Original Research Article

Is Angiotensin Converting Enzyme Insertion/Deletion (rs1799752) Polymorphism Associated with Breast Cancer Risk in Egyptian Population?

ABSTRACT

Background: According to GLOBOCAN estimates, breast cancer was found to be the most often diagnosed cancer in women worldwide, (11.7 %) and the fourth leading cause of cancer mortality (6.9 %).

Aim: The purpose of this study is to investigate the role of the Angiotensin I-converting enzyme (ACE) gene polymorphism in breast cancer prediction risk in Egyptian population.

Methods: Polymorphism detection analysis was performed on 163 subjects from breast cancer (**BC**) patients, 79 with Benign Breast Disease group (**BBD**) patients and 202 controls (**C**). ACE I/D (rs1799752) polymorphism were detected using polymerase chain reaction (PCR).

Results: The observed genotype frequencies were II 10.9%, ID 78.2% and DD 10.9% in healthy control, II 8.6%, ID 79.1% and DD 12.3% in BC patients and II 12.6%, ID 78.4% and DD 9% in BBD patients. There were no association between ACE gene polymorphisms, between the BC or BBD groups when compared to the control group (OR_{DD}= 1.43, 95 % CI= (0.58-3.52), P= 0.29) and (OR_{DD}= 1.29, 95 % CI= (0.57-2.95), P= 0.37) respectively. There was no risk estimate in BC or BBD when DD vs II + ID (Recessive) or ID vs II+ DD (Over-dominant) were compared to control. Allele frequencies show the same figure. From the different histological BC hormonal markers the Her2 was showing significant association in ID genotype of ACE I/D (rs1799752) (P= 0.04) and dominant model (II vs ID + DD, P= 0.03). Concerning different BC prognostic models, the poor prognostic one of **Her2 enriched model (ER^{-ve} PR^{-ve} $Her2^{+ve}$)** show significant association in ACE genotype ID and dominant model (II vs ID + DD), (P= 0.01) when compared to the good prognostic hormonal status**.**

Conclusion: It seems that this is the first study that interested in correlate the ACE gene polymorphisms in different BC variants characters in Egyptian patients. ACE I/D (rs1799752) polymorphism ID genotype have strong association to breast cancer carcinogenesis, poor prognosis and metastasis. It may be used as practical biomarker to guide the BC carcinogenesis and risk process.

Keywords: Breast cancer, ACE, Polymorphism, Genotypes, risk factor.

1. INTRODUCTION

Breast cancer (BC), worldwide is showing persistent diagnosing type of cancer which growing by about more than two million new cases each year presented over (11.7%) of all diagnosed cancer, and found to be the primarily cause of women death which accounts for (6.9%) of total cancer deaths. The death rate in female BC was found more in transitioning countries when compared to transitioned countries (15.0 to 12.8 per 100,000 cases), **[1].** In Egypt, breast cancer represents the highest incidence female's cancer types; with more than (32%) and a three-fold increase is predicted by 2050 as recently reported by the National Cancer Institute (NCI), Egypt **[2].**Comparing to USA and other Western societies, Egypt show lower incidence in BC, while Egyptian BC patients shows higher mortality rate. BC is the second-leading cause of death from cancer in Egyptian women. Egyptian BC patients with no family history of BC shows 85% of all diagnosed BC. This may explained by the genetic mutations that happen as a result of the aging or life style with a tendency to occur in younger age groups with advanced stages **[3- 5].**

BC develops due to complex interactions between genetic and different risk factors. Different classical pathological markers was used to conform patients clinical character like tumor size, as well as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) statuses. The high-risk patients should be identified at the earliest stage by applying a novel diagnostic and therapeutic regimen. Regarding chemotherapy, increased response to neo-adjuvant and adjuvant treatment with high immune infiltration has been documented **[6, 7].**

Single nucleotide polymorphism (SNP) is a common genetic variation that affects biological function **[8].** Renin–angiotensin system (RAS) is mainly influences sodium balance, extracellular fluid volume and systemic vascular resistance **[9, 10],** also it is involved with systemic regulation of cardiovascular system and homeostasis and is also known to be expressed in multiple cancer types including BC **[11, 12].** ACE is differentially regulated in several malignancies and affects tumor cell proliferation, invasion, angiogenesis, and aggressive behavior. Overexpression of ACE gene often reported in most of the neoplastic transformation and angiogenesis **[13- 15].**

The ACE, a cell surface zinc metalloenzyme, dipeptidyl carboxypeptidase is considering a member of RAS system. It involves in catalyzing the conversion of angiotensin I (Ang I) into a physiologically active octa-peptide angiotensin II (Ang II) is another emerging candidate marker for tumorigenesis **[16, 17].** The ACE gene (Gene ID: 1636; also known as: DCP; ACE1; DCP1; CD143) is localized in human chromosomes 17q23, and composed of 26 exons and 25 introns, spans about 21 kb and more than 13 polymorphisms in this gene have been identified with susceptibility to different disease such as ACE I/D (rs1799752), A240A>T (rs4291), 2350G>A (rs4344), and 17888C>T (rs4359) **[18].** The ACE insertion/deletion (I/D) polymorphism is a nonsense and 287 bp Alu repetitive sequence of DNA in the intron 16 of ACE gene, which represented by "Insertion" or "I", and absence of the same denotes "Deletion" or "D" **[19].** Thus, patients can be of three genotypes with regard to ACE, namely, II, ID and DD. In terms of ACE plasma levels, homozygotes for the D allele have the highest, followed by ID heterozygotes and homozygotes for the I allele **[20, 21].**

To date, several studies have evaluated the association between ACE I/D polymorphism and breast cancer risk. However, the results are inconsistent and inconclusive for the low sample size, with some studies found significant association, while others were not. Therefore, this study is performed to investigate the role of the ACE I/D (rs1799752) gene polymorphism in breast cancer prediction risk in Egyptian population.

2. MATERIAL AND METHODS

2.1. Ethical declaration:

The patients were admitted to Mansoura University Oncology Center Hospitals, Mansoura, Egypt, over the years 2019 and 2020. The protocol approval was allowed by the Institutional Review Board (IRB) at Mansoura University before starting the study. All methods were performed in accordance to the guidelines and regulations proposed in the 1975 Declaration of Helsinki. Informed consent letter

was obtained from all the participants. All the patient related data including biological samples were anonymized to ensure confidentiality.

