

## 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

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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 histopathological 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., [21]. 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 (vivantis, 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:

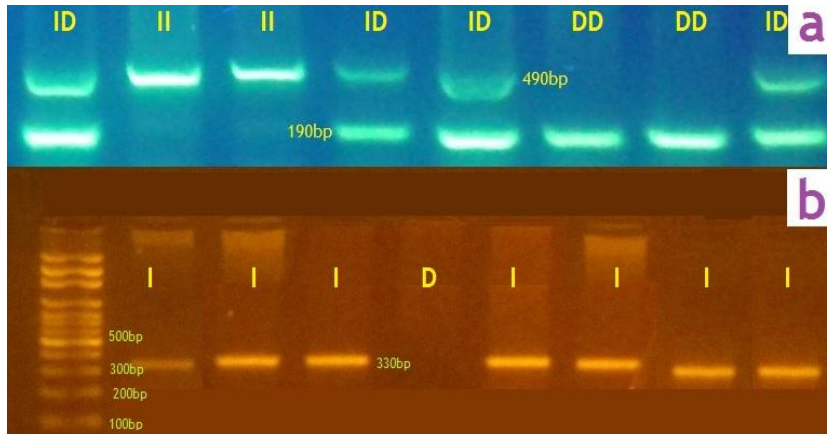
The gene counting method was used to calculate allelic frequencies in all subjects participating in the study. The genotypes of ACE I/D (rs1799752) and allele frequencies in BC patients were compared to BBD and controls using chi-square test. Relative risk for disease were calculated by Odds ratios

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(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, Illinois, 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 1<sup>st</sup> 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 lack 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  $\geq 2$  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 ( $<2$ cm) 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 ( $5.05 \pm 0.2$  for II,  $4.68 \pm 0.07$  for ID and  $4.49 \pm 0.16$  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
95% CI		(0.64- 2.63)	(0.65- 1.96)
Sig. (P)		0.29	0.4
DD vs II + ID (Recessive) BC vs BBD			
OR		0.87	1.26
95% CI		(0.46- 1.66)	(0.51- 3.07)
Sig. (P)		0.4	0.4
ID vs II + DD (Over-dominant) BC vs BBD			
OR		0.95	0.98
95% CI		(0.57- 1.58)	(0.52- 1.85)
Sig. (P)		0.47	0.55
D allele vs I allele BC vs BBD			
OR		1.07	1.05
95% CI		(0.8- 1.44)	(0.81- 1.38)
Sig. (P)		0.34	0.38
II vs ID (Co-dominant) BC vs BBD			
OR		1.28	0.86
95% CI		(0.63- 2.61)	(0.39- 1.93)
Sig. (P)		0.3	0.43
II vs DD BC vs BBD			
OR		1.43	1.29
95% CI		(0.58- 3.52)	(0.57- 2.95)
Sig. (P)		0.29	0.37

**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)
<b>Cancer stage</b>			
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)
<b>Node Status</b>			
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)
<b>Overall grade</b>			
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)
<b>Tumor size</b>			
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)
<b>NPI</b>			
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)
<b>Estrogen receptor</b>			
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)
<b>Progesterone receptor</b>			
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)
<b>Her2/neu expression</b>			
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)
<b>Metastasis</b>			
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)
<b>Operation Type</b>			
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)
<b>Co-dominant</b>	<b>Negative 89 (54.6)</b>	<b>Positive 74 (45.4)</b>		
<b>II</b>	4 (4.5)	10 (13.5)	1	
<b>ID</b>	73 (82)	56 (75.7)	1.64 (1.12- 2.42)	0.04
<b>DD</b>	12 (13.5)	8 (10.8)	1.78 (0.95- 3.35)	0.07
<b>Dominant</b>	II vs ID+ DD		1.66 (1.14- 2.43)	0.03
<b>Recessive</b>	II+ ID vs DD		1.28 (0.49- 3.33)	0.39
<b>Over-dominant</b>	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
<b>Co-dominant</b>	<b>Lt MRM 100 (61.4)</b>	<b>Rt MRM 63 (38.6)</b>		
<b>II</b>	5 (5)	9 (14.3)	1	
<b>ID</b>	80 (80)	49 (77.8)	1.69 (1.08- 2.65)	0.05
<b>DD</b>	15 (15)	5 (7.9)	2.57 (1.09- 6.03)	0.02
<b>Dominant</b>	II vs ID+ DD		1.77 (1.13- 2.77)	0.04
<b>Recessive</b>	II+ ID vs DD		2.05 (0.71- 5.94)	0.13
<b>Over-dominant</b>	II+ DD vs ID		1.14 (0.53- 2.47)	0.44

