significantly higher in patients carrying this polymorphism either CC or C/A. The relation between genetic polymorphism to the incidence of early relapse. The occurrence of relapse earlier in patients with CYP1A2 carrying C allele and earlier among CC compared to C/A and wild AA. There was significantly higher in positive family history

than in negative ones, with about 85.0% of positive family patients carry C/A polymorphism and 77.8% in CC polymorphism.**Table 3**

The incidence of BC events was studied using Kaplan-Meier scale to study event free survival in relation to CYP1A2 rs 762551 in postmenopausal BC patients treated with AI along five years treatment. **Figure 1**

Discussion

Our research addressed a significant association between early onset of menarche and late onset of menopause developing breast cancer in the female cases included in the investigation. This finding is in line with the international understanding of the hormones' role in BC, although some studies suggest that the impact of both factors is not equal. A comprehensive study published in the Lancet in 2012 revealed that breast cancer risk increases more with each year of onset at earlier menarche compared to each year of onset at later menopause, implying that menarche as well as menopause could not solely influence the risk of BC by prolonging the women's total reproductive years.

The study also showed that the use of hormonal contraceptives was more prevalent among BC patients and the difference was statistically significant. Jenny et al.[9]suggest that women aged 15-34 using progestogen-only methods exhibit a slight rise as regards relative risk of developing BC or BC in situ in comparison to women who have never utilized hormonal contraceptives. The risk was greatest during the first 5 years of combined hormonal contraceptive (HC) use, and up to ten years after discontinuing its usage. The BCrisk was highest between current HC consumers. Additionally, the HC usage has been linked to decreased activity of CYP1A2 [10].

Finally, the study supported the positive association of family history of BC with a higher incidence of BC in the studied patient population. A family history of BC is widely

recognized as the greatest risk factor for BC incidence, and paying attention to proper follow-up and medical care for women with a relative with BC can lead to improved outcomes [11].

The link between tobacco smoking and many cancer types, involvingBC, has been established in numerous studies [12].However, the current study does not find a significant correlation between smoking and the BC occurrence, which contradicts previous findings, which have shown a significantly rise as regards the BC riskbetween smokers, particularly among women who began to smoke during adolescence or peri-menarchial ages [13].

In our research, the expression of the CYP1A2 genetic polymorphism was found to be higher in BC patients than within the control group, and the varianceexhibited a statistical significance. This is consistent with Rebbeck, T. R. et al. [14] and Shimada N et al. [15] reported an association between the CYP1A21f* C/A genetic polymorphism and BC incidence. However, IMENE A et al.[16]reported different results, highlighting the complex and varied role of CYP1A2 in BC risk. The current study also found a higher expression of the homozygous minor allele polymorphism within cases developing early relapse than in others developing late relapse, a difference that was statistically significant.

The CYP genes, including CYP1A2, have been implicated in cancer formation and development as they exhibit a role in drug metabolism, oxidative stress, as well as activation of procarcinogens [17]. Other CYP genes, such as CYP19A1, have been found to modulate estrogen levels and impact BC treatment outcomes [18, 19]. CYP1A2 metabolizes several procarcinogens and anticancer drugs, and its activity is influenced by dietary patterns[5]. A study of fifty-nine caseswithin the current cohort addressed that the CYP1A2 rs762551 C-allele was associated with a low 2OHE-to-16alphaOHE1 plasma ratio. However, the AIs absence during blood draw raises questions about the validity of these findings.

Limitations of the study included that difficulty in obtaining accurate smoking histories and a small sample size of women who reported active smoking, leading to the inclusion of passive smokers in the group of smokers.

Conclusions:

CYP1A2genetic polymorphism is linked to early treatment events among female cases with BC treated with aromatase inhibitors.

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Table 1: Age, age of menarche, age at menopause, history of HC, family history of BC and history of active and passive smoking of patient groups and control

		Group I A (n=20)	Group IB (n=30)	Control (n=20)	P
Age (years)		57.7±2.68	58±3.27	55.3±3.63	0.113
		P1=0.748, P2=0.102, P3=0.075			
Menarche age (years)		9.45±1.19	9.84±1.03	11.26±0.99	**0.001***
		P1=0.230, **P2=0.001*, P3=0.001***			
Age at menopause		50.8±1.24	51±1.05	47.4±1.57	**0.013***
		P1=0.764, **P2=0.013*, P3=0.013***			
OCP	**+Ve**	13(65.0%)	19(63.3%)	5(25.0%)	**0.013***
	-Ve	7(35.0%)	11(36.7%)	15(75.0%)	
Family history	**+Ve**	8(40.0%)	14(46.6%)	1(5%)	**0.019***
	-Ve	12(60.0%)	16(53.4%)	19(95.0%)	
Smoker		10(50.0%)	19(63.3%)	8(40.0%)	0.685

Data are presented as mean± SD or frequency (%). * Significant p value <0.05, P1: significance bet. I A and I B, P2: significant bet. I A and II, P3: significant bet. I B and II, OCP: Oral contraceptive pills, HC: Hormonal contraception, BC: Breast Cancer.