2.2. Patients and controls:

BC female patients 163 the median age = 52.7 years, (age range = 27– 80 years). BC patients are classified by different grading systems which influence the prognosis and different factors for histo-pathological diagnosis. Histological appearance is usually used to classify BC which is derived from the lobules or epithelium lining the ducts and these cancers are classified according grade, stage, node status and metastasis as well operation type **[12].** For each patient, tumor size, as well as ER, PR and Her2 statuses were detected by which the BC group was further correlate these separate individual prognostic factors to the ACE I/D polymorphism genotypes. BC patients group have been recently diagnosed as having breast cancer with no chemo/radiotherapy involvements. NPI, the mandatory Nottingham prognostic index accurately predicts survival in BC patients **[22],** was calculated for each BC patient. Three prognostic groups were cut-off points separated. They were (NPI of < 3.4) represent the good prognostic index (GPI), (NPI of 3.41–5.4) was performed as the moderate prognostic index (MPI) and finally the (NPI of > 5.41) were illustrating the poor prognostic index (PPI). The equation used in NPI quantitation is:

$$NPI = (0.2 \times \text{tumor size}) + \text{Node status} + \text{Grade status}.$$

Another two groups were recruited, Benign Breast Disease group (**BBD**) of 79 patients and 202 volunteer of control group (**C**) were recruited as cancer-free and donors of solid organ from Mansoura University with median age of 45.9 years, (age range 36– 63 years).

2.3. DNA extraction and ACE (rs1799752) gene I/D polymorphism Genotyping:

EDTA containing tubes were used to collect blood samples. DNA was extracted from puffy coats of EDTA samples were it can be collected after spin at 2500 g for 9 min at RT. DNA extraction was performed according to the commercial kit procedure Promega DNA extraction kit (Promega. USA. A1120).

The ACE I/D (rs1799752) genotypes were determined using the polymerase chain reaction method (PCR) according to the method of **Rigat et al., [2**1]**.** The sequences of the sense (F) and antisense (R) primers were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', respectively prepared by (Eurofins, genomics, Germany). PCR was performed in a final volume of 20 µl that contained 10 µl 2X ViRed Taq Master Mix (v*i*vant*i*s, Malaysia), ≈500 ng of genomic DNA, 12.5 pmol of each primer and 5% dimethylsulphoxide (DMSO). Amplification was performed using a Gene Amp PCR system (Thermo Scientific ARKTIK thermal cycler). Samples were denatured for 7 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 minute at 72°C. PCR products (490-bp insertion and 190-bp deletion) were visualized on a 1.5 % agarose- gel containing GelStar™ Nucleic Acid Gel Stain (LONZA, Rockland, ME, USA, Cat No: 50535) **(Figure 1a).**

A second PCR amplification was performed for each DD type with a primer pair that recognizes an insertion-specific sequence (sense and antisense primers were 5'- TGG GAC CAC AGC GCC CGC CAC TAC-3'; 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3' respectively), with identical PCR conditions except for an annealing temperature of 67°C and the absence of 5% DMSO. The PCR product was detected at 330- base pair **(Figure 1b).** The test consistency and reproducibility were confirmed by randomly selecting 15% of the DNA samples to repeat the PCR for second time and where no misapprehension in the genotyping. The results were totally concordant with the former ones.

2.4. Statistics:

The gene counting method was used to calculate allelic frequencies in all subjects participating in the study. The genotypes of ACE I/D (rs1799752) and allele frequencies in BC patients were compared to BBD and controls using chi-square test. Relative risk for disease were calculated by Odds ratios (OR)

and 95% confidence intervals (CI). The correlation values of histological and clinical data with the ACE I/D (rs1799752) genotypes in BC patients were calculated using the same tests. NPI quantitatively compared to the ACE I/D (rs1799752) genotypes using two-tailed Student's t-test. Statistical significance was assumed at the $P<0.05$ level. The SPSS statistical software package version 21.0 for Windows (Chicago, Illiniois, USA) was used for the statistical analysis.

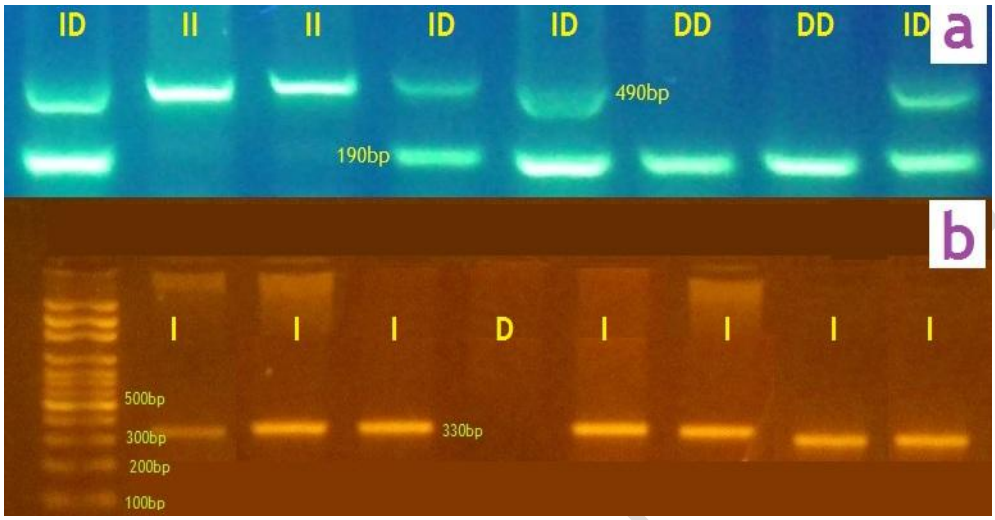

Figure 1: Agarose gel electrophoresis of the ACE I/D (rs1799752) polymorphism showing different ACE genotypes which representative by 1.5 % agarose gel stained with GelStar™ Nucleic Acid Gel Stain and photographed under ultraviolet trans-illumination after PCR amplification with specific primers, **a) ACE1,** The upper band of 490 bp is representing the (I) allele and the lower band of 190 bp is representing the (D) allele. The II genotype is shown as a single upper band, the DD genotype as a single lower band, and the DI type as a double band. **b) ACE2,** shows the results of different samples from the 1st PCR identified as DD genotype, by using an insertion-specific primer to differentiate if it is real DD or mis-genotype from ID. The sample in lane 1 is the Ladder, the band of 330bp present the I allele in the former mis-typed DD, while the true DD genotype show no band.

