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Frequency of ABCB1 Polymorphisms in Breast Cancer Patients in South Egypt Cancer Institute by High-Resolution Melting Curve Analysis (Pilot Study)

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Authors' contributions

This work was carried out in collaboration between all authors. Author HB designed the study, wrote the protocol and managed the analyses of the study. Author AI collected patients sample and wrote the draft of manuscript. Author AM managed the analyses of the study and the literature searches and did the experimental part herself. All authors read and approved the final manuscript.

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ABSTRACT

Introduction and Aim of the Work: The ABC (ATP-binding cassette) transporter family contributes to the multidrug resistance the genetic variants of this gene have been intensively studied so far. Some single nucleotide polymorphism (SNPs) of ABCB1 has a correlation with the risk of breast cancer. So this study was conducted to assess value of using of 2 SNPs gene as predicator of risk in Egyptian breast.
Study Design: It is a pilot study to evaluate the value of using 2 SNPs gene as predicator of risk in Egyptian breast.
Patients and Methods: Between September 2016-2017, 50 female breast cancer patients and 20

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healthy females control group were included. Peripheral blood samples from patients and control were taken for genomic DNA extraction. And HRM experiment was designed to assess (rs2214102) and (rs2032582) (single nucleotide polymorphism) SNPs of ABCB1 gene this in South Egypt Cancer Institute.

Results: Regarding (rs2214102), all samples have the same heterozygous genotype (GA) with no significant difference between cases and controls. On the other hand, samples of (rs2032582) revealed that samples are divided into 3 groups one is wild homozygous genotype (GG) and other 2 homozygous variants (TT) and (AA). The frequency of the 3 variants in patients and control group showed no statistical difference P=.057.

Conclusion: Using SNPs rs2214102 and rs2032582 are not good predictive factors for breast cancer risk or development.

Keywords: Breast cancer; single nucleotide polymorphism; multidrug resistance-associated protein; genes polymorphism; DNA samples; high resolution melting.

1. INTRODUCTION

The ABC (ATP-binding cassette) transporter family contributes to the multidrug resistance (MDR) mechanisms. Glycoprotein P (PgP), multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP) are the most widely expressed, thus, they are most often responsible for this phenomenon [1,2].

The hypothesis assumes that bearing a mutation/polymorphism in one of these genes (resulting in protein dysfunction) may contribute to the accumulation of mutagens and increased risk of cancer development.

Consequently, long-term exposition and accumulation might result in faster development of cancer [3,4].

The carcinogenesis processes can be related to the ABC protein's dysfunction when provoked by mutagens or their metabolites and/or the absorption and accumulation of conjugates [5,6].

The genetic variants of the ABC family members have been intensively studied so far. Some single nucleotide polymorphism (SNPs) of ABCB1 has a correlation with the risk of breast cancer, while others have failed to observe an association [7,8]. In the ABCB1gene, more than 50 SNPs were identified. Some studies suggested that significant associations of ABCB1 rs2032582 and rs2214102 SNPs with prognostic factors and survival of patients [9].

This study was conducted to assess the value of using of two SNPs gene (rs2214102) and (rs2032582) as a predictor of risk in Egyptian breast cancer as some studies suggested its use as a good predictor of cancer development.

2. PATIENTS AND METHODS

The study population consisted of 50 female patients with histologically confirmed infiltrating duct carcinoma in the breast. Their mean ages were 49.90 ± 11.62 years. A control group of 20 apparently healthy females was also included, with a mean of 46.80 ± 9.64 years. Peripheral blood samples from patients and control were taken.

2.1 Ethical Consideration

The research is approved from south Egypt cancer institute ethical committee under the number of SECI-IRB IORG0006563 No (231 /2015). All cases were collected from the medical oncology outpatient clinic of South Egypt Cancer Institute. All patients provided informed written consent from all patients the following data was taken: Age at diagnosis, menopausal status, personal medical history, family history.

2.2 DNA Extraction

Genomic DNA was isolated from peripheral venous blood by using the DNA blood mini kit from Qiagen, Hilden, Germany. DNA samples were stored at – 20°C prior to analysis.

2.3 High Resolution Melting (HRM) Analysis

HRM experiment was designed to assess (rs2214102) and (rs2032582) (single nucleotide polymorphism) SNPs of the ABCB1 gene in genomic DNA samples of the breast carcinoma patients and controls.