Table 2: The prevalence of CYP1A2 polymorphism in (patients and control groups) and (subgroups of 1B in comparison with control group)

		Group I A (n=20)	Group IB (n=30)	Control (n=20)	P
Polymorphism	**Wild**	4(20.0%)	13(43.3%)	16(80.0%)	**0.002***
	Homozygous	7(35.0%)	4(13.3%)	1(5.0%)	
	Heterozygous	9(45.0%)	13(43.3%)	3(15.0%)	
		Relapse	**No Relapse**	**Control**	
Polymorphism	**Wild**	4(21.1%)	9(81.8%)	16(80.0%)	**0.001***
	Homozygous	4(21.1%)	0(0.0%)	1(5.0%)	
	Heterozygous	11(57.9%)	2(18.2%)	3(15.0%)	

Data are presented as frequency (%). * Significant p value <0.05.

Table 3: The relation between CYP1A2 polymorphism and smoking history, age and time to relapse in BC patients

		Polymorphism			P
		AA	**CC**	**C/A**	
Smoking	**Non-smokers**	12(57.1%)	3(33.3%)	6(30.0%)	0.436
	smokers	9(42.9%)	6(66.7 %)	14(70 .0%)	
Age		55.33±1.06	59.56±2.55	59.80±2.69	**0.001***
		P1=0.001*, P2=0.001*, P3=0.766			
Time to relapse		14.0±6.90	9.67±4.21	12.94±4.67	0.192
Family history	**+Ve**	7(33.3%)	7(77.8%)	17(85.0%)	**0.002***
	-Ve	14(66.7%)	2(22.2%)	3(15.0%)	

Data are presented as mean± SD or frequency (%). * Significant p value <0.05, P1: significance bet. No and Homo, P2: significant bet. No and hetero, P3: significant bet. homo and hetero. AA: Wild allele, CC: Homozygous allele, C/A: Heterozygous allele.

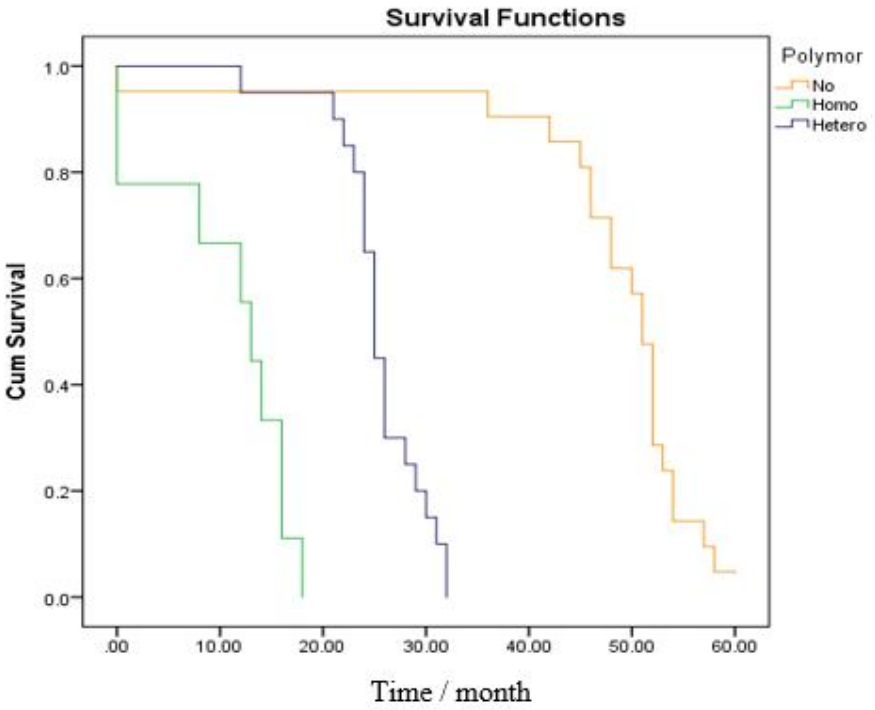

Figure 1: Kaplan-Meier scale of breast cancer event during treatment period in patients with CYP1A2 genetic polymorphism

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