3. RESULTS

3.1. Distribution of ACE I/D genotypes (rs1799752) in different studied groups:

A total of 242 female breast patients were participated in the study in addition to 202 healthy unrelated individuals from the same locality. The amplified PCR product for ACE I/D (rs1799752) were detected at 490- base pair for insertion and 190- base pair for deletion as shown in **(Fig. 1)**, second PCR amplification was performed for each DD genotype with an insertion-specific sequence detected at 330- base pair **(Fig. 2)**. Based on these results, in different studied groups, the genotypes and the alleles of the ACE I/D genes polymorphism were determined and evaluated in comparison with their respective healthy controls. Results shown in **(Table 1)**, pointed out the frequencies of different genotypes as well as different genetic models which revealed the same frequencies in different genotypes (II, ID and DD) within different studied groups (BC, BBD and C). These frequencies were C_{II} (10.9%), C_{ID} (78.2%) and C_{DD} (10.9%); BC_{II} (8.6%), BC_{ID} (79.1%) and BC_{DD} (12.3%) and BBD_{II} (12.6%), BBD_{ID} (78.4%) and BBD_{DD} (9%). Similarly, the allele frequencies also have the same figure in different studied groups C_I (50%) and C_D (50%); BC_I (48.2%) and BC_D (51.8%) and BBD_I (51.9%) and BBD_D (48.1%).

The data shows no significant differences in BC or BBD groups in different genetic models of ACE I/D (rs1799752) genotype when compared to control group or when both groups were compared together **(Table 1)**. This observation was seen in all genetic models (II vs ID, II vs DD; Co-dominant), (DD+ID vs II; Dominant), (ID vs II + DD; Over-dominant) as well as (DD vs II + ID;

Recessive). All these models shows the same OR (95% CI) within different studied groups which gives no significant probability (P) which reveals the lake of the ACE I/D (rs1799752) genes polymorphism in the development of breast cancer.

3.2. Distribution of ACE I/D genotypes (rs1799752) in different variant of BC Group:

Demographic, clinico-pathological data and biomarker parameters of the study participants which have been gathered from patients' medical records were shown in **(Table 2, First Column)**. Different features listed in the table represent the number and percentage of each variant in relation to the BC group, among these features the predominant cancer stage was stage II (67.5%), node status was N0 (34.4%), cancer grade was grade II (71.2%), tumor size was ≥ 2 cm- 5 cm (74.2%), NPI was >3.4- 5.4 (74.2%), positive ER was (79.9%), positive PR was (76.1%), negative Her2/neu expression was (56.4%), negative metastasis was (85.3%) and left operated breast was (61.3%). Different ACE I/D (rs1799752) genotypes in BC group was 14 (8.6%), 129 (79.1%) and 20 (12.3%) for II, ID and DD genotype respectively.

The distribution of different genotype of ACE I/D (rs1799752) gene in different variables of tumor in breast cancer patients (163 Patients) were detailed in **(Table 2).** among the predominant of these features ID genotype show the most prevalence genotype in cancer stage was in stage T2 (67.4%), in node status was N0 (37.2%), in cancer grade was grade II (71.3%), in tumor size was (2- 5cm, 73.6%), in NPI was (>3.4- 5.4, MPI) (73.6%), in positive ER was (82.1%), in positive PR was (76.7%), in negative Her2/neu expression was (56.6%), in negative metastasis was (84.5%) and right operated breast was (62%). The II and DD genotypes show mostly the same presentation in different BC characteristic variables of the tumor. II genotype tends to be predominant in the worse variable of T3, N3, G3, negative ER and PR, positive Her2new, (2- 5cm) tumor size and PPI. Inversely, DD genotype tends to be predominant in the initial variable of T1, N1, G1 tumor size (<2cm) and MPI. Detailed distribution of different genotype of ACE I/D (rs1799752) gene in different variables of tumor in breast cancer patients (163 Patients) were presented in **(Table 2).**

3.3. Association of ACE I/D genotypes (rs1799752) in response to hormonal status of BC Group:

By comparing the different models of ACE I/D (rs1799752) genotype as a risk estimate with different variables of tumor in BC group, results revealed no association with ER, PR, metastasis or operation type **(Supplement Tables,** (ER) **1,** (PR) **2** and (Metastasis) **3)**. While a significant association in the host ACE I/D (rs1799752) genotype with **Her2/neu** expression marker, in the co-dominant model (II vs. ID, P= 0.04, II vs. DD, P= 0.07) as well as dominant model (II versus ID+DD, P= 0.03) with the negative Her2/neu expression marker, (**Table 3).** Same figure was noted when look at the Operation Type, where significant association in the host ACE I/D (rs1799752) genotype with Lt MRM the co-dominant model (II vs. ID, P= 0.05, II vs. DD, P= 0.02) as well as dominant model (II versus ID+DD, P= 0.04) (**Table 4).**

When testing the host ACE I/D (rs1799752) genotype in different BC prognostic models **(Salimifard et al., 2020)** the very poor prognostic model **(Triple –ve model)** which show negative expression for different hormonal status (10 cases) as well as other poor prognostic model of **luminal B model, (ER^{+ve} PR^{+ve} $Her2^{+ve}$)** of hormonal status (55 cases), we found no statistical significant differences within different host ACE I/D (rs1799752) genotype when compared to the good prognostic model (64 cases) hormonal status **luminal A model, (ER^{+ve} PR^{+ve} $Her2^{-ve}$), (Supplement Tables 4 and 5)** respectively**.** The same figure with no association of ACE I/D (rs1799752) genotype was noted when **Triple –ve model** was compared to the poor prognostic hormonal status **Her2 enriched model (ER^{-ve} PR^{-ve} $Her2^{+ve}$)** model (14 cases), **(Supplement Table 6).** While a significant association in the host ACE I/D (rs1799752) genotype was noted in the co-dominant model (II vs. ID, P= 0.01) as well as dominant model (II versus ID+DD, P= 0.02) when the poor prognostic hormonal status **Her2 enriched model (ER^{-ve} PR^{-ve} $Her2^{+ve}$)** model (14 cases) compared to the good prognostic hormonal status **luminal A model, (ER^{+ve} PR^{+ve} $Her2^{-ve}$), (Table 5).**