Real-time PCR and HRM analysis of genomic DNA samples were carried out using Applied Biosystem, 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA).

All experiments were performed with MeltDoctor™ HRM Master Mix (Thermo Fisher Scientific, USA).

2.4 The Optimized Reaction Mixture for HRM Analysis

10 µ L of 2 × MeltDoctor™ HRM Master Mix,1.0µ L of both forward and reverse primer for each of (rs2214102 and rs2032582), 6.0 µ L of nuclease-free water, 2.0 µ L of genomic DNA.

Primers (Invitrogen, Thermo Fisher Scientific, USA)

Primer	Sequence (5′ -3′)	Annealing temperature
rs2214102	F:5′CTTACTGCTCTCTGGCTTCG3′	64°C
	R:5′TTCAGAGCTGGAGGCTAGAA3′	64°C
rs2032582	F: 5′TGTTGTCTGGACAAGCACTGA3′	64°C
	R: 5′TGTTGTCTGGACAAGCACTGA3′	64°C

2.5 PCR Program

Holding stage (initial denaturation): 95°C for 10 minutes.

Pcr stage: 40 cycles include 95°C for 15 sec and 64°C for 1 minute.

HRM stage: Continuous mode, 95°C for 15 sec and 64°C for 1 minute.

2.6 Interpretation

All samples of patients and controls were the heterozygous variant of rs2214102 (GA) with 2 peaks, each peak has a specific Tm TM1 (82.78 ± 0.15) and TM2 (75.38 ± 0.14) in the patient group and TM1 (82.84 ± 0.11) and TM2 (75.39 ± 0.14) in control group. Melting curves and data obtained by this software are shown in Figs. 1 and Table 2. As for rs2032582, HRM curves showed that patients and control samples are divided into 3 homozygous variants in both groups with different TMs. Tm was (74.09 ± 0.09), (74.36 ± 0.05), (74.76 ± 0.08) for GG, TT, AA genotypes receptively in patients samples. Tm was (74.10 ± 0.09), (74.37 ± 0.05), (74.74 ± 0.05) for GG, TT, AA genotypes receptively in control samples as shown in Table 2 and Fig. 2.

Results of HRM analysis were confirmed with the results of direct sequencing.

3. RESULTS

Clinical and demographic characteristics of patients and controls involved in the study are described in Table 1.

ABCB1 (rs2214102) and (rs2032582) SNP was estimated using HRM and the shape of the melting curves are shown in Figs. 1,2,3,4.

Regarding (rs2214102), all samples have the same heterozygous variant with 2 peaks, each peak has a specific Tm. No significant difference between cases and controls, as shown in Tables 2, 3 and Figs. 1, 2.

Table 1. Demographic and clinical data between patients and control groups

Age: (years)	Patients (n= 50)		Control (n= 20)		P-value
	No.	%	No.	%	
< 40	8	16.0	6	30.0	0.415
40 - < 50	16	32.0	5	25.0	
50 - < 60	15	30.0	7	35.0	
≥ 60	11	22.0	2	10.0	
Mean ± SD	49.90 ± 11.62		46.80 ± 9.64		0.283
Range	28.0 – 74.0		31.0 – 62.0		
Type of disease:					--
Metastatic	24	48.0	--	--	
Locally advanced	26	52.0	--	--	