3.4. Distribution of ACE I/D (rs1799752) genotype according NPI in BC Group:

Regarding NPI, the frequency among different ACE I/D (rs1799752) genotype was listed in (**Table 6).** The significant differences have been noted within different genotypes when using student t- Test. The different NPI were (5.05 ± 0.2 for II, 4.68 ± 0.07 for ID and 4.49 ± 0.16 for DD) respectively and the significant were (P= 0.10 for II vs ID, **P= 0.03** for II vs DD and P= 0.3 for ID vs DD) respectively. When ACE I/D (rs1799752) genotype where tested in response to different hormonal markers, no significance differences were noted in NPI in both ER and PR **(Supplement Tables** (ER) **7**, (PR) **8).** While in **Her2/neu** expression marker, it shows a significant increase in NPI in positive one than the negative (P= 0.02) in ID genotype (**Table 7)**. When different ACE I/D (rs1799752) genotypes were tested within different NPI groups MPI and PPI **(Supplement Table 9),** no significant differences were observed between different ACE I/D (rs1799752) genotypes in each NPI groups. For different hormonal markers, (**Table 8)** similarly, no significant differences in NPI when negative hormonal markers were compared to positive ones for ER and PR while **Her2/neu** expression marker show a significant increase in NPI in positive one than the negative (P= 0.05).

3.5. Distribution of ACE I/D (rs1799752) genotype according Metastasis in BC Group:

Metastasis the most worth complication in BC was detected in 24 patients, where 2 patients show ACE I/D (rs1799752) II genotype (bone metastasis), 20 patients show ID genotype and 2 patients show DD genotype (1 bone and 1 bone & lung metastasis). The most presented metastasis was in bone metastasis presented in 8 cases, bone and LN in 5 cases, lung in 5 cases, bone and liver in 3 cases, bone and lung in 2 cases and another case the metastasis goes to brain, bone and LN. Detailed presentation of different ACE I/D (rs1799752) genotype showing metastasis were presented in (**Table 9).**

Table 1: Distribution of different genotype of ACE I/D (rs1799752) with risk estimate and allele frequencies in control, BC and BBD groups in different ACE genetic models.

ACE Genotype	Group's # (%)		
	Control (202)	BC (163)	BBD (79)
II	22 (10.9)	14 (8.6)	10 (12.6)
ID	158 (78.2)	129 (79.1)	62 (78.4)
DD	22 (10.9)	20 (12.3)	7 (9)
Allele			
I	202 (50)	157 (48.2)	82 (51.9)
D	202 (50)	169 (51.8)	76 (48.1)
Statistics	DD+ID vs II (Dominant)		BC vs BBD
OR		1.3	1.13	1.31
95% CI		(0.64- 2.63)	(0.65- 1.96)	(0.79- 2.2)
Sig. (P)		0.29	0.4	0.22
	DD vs II + ID (Recessive)			BC vs BBD
OR		0.87	1.26	1.38
95% CI		(0.46- 1.66)	(0.51- 3.07)	(0.55- 3.42)
Sig. (P)		0.4	0.4	0.32
	ID vs II + DD (Over-dominant)			BC vs BBD
OR		0.95	0.98	1.04
95% CI		(0.57- 1.58)	(0.52- 1.85)	(0.54- 2)
Sig. (P)		0.47	0.55	0.51
	D allele vs I allele			BC vs BBD
OR		1.07	1.05	1.11
95% CI		(0.8- 1.44)	(0.81- 1.38)	(0.85- 1.43)
Sig. (P)		0.34	0.38	0.25
	II vs ID (Co-dominant)			BC vs BBD
OR		1.28	0.86	1.28
95% CI		(0.63- 2.61)	(0.39- 1.93)	(0.76- 2.15)
Sig. (P)		0.3	0.43	0.25
	II vs DD			BC vs BBD
OR		1.43	1.29	1.6
95% CI		(0.58- 3.52)	(0.57- 2.95)	(0.73- 3.55)
Sig. (P)		0.29	0.37	0.18

Table 2: Characteristic frequency of tumor characters in breast cancer patients (163 Patients, first column). Distribution of different genotype of ACE I/D (rs1799752) gene in different variables.

Variables	Patient number (percentage)		
Genotype	II	ID	DD
(163 Patients)	14 (8.6)	129 (79.1)	20 (12.3)
Cancer stage			
26 (15.9) T1	1 (3.8)	20 (76.9)	5 (19.3)
110 (67.5) T2	8 (7.3)	87 (79.1)	15 (13.6)
21 (12.9) T3	5 (23.8)	16 (76.2)	0 (0)
6 (3.7) T4	0 (0)	6 (100)	0 (0)
Node Status			
56 (34.4) N0	2 (3.6)	48 (85.7)	6 (10.7)
42 (25.7) N1	3 (7.1)	33 (78.6)	6 (14.3)
40 (24.5) N2	5 (12.5)	29 (72.5)	6 (15)
25 (15.4) N3	4 (16)	19 (76)	2 (8)
Overall grade			
3 (1.8) G1	0 (0)	2 (66.7)	1 (33.3)
116 (71.2) G2	9 (7.8)	92 (79.3)	15 (12.9)
44 (27) G3	5 (11.4)	35 (79.5)	4 (9.1)
Tumor size			
14 (8.6) <2cm	1 (7.1)	11 (78.6)	2 (14.3)
121 (74.2) 2- 5cm	10 (21.7)	95 (78.5)	16 (13.2)
28 (17.2) >5 cm	3 (10.7)	23 (82.1)	2 (7.2)
NPI			
9 (5.5) >2.4- 3.4	0 (0)	8 (88.9)	1 (11.1)
121 (74.2) >3.4- 5.4	9 (7.4)	95 (78.5)	17 (14.1)
33 (20.3) >5.4	5 (15.2)	26 (78.8)	2 (6)
Estrogen receptor			
33 (20.3) Negative	4 (12.1)	23 (69.7)	6 (18.2)
130 (79.7) Positive	10 (7.7)	106 (81.5)	14 (10.8)
Progesterone receptor			
39 (23.9) Negative	5 (12.8)	30 (76.9)	4 (10.3)
124 (76.1) Positive	9 (7.3)	99 (79.8)	16 (12.9)
Her2/neu expression			
89 (54.6) Negative	4 (4.5)	73 (82)	12 (13.5)
74 (45.4) Positive	10 (13.5)	56 (75.7)	8 (10.8)
Metastasis			
139 (85.3) Negative	12 (8.6)	109 (78.5)	18 (12.9)
24 (14.7) Positive	2 (8.3)	20 (83.4)	2 (8.3)
Operation Type			
100 (61.4) Lt MRM	5 (5)	80 (80)	15 (15)
63 (38.6) Rt MRM	9 (14.3)	49 (77.8)	5 (7.9)

Table 3: Distribution of different genotype of ACE I/D (rs1799752) with risk estimate in response to Her2/neu expression marker in BC group.

Model	Genotype # (%) Her2/neu		OR (95% CI)	Sig. (P)
Co-dominant	**Negative 89 (54.6)**	**Positive 74 (45.4)**		
II	4 (4.5)	10 (13.5)	1	
ID	73 (82)	56 (75.7)	1.64 (1.12- 2.42)	0.04
DD	12 (13.5)	8 (10.8)	1.78 (0.95- 3.35)	0.07
Dominant	II vs ID+ DD		1.66 (1.14- 2.43)	0.03
Recessive	II+ ID vs DD		1.28 (0.49- 3.33)	0.39
Over-dominant	II+ DD vs ID		1.47 (0.69- 3.13)	0.21

Table 4: Distribution of different genotype of ACE I/D (rs1799752) with risk estimate in response to Operation Type in BC group.

Model	Genotype # (%) Op. Type		OR (95% CI)	P
Co-dominant	**Lt MRM 100 (61.4)**	**Rt MRM 63 (38.6)**		
II	5 (5)	9 (14.3)	1	
ID	80 (80)	49 (77.8)	1.69 (1.08- 2.65)	0.05
DD	15 (15)	5 (7.9)	2.57 (1.09- 6.03)	0.02
Dominant	II vs ID+ DD		1.77 (1.13- 2.77)	0.04
Recessive	II+ ID vs DD		2.05 (0.71- 5.94)	0.13
Over-dominant	II+ DD vs ID		1.14 (0.53- 2.47)	0.44

Table 5: Distribution of different genotype of ACE I/D (rs1799752) with risk estimate in poor prognostic hormonal status Her2 enriched model (ER^{-ve} PR^{-ve} $Her2^{+ve}$) vs good prognostic hormonal status luminal A model, (ER^{+ve} PR^{+ve} $Her2^{-ve}$) in BC group.

Model	Genotype # (%)		OR (95% CI)	P
Co-dominant	**ER^{+ve} PR^{+ve} $Her2^{-ve}$ 64 cases**	**Her2 enriched 14 cases**		
II	3 (4.7)	4 (28.6)	1	
ID	52 (81.2)	8 (57.1)	4.28 (1.72- 10.64)	0.01
DD	9 (14.1)	2 (14.3)	3.14 (0.77- 12.85)	0.11
Dominant	II vs ID+ DD		4.06 (1.71- 9.6)	0.01
Recessive	II+ ID vs DD		0.98 (0.18- 5.13)	0.63
Over-dominant	II+ DD vs ID		3.25 (0.95- 11.12)	0.06

Table 6: Means and standard error of the mean of NPI for different genotype of ACE I/D (rs1799752) in BC group.

	ACE	N	Mean	Std. Error	Sig.[a]
NPI	II	14	5.0571	.20481	
	ID	129	4.6819	.07228	.103
	DD	20	4.4950	.16391	.038
					.306[b]

a= significance of II genotype vs other genotype, b= significance of ID genotype vs DD genotype.

Table 7: Means and standard error of the mean of NPI for different genotype of ACE I/D (rs1799752) in response to Her2/neu expression marker in BC group.

ACE	Her2neu	N	Mean	Std. Error	Sig
II	negative	4	5.2250	.17500	
	positive	10	4.9900	.28105	.491
ID	negative	73	4.5408	.09329	
	positive	56	4.8657	.10987	.025
DD	negative	12	4.5917	.23305	
	positive	8	4.3500	.22200	.463

Table 8: Means and standard error of the mean of NPI for different hormonal marker status in BC group.

NPI		N	Mean	Std. Error	Sig.
ER	negative	33	4.6176	.14622	
	positive	130	4.7098	.07076	.561
PR	negative	39	4.7718	.14180	
	positive	124	4.6658	.07088	.479
Her2neu	negative	89	4.5784	.08384	
	positive	74	4.8268	.09545	.052

Table 9: Distribution of different metastasis sites in different ACE I/D (rs1799752) genotype.

Site of Metastasis	II	ID	DD
Bone	2	5	1
Bone & LN	0	5	0
Bone & Liver	0	3	0
Bone & Lung	0	1	1
Bone & Brain & LN	0	1	0
Lung	0	5	0

4. DISCUSSION

Breast cancer is known as complex and multifactorial disease, the interaction between environmental and genetic factors probably important in initiation and development of the disease. It is now well established that breast cancer is the most persistent diagnosed cancer worldwide and considered a chief reason of cancer mortality among females worldwide **[1].** In Egypt, the dispersal of BC is growing and it remains a major health problem of the country with no solution. It constitutes 33% of female cancer cases and more than 22,000 new cases diagnosed each year **[23].** This is expected to rise exponentially over the next years given the enlarging population. A three-fold increase is predicted by 2050 as recently reported by the National Cancer Institute (NCI), Egypt **[2].**

RAS is represented by the system of enzymes and hormones which regulatearterial pressure, electrolytic and fluid balance. RAS activation directly or indirectly leads to activation of angiogenesis processes**.** As far as cancer development, progression and metastasis are associated with angiogenesis and proliferative processes, one may suppose that RAS could be related to cancer development. ACE is well known to be a key part of RAS, the polymorphisms especially I/D in ACE gene has been found to be associated with different diseases including cancer **[24, 25].** This study aimed to determine the association of the ACE I/D (rs1799752) gene polymorphism in breast cancer prediction risk in Egyptian population.

Analysis of ACE I/D (rs1799752) polymorphism on 163 Egyptian patients with **BC**, 79 **BBD** and 202 healthy controls from the same area, showed that the frequencies of different genotypes as well as different genetic models were revealed the same frequencies in different genotypes (II, ID and DD) within different studied groups (BC, BBD and C). the most present predominant genotype is ID where its frequencies was over 75% while the other two genotypes (II and DD) ware shared the (20%) left frequency. This finding in concise with **Sharma and coworker**, **[26]**, where they found that ID genotype was conferring approximately 2.5 folds risk for BBD and ACE polymorphism was projecting a protective role towards BC susceptibility.

A number of meta-analysis studies were conducted to investigate the association of angiotensin converting enzyme insertion/deletion (ACE I/D) polymorphism with breast cancer. But the results remain controversial. Some suggests that ACE I/D polymorphism may be associated with increased risk of breast cancer. ACE I/D might be associated with BC in overall and by ethnicity **[24, 25]**, especially among Asian and Caucasians. However, well-designed studies with larger sample size and more ethnic groups are needed to further validate the suspected association.