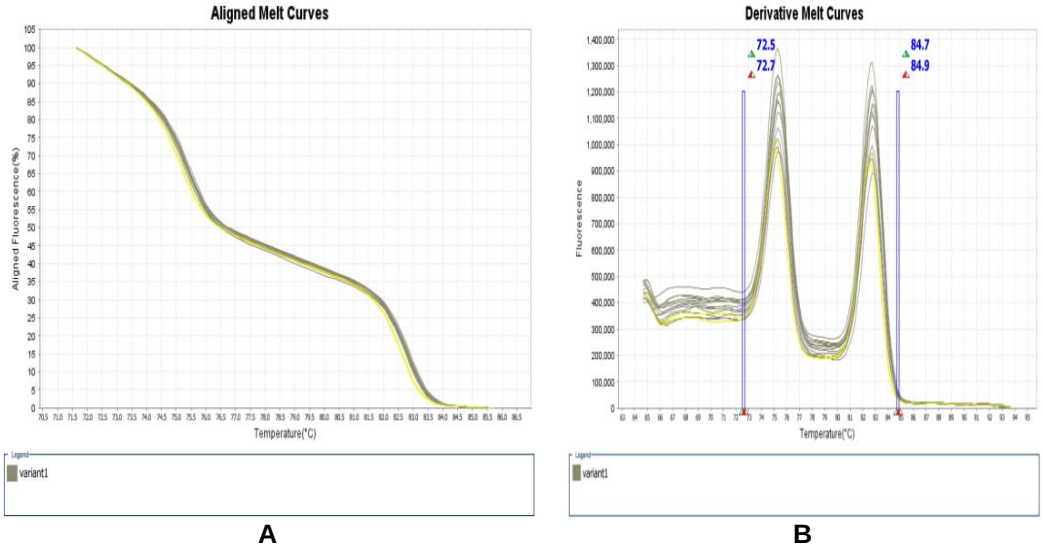

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Fig. 1(A,B). High resolution melting curve of 20 controls for rs1 rs(rs2214102), the curve shows 2obvious peaks with 2 TM, all sample were heterozygous for this rs and this was confirmed with sequencing results

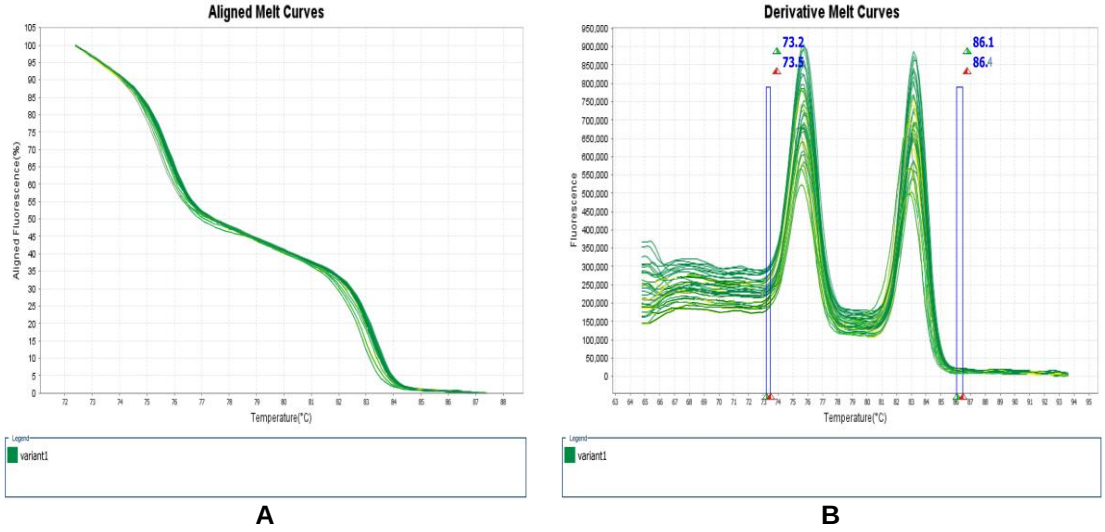

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Fig. 2(A,B). High resolution melting curve of breast cancer patients for rs1 rs1(rs2214102), the curve shows 2obvious peaks with 2 TM, all samples were heterozygous for this rs and this was confirmed with sequencing results

On the other hand , HRM analysis of 50 DNA samples of 50 patients and 20 DNA samples of the controls for (rs2032582) 2677G > T/A revealed that samples are divided into 3 groups, first one is wild homozygous genotype (GG) 22 sample out of 50 patient and 6 out of 20 control. Another 2 homozygous variants (TT) or (AA), each variant has a different Tm. (TT) variant was 21 sample out of 50 patient as shown in Tables 2, 3 and Figs. 3, 4. The frequency of the 3 variants in Patients samples were 44%, 42%, 14% receptively. The frequency of the 3 variants in control group was 30%, 30%, 40% receptively, with no statistical difference between patients and control P =.057.

The results of HRM analysis were confirmed by the results of direct sequencing of the corresponding (rs2214102) and (rs2032582) amplicons within the ABCB1 gene, obtained from independent PCR reactions. The results were compared to the wild ABCb1 sequence using Basic Local Alignment Search Tool (www.Blast.ncbi.nlm.nih.gov/Blast.cgi).