In agreement of the present results, a lack of association between ACE I/D (rs1799752) gene Polymorphism and breast cancer risk was reported from different ethnic background like Ukraine **[27],** Pakistani **[13],** Indian **[12, 26]** as well as Egyptians **[11].** Although all these studies were in agree with the present results, all were lacking a good statistically participating numbers. Within the BC group, we try to make different correlations between ACE I/D (rs1799752) gene Polymorphism and hormonal analysis and Predictive Index (NPI). DD genotype was found to be more present in the initially primitive cancer characters like cancer stage, grade and node status. Inversely it was more present in the worth hormonal receptor status. Where this study observes the ID genotype of ACE I/D (rs1799752) polymorphism is the most predominant in different BC variant like grad and stage, the different ethnic Brazilian observes that DD genotype is the most predominant **[28],** this may be the did not perform the second PCR to differentiate the mistyping DD genotype.

No association has been noted with ACE I/D (rs1799752) gene polymorphism in response to negative vs positive **ER** or **PR** hormonal status or metastasis, while the human epidermal growth factor receptor 2 (**Her2**) show a significant association to ACE I/D (rs1799752) genotype (P= 0.04, 0.03) in

the co-dominant model as well as dominant model (II vs. ID and II versus ID+DD) respectively. This confirms the association of ID genotype with the aggressiveness type of BC. When analyzing different prognostic model a significant association in ACE I/D (rs1799752) genotype (P= 0.01) with the poor prognostic model of **Her2 enriched model, (ER^{-ve} PR^{-ve} $Her2^{+ve}$)** for both the co-dominant model (II vs. ID) as well as dominant model (II versus ID+DD) when compared to the good prognostic hormonal status **luminal A model, (ER^{+ve} PR^{+ve} $Her2^{-ve}$).** This again confirms the association of ID genotype with the aggressiveness type of BC. We found no studies concerning these different models to share their results with them.

The **NPI** frequency among different ACE I/D (rs1799752) genotype show no significant differences when different genotypes were tested within different NPI groups GPI, MPI and PPI**.** Significant differences were observed in NPI between II and DD genotype of ACE I/D (rs1799752) polymorphism where **II** shows the most worth NPIwhen compared to DD genotype. A significant difference in NPI was noted in response to **Her2/neu** expression marker **in ID** genotype of ACE I/D (rs1799752) polymorphism. When different markers have been analyzed in response to NPI only **Her2/neu** expression marker is show significant decrease NPI in negative expression individuals when compared to positive ones. These results can give us the chance to confirm the association between ACE I/D (rs1799752) ID genotype, **NPI** and **Her2/neu** expression marker. We found no studies concerning this association to share their results with them.

Metastasis is a complex process that involves tumor spread to distant parts of the body from its original site. The exact initiation process of breast cancer metastasis is unknown. The most metastatic patients were observed in ID genotype of ACE I/D (rs1799752) polymorphism. We found no studies concerning these different models to share their results with them.

5. CONCLUSION

It seems that this is the first study that interested in correlate the most functional important gene polymorphisms of ACE I/D (rs1799752) with different BC characteristic variants in Egyptian women. The study demonstrated no association in BC group in response to DD genotype or D allele of ACE I/D (rs1799752) polymorphism when compared to either BBD or control group. The ID genotype show the significantly correlated with the aggressive carcinogenesis of BC, suggesting its role in the pathogenesis of BC, this may explain the spread of this ethnic patients where ID genotype have the most frequency among different ACE I/D (rs1799752) polymorphism. This study confirm also that ID genotype have association with **NPI**, **Her2/neu** expression marker and metastatic distribution in BC patient. ACE I/D (rs1799752) polymorphism ID genotype have strong association to breast cancer carcinogenesis, poor prognosis and metastasis. It may be used as practical biomarker to guide the BC carcinogenesis and risk process. This may explain the high incidence of breast cancer in Egyptian population as it possesses the frequency for ACE I/D (rs1799752) genotype.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021**;** 0:1-41. doi: 10.3322/caac.21660.
2. National Cancer Institute, Cairo University. Available at: http://www. nci. cu. edu. eg/. Accessed 10 Nov **2020**.
3. Feng, Y.; Spezia, M.; Huang, S.; Yuan, C.; Zeng, Z.; Zhang, L.; Ji, X.; Liu, W.; Huang, B.; Luo, W.; et al. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis. **2018;** 5, 77–106.
4. National Cancer Registry Program of Egypt. Available at: http://www. Cancer registry. gov. eg/. Accessed 10 Nov **2020**.
5. Saleh B, Elhawary MA, Mohamed ME, El Zayat MS, Mohamed H. Gail model utilization in predicting breast cancer risk in Egyptian women: a cross‐sectional study. Breast Cancer Research and Treatment. **2021;** https://doi.org/10.1007/s10549-021-06200-z.
6. Pruneri G, Vingiani A, Denkert C. **(2018):** Tumor infiltrating lymphocytes in early breast cancer. Breast. 37: 207–214. doi:10.1016/j.breast.2017.03.010.
7. Dannenfelser R, Nome M, Tahiri A, Ursini-Siegel J, Vollan HKM, et al. **(2017):** Data-driven analysis of immune infiltrate in a large cohort of breast cancer and its association with disease progression, ER activity, and genomic complexity. Oncotarget. 8: 57121– 57133. doi:10.18632/oncotarget.19078.
8. Mituś JW, Blecharz P, Reinfuss M, et al. **(2013):** Changes in the clinical characteristics, treatment options, and therapy outcomes in patients with phyllodes tumor of the breast during 55 years of experience. Med Sci Monit; 19: 1183–1187.
9. Nishiyama, A., Kobori, H. **(2018):** Independent regulation of renin–angiotensin–aldosterone system in the kidney. Clin Exp Nephrol 22, 1231–1239. https://doi.org/10.1007/s10157-018-1567-1.
10. Smyth LJ, Ca ̃adas- Garre M, Cappa RC, et al. **(2019):** Genetic associations between genes in the renin-angiotensinaldosterone system and renal disease: a systematic review and meta-analysis. BMJ Open.9:e026777. doi:10.1136/bmjopen-2018-026777.
11. El Sharkawy RM, Zaki AM, Kamel AAEF, Bedair RN, and Ahmed AS. **(2014):** Association between the polymorphisms of angiotensin converting enzyme (Peptidyl-Dipeptidase A) INDEL mutation (I/D) and Angiotensin II type I receptor (A1166C) and breast cancer among post menopausal Egyptian females.Alex J Med.; 50: 267–274.
12. Khan S, Ahmad S **(2019):** Role of Angiotensin Converting Enzyme (ACE) Gene Polymorphism in Breast Cancer among North Indian Population. Annals of International Medical and Dental Research. 5, (6): 17- 23. DOI: 10.21276/aimdr.2019.5.6.PT3
13. Annum I, Ejaz A, Warda F and Nageen H **(2020):** Genetic Association of Angiotensin Converting Enzyme I/D Gene Polymorphisms with Breast Cancer. European Journal of Biology and Biotechnology. 1 (4): DOI: http://dx.doi.org/10.24018/ejbio.2020.1.4.41
14. Afsar B, Afsar RE, Ertuglu LA, Kuwabara M, Ortiz A, Covic A and Kanbay M **(2021):** Renin‐angiotensin system and cancer: epidemiology, cell signaling, genetics and epigenetics. Clinical and Translational Oncology. 23:682–696. https://doi.org/10.1007/s12094-020-02488-3.
15. Yusof HA and Ahmad Muhamed MC **(2021):** Angiotensin‐converting enzyme (ACE) insertion/deletion gene polymorphism across ethnicity: a narrative review of performance gene. Sport Sciences for Health 17:57–77. https://doi.org/10.1007/s11332-020-00712-9
16. Fagyas M, Úri K, Siket IM, et al **(2014):** New perspectives in the Renin-Angiotensin-Aldosterone System (RAAS) I: Endogenous Angiotensin Converting Enzyme (ACE) Inhibition. Karamyan V, ed. *PLoS One*, **9**, e87843.
17. Zhang K, Cheng D, Yi L, Shi H, Zhen G **(2014):** Association between angiotensin I-converting enzyme gene polymorphism and susceptibility to cancer: A metaanalysis. *Int J Clin Exp Pathol*, 7(9): 6291-6300.

18. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JCM **(2006):** ACE polymorphisms. *Circ Res*, **98**, 1123-33.
19. Mahjoub SA, Abdelrhman E, El-Deen MEM, Mustafa MSE, Ali EW **(2016):** Angiotensin-converting enzyme insertion/ deletion polymorphism is not associated with vasoocclusive complications of sickle cell anemia. *Int J Appl basic Med Res*, **6**, 267-70.
20. Rigat B, Hubert C, Alhenc-Gelas F, et al. **(1990):** An insertion/deletion polymorphism in the angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest*; 86: 1343–1346.
21. Rigat B, Hubert C, Corvol P, Soubrier F **(1992):** PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res 20(6):1433.
22. Todd J.H., C. Dowle, M.R. Williams, C.W. Elston, I.O. Ellis, et al., **(1987):** Confirmation of a prognostic index in primary breast cancer. Br. J. Cancer. 56, 489-492
23. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. **(2014):** Cancer incidence in Egypt: Results of the National Population-Based Cancer Registry Program. J Cancer Epidemiol.; 2014: 437971.
24. Moghimi M, Kargar S, Jafari MA, Ahrar H, Jarahzadeh MH, Neamatzadeh H, Sadeghizadeh-Yazdi J **(2018):** Angiotensin Converting Enzyme Insertion/Deletion Polymorphism is Associated with Breast Cancer Risk: A Meta-Analysis. Asian Pac J Cancer Prev, 19 (11), 3225-3231. DOI:10.31557/APJCP.2018.19.11.3225
25. Dastgheib SA, Asadian F, Farbod M, Karimi-Zarchi M, Meibodi B, Akbarian E & Neamatzadeh H **(2020):** Association of ACE I/D, -240A > T and AT1R A1166C polymorphisms with susceptibility to breast cancer: a systematic review and meta-analysis based on 35 case-control studies, Nucleosides, Nucleotides & Nucleic Acids. October 2020. https://doi.org/10.1080/15257770.2020.1826515
26. Sharma R, Raina JK, Azad T, Kumar P and Panjaliya RK **(2018):** Methylene-tetrahydrofolate Reductase (MTHFR) and Angiotensin Converting Enzyme (ACE) Gene Variations in Link with Breast Cancer in Jammu Region of Jammu and Kashmir State. Int J Hum Genet, 18(3): 219-227. DOI: 10.31901/24566330.2018/18.3.669
27. Fishchuk LE and Gorovenko NG **(2013):** Genetic Polymorphisms of the Renin-Angiotensin System in Breast Cancer Patients. Exp Oncol. 35, 2, 101–104.
28. Corrêa SAA, de Noronha SMR, Nogueira-de-Souza NC, de Carvalho CV, Costa AMM, Linhares JJ, Gomes MTV, da Silva IDCG **(2009):** Association between the angiotensin-converting enzyme (insertion/deletion) and angiotensin II type 1 receptor (A1166C) polymorph hisms and breast cancer among Brazilian women. J Renin-Angiotensin- Aldosterone System. 10, (1): 51- 58.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Abbreviations:

BC- Breast Cancer, BBD- Benign Breast Disease, C- Controls, PCR- Polymerase Chain Reaction method, OR- Odds Ratio, CI- 95% Confidence Intervals, Her2- Human epidermal growth factor receptor 2, ER- estrogen receptor, PR- progesterone receptor, RAS- Renin–Angiotensin System, ACE- angiotensin converting enzyme, SNPs- single nucleotide polymorphism, IRB- Institutional Review Board, NPI- the Nottingham Prognostic Index, GPI- Good Prognostic Index, MPI- Moderate Prognostic Index, PPI- Poor Prognostic Index, TNBC- Triple Negative BC.

APPENDIX

S-Table 1: Distribution of different genotype of ACE (rs 1799752) with risk estimate in response to Estrogen receptor (ER) marker in BC group.

Model	Genotype # (%) ER		OR (95% CI)	Sig. (P)
Co-dominant	**Negative 33 (20.3)**	**Positive 130 (79.7)**		
II	4 (12.1)	10 (7.7)	1	

ID	23 (69.7)	106 (81.5)	1.84 (0.53- 6.39)	0.26
DD	6 (18.2)	14 (10.8)	0.93 (0.21- 4.19)	0.62
Dominant	II vs ID+ DD		1.65 (0.48- 5.65)	0.31
Recessive	II+ ID vs DD		1.84 (0.65- 5.23)	0.19
Over-dominant	II+ DD vs ID		1.16 (0.92- 1.47)	0.11

S-Table 2: Distribution of different genotype of ACE (rs 1799752) with risk estimate in response to Progesterone receptor (PR) marker in BC group.