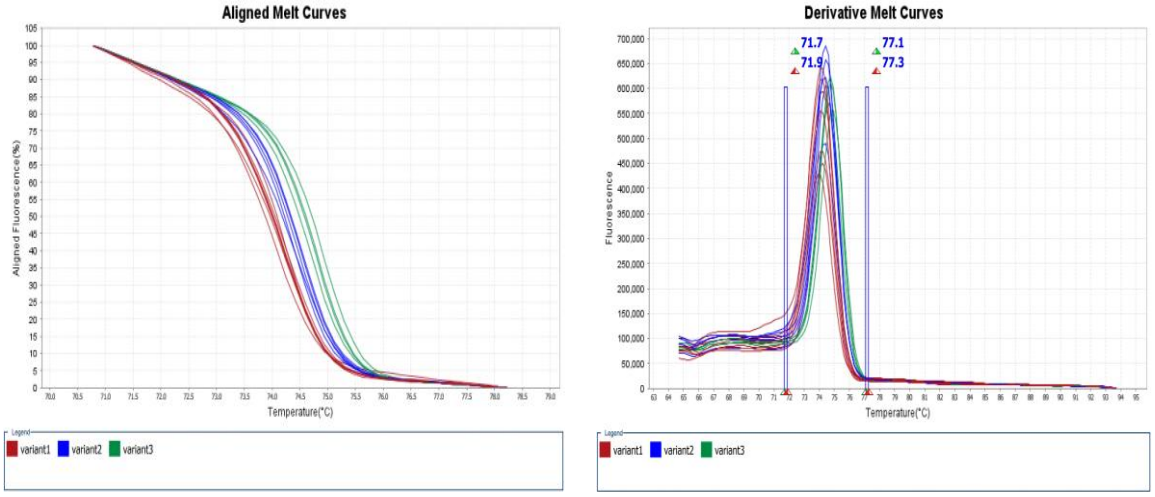

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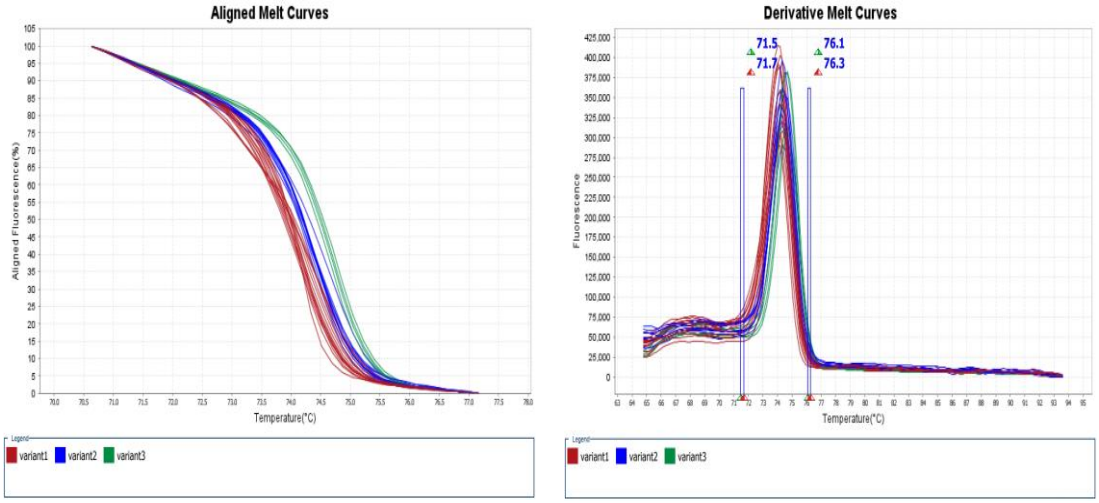
Fig. 3(A,B). High resolution melting curve of 20 controls for rs2 rs2032582, curves shows adjacent peaks with 3different TM .samples divided into 3 varients and this was confirmed with sequencing results

Table 2. Comparison between Tm values of (rs2214102) and (rs2032582) in patients and control results

TM result	Patients (n= 50)	Control (n= 20)	P-value
TM1 Rs1(rs2214102):GA			0.114
Mean ± SD	82.78 ± 0.15	82.84 ± 0.11	
Range	82.6 - 83.0	82.6 - 83.0	
TM2 Rs1(rs2214102):GA			0.878
Mean ± SD	75.38 ± 0.14	75.39 ± 0.14	
Range	75.2 - 75.6	75.2 - 75.6	
TM (rs2032582) wild allele (GG)			0.812
Mean ± SD	74.09 ± 0.09	74.10 ± 0.09	
Range	74.0 - 74.2	74.0 - 74.2	
TM (rs2032582) Varient 2 (TT)			0.681
Mean ± SD	74.36 ± 0.05	74.37 ± 0.05	
Range	74.3 - 74.4	74.3 - 74.4	
TM(rs2032582) Varient 3(AA)			0.688
Mean ± SD	74.76 ± 0.08	74.74 ± 0.05	
Range	74.7 - 74.9	74.7 - 74.8	