Model	**Genotype # (%) PR**		**OR (95% CI)**	**Sig. (P)**
Co-dominant	**Negative 39 (23.9)**	**Positive 124 (76.1)**		
II	5 (12.8)	9 (7.3)	1	
ID	30 (76.9)	99 (79.8)	1.83 (0.57- 5.89)	0.23
DD	4 (10.3)	16 (12.9)	2.22 (0.47- 10.45)	0.26
Dominant	II vs ID+ DD		2.04 (0.64- 6.49)	0.18
Recessive	II+ ID vs DD		0.77 (0.24- 2.46)	0.45
Over-dominant	II+ DD vs ID		0.84 (0.35- 2)	0.42

S-Table 3: Distribution of different genotype of ACE (rs 1799752) with risk estimate in response to Metastasis status in BC group.

Model	**Genotype # (%) Metast.**		**OR (95% CI)**	**Sig. (P)**
Co-dominant	**Negative 139 (85.3)**	**Positive 24 (14.7)**		
II	12 (8.6)	2 (8.3)	1	
ID	109 (78.5)	20 (83.4)	1.1 (0.23- 5.3)	0.63
DD	18 (12.9)	2 (8.3)	1.43 (0.23- 8.97)	0.55
Dominant	II vs ID+ DD		1.04 (0.22- 4.96)	0.66
Recessive	II+ ID vs DD		1.63 (0.35- 7.55)	0.4
Over-dominant	II+ DD vs ID		1.32 (0.48- 3.6)	0.4

S-Table 4: Distribution of different genotype of ACE (rs 1799752) with risk estimate in Triple –ve (very poor prognostic model) of hormonal status vs good prognostic hormonal status luminal A model, (ER^{+ve} PR^{+ve} $Her2^{-ve}$) in BC group.

Model	**Genotype # (%)**		**OR (95% CI)**	**Sig. (P)**
Co-dominant	**ER^{+ve} PR^{+ve} $Her2^{-ve}$ 64 cases**	**Triple –ve 10 cases**		
II	3 (4.7)	0 (0)	1	
ID	52 (81.2)	8 (80)	1.15 (1.04- 1.27)	0.66
DD	9 (14.1)	2 (20)	1.22 (0.92- 1.61)	0.6
Dominant	II vs ID+ DD		1.16 (1.06- 1.28)	0.64
Recessive	II+ ID vs DD		1.43 (0.35- 5.87)	0.46
Over-dominant	II+ DD vs ID		1.08 (0.2- 5.76)	0.6

S-Table 5: Distribution of different genotype of ACE- (rs 1799752) with risk estimate in the poor prognosis luminal B model (ER^{+ve} PR^{+ve} $Her2^{+ve}$) of hormonal status vs good prognostic hormonal status luminal A model (ER^{+ve} PR^{+ve} $Her2^{-ve}$) in BC group.

Model	Genotype # (%)		OR (95% CI)	Sig. (P)
Co-dominant	**ER^{+ve} PR^{+ve} $Her2^{-ve}$ 64 cases**	**ER^{+ve} PR^{+ve} $Her2^{+ve}$ 55 cases**		
II	3 (4.7)	6 (10.9)	1	
ID	52 (81.2)	44 (80)	1.45 (0.87- 2.42)	0.2
DD	9 (14.1)	5 (9.1)	1.86 (0.8- 4.33)	0.15
Dominant	II vs ID+ DD		1.49 (0.9- 2.48)	0.17
Recessive	II+ ID vs DD		1.63 (0.51- 5.21)	0.29
Over-dominant	II+ DD vs ID		1.08 (0.43- 2.69)	0.52

S-Table 6: Distribution of different genotype of ACE (rs 1799752) with risk estimate in Triple –ve (very poor prognostic model) of hormonal status vs poor prognostic hormonal status Her2 enriched model (ER^{-ve} PR^{-ve} $Her2^{+ve}$) in BC group.

Model	Genotype # (%)		OR (95% CI)	Sig. (P)
Co-dominant	**Triple –ve 10 cases**	**Her2 enriched 14 cases**		
II	0 (0)	4 (28.6)	1	
ID	8 (80)	8 (57.1)	2.0 (1.22- 3.26)	0.1
DD	2 (20)	2 (14.3)	2.0 (0.75- 5.33)	0.21
Dominant	II vs ID+ DD		2.0 (1.29- 3.1)	0.09
Recessive	II+ ID vs DD		1.5 (0.17- 12.93)	0.56
Over-dominant	II+ DD vs ID		3.0 (0.46- 19.59)	0.23

S-Table 7: Means and standard error of the mean of NPI for different genotype of ACE (rs 1799752) in response to Estrogen receptor (ER) expression marker in BC group.

ACE	ER	N	Mean	Std. Error	Sig
II	negative	4	5.1500	.27839	
	positive	10	5.0200	.27195	.746
ID	negative	23	4.5861	.17868	
	positive	106	4.7026	.07922	.539
DD	negative	6	4.3833	.36553	
	positive	14	4.5429	.18299	.668

S-Table 8: Means and standard error of the mean of NPI for different genotype of ACE (rs 1799752) in response to Progesterone receptor (PR) expression marker in BC group.

ACE	PR	N	Mean	Std. Error	Sig
II	negative	5	5.2400	.23367	
	positive	9	4.9556	.29539	.465
ID	negative	30	4.6933	.17200	
	positive	99	4.6784	.07898	.931
DD	negative	4	4.7750	.37500	
	positive	16	4.4250	.18405	.408

S-Table 9: Means and standard error of the mean of NPI for different genotype of ACE I/D (rs1799752) within different prognostic groups in BC group.

Group	ACE	N	Mean	Std. Error	Sig.[a]
MPI	II	9	4.6444	.20556	
	ID	95	4.4815	.05216	.369
	DD	17	4.3941	.13380	.301
					.521[b]
PPI	II	5	5.8000	.12247	
	ID	26	5.8808	.04804	.512
	DD	2	4.700	13.000	.682
					.883[b]

a= significance of II genotype vs other genotype, b= significance of ID genotype vs DD genotype. MPI= Moderate Prognosis. PPI= Poor Prognosis