Table 3. Comparison of HRM results between 50 breast cancer patients and 20 normal controls

SNP result	Patients (n= 50)		Control (n= 20)		P-value
	No.	%	No.	%	
Rs1 (rs2214102) result:					
Heterozygos variant **(GA)**	50	100.0	20	100.0	--
Rs2(rs2032582)result:					0.057
Variant 1,homozygous**(GG)** Wild allele	22	44.0	6	30.0	
Variant 2, homozygous**(TT)**	21	42.0	6	30.0	
Variant 3, homozygous**(AA)**	7	14.0	8	40.0	



A B

Fig. 4(A,B). High resolution melting curve of breast cancer samples for rs2 rs2032582, curves shows adjacent peaks with 3different TM .samples divided into 3 varients and this was confirmed with sequencing results

4. DISCUSSION

The ABCB1 gene is located on chromosome 7 at 21q, with 28 exons, encoding for p_glycoprotien (Pgp). Pgp is an active ATP dependant drug‑efflux pump [10-12].

Increased expression of the ABCB1 gene product, P-glycoprotein (Pgp) in cells is usually connected with increased risk of breast cancer, occurrence of multidrug resistance (MDR) and poor prognosis. It was suggested that the P-gp mediated efflux decreases the drug concentration in cancer cells which results in the failure of chemotherapy [13,14].

The ABCB1 polymorphism contribution to MDR and breast cancer development has been intensively studied over the last few years. However, the data are still controversial and show dependence on the ethnic group and population studied [15].

In this study, we analyzed the frequency of 2 SNP in the ABCB1 gene (rs2214102) and (rs2032582).

Firstly, regarding ABCB1 rs2214102, our data revealed that all samples of patients and control have the same heterozygous variant of rs (rs2214102) with 100% (GA) in both groups.

Our results agree with Kroetz et al. [15] who performed a study on 247 ethnically diverse DNA samples and aimed to identify and describe novel variants in the ABCB1 gene, also to understand the extent of variation in ABCB1 among different ethnic populations. They found that variation in the ABCB1 gene is not evenly distributed among different ethnic populations [15,16].

Also, a recent meta-analysis of the ABCB1 polymorphisms concluded lack of association between the ABCB1 rs2214102 G>A polymorphisms and the risk of breast cancer [17].

On the other hand, we also studied the frequency of (rs2032582) on both patients and control group we found that samples are divided into 3 homozygous variants for (rs2032582) 2677G > T/A, each variant has a different Tm. The frequency of the 3 variants in Patients samples were 44%, 42%, 14% receptively. The frequency of the 3 variants in the control group was 30%, 30%, 40% receptively. Despite the difference in the percentage but we didn't find any statistically significant difference (P value 0.057) in the variants frequencies between the patients and control. We confirmed our results of HRM analysis by direct sequencing of the corresponding (rs2032582) amplicons. Comparing sequencing results to the wild ABCb1 sequence using Basic Local Alignment Search Tool (www.Blast.ncbi.nlm.nih.gov/Blast.cgi) showed that variant 1 samples had 100% similarity with ABCb1 gene, this will be consistent with marking variant 1 in (rs 2032582) as the wild homozygous genotype GG.

Our results are matched with the finding of the previous study done by Rubis et al. [3] who assessed the frequency of (rs2032582), 2677 G>A, T polymorphism in Polish population breast cancer patients and estimated their contribution to cancer development using allele-specific amplification. The study revealed no significant differences in the polymorphism frequencies between patients and controls. So they suggested (rs2032582), 2677 G>A, T SNPs are not good predictive factors in breast cancer risk or development in Caucasians.

Similarly, Tatari et al. [18] study revealed no significant differences in the genotype frequencies between the patients and control subjects for (rs2032582), 2677 G>A, T.

5. CONCLUSION

In conclusion, our pilot study revealed no significant differences in the studied polymorphism frequencies between patients and controls. So they might not be good predictive factors for the value of money in breast cancer risk or development.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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