**Comment [DRM5]:** What are the different operative interventions . Right and left MRM cannot be considered as different operation types. BCS and MRM can be considered

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

Model	Genotype # (%)		OR (95% CI)	P
<b>Co-dominant</b>	<b>ER<sup>+</sup> PR<sup>+</sup> Her2<sup>-</sup> 64 cases</b>	<b>Her2 enriched 14 cases</b>		
<b>II</b>	3 (4.7)	4 (28.6)	1	
<b>ID</b>	52 (81.2)	8 (57.1)	4.28 (1.72- 10.64)	0.01
<b>DD</b>	9 (14.1)	2 (14.3)	3.14 (0.77- 12.85)	0.11
<b>Dominant</b>	II vs ID+ DD		4.06 (1.71- 9.6)	0.01
<b>Recessive</b>	II+ ID vs DD		0.98 (0.18- 5.13)	0.63
<b>Over-dominant</b>	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. <sup>a</sup>
NPI	II	14	5.0571	.20481	
	ID	129	4.6819	.07228	.103
	DD	20	4.4950	.16391	.038
					.306 <sup>b</sup>

**Comment [DRM6]:** These tables seem to have been copied and pasted directly from the SPSS and are not representative of the idea. Please change the tables

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].

**Comment [DRM7]:** Meaning unclear

RAS is represented by the system of enzymes and hormones which regulate arterial 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.

**Comment [DRM8]:** Many of the sentences are being duplicated in the introduction and the discussion

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) were 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.

**Comment [DRM9]:** Deduction of the idea from the data is unclear

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<sup>-ve</sup> PR<sup>-ve</sup> Her2<sup>++ve</sup>)** 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<sup>++ve</sup> PR<sup>++ve</sup> Her2<sup>-ve</sup>)**. 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 NPI when 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.

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## 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	

<b>ID</b>	23 (69.7)	106 (81.5)	1.84 (0.53- 6.39)	0.26
<b>DD</b>	6 (18.2)	14 (10.8)	0.93 (0.21- 4.19)	0.62
<b>Dominant</b>	II vs ID+ DD		1.65 (0.48- 5.65)	0.31
<b>Recessive</b>	II+ ID vs DD		1.84 (0.65- 5.23)	0.19
<b>Over-dominant</b>	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)
<b>Co-dominant</b>	<b>Negative 39 (23.9)</b>	<b>Positive 124 (76.1)</b>		
<b>II</b>	5 (12.8)	9 (7.3)	1	
<b>ID</b>	30 (76.9)	99 (79.8)	1.83 (0.57- 5.89)	0.23
<b>DD</b>	4 (10.3)	16 (12.9)	2.22 (0.47- 10.45)	0.26
<b>Dominant</b>	II vs ID+ DD		2.04 (0.64- 6.49)	0.18
<b>Recessive</b>	II+ ID vs DD		0.77 (0.24- 2.46)	0.45
<b>Over-dominant</b>	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)
<b>Co-dominant</b>	<b>Negative 139 (85.3)</b>	<b>Positive 24 (14.7)</b>		
<b>II</b>	12 (8.6)	2 (8.3)	1	
<b>ID</b>	109 (78.5)	20 (83.4)	1.1 (0.23- 5.3)	0.63
<b>DD</b>	18 (12.9)	2 (8.3)	1.43 (0.23- 8.97)	0.55
<b>Dominant</b>	II vs ID+ DD		1.04 (0.22- 4.96)	0.66
<b>Recessive</b>	II+ ID vs DD		1.63 (0.35- 7.55)	0.4
<b>Over-dominant</b>	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<sup>+ve</sup> PR<sup>+ve</sup> Her2<sup>-ve</sup>) in BC group.**

Model	Genotype # (%)		OR (95% CI)	Sig. (P)
<b>Co-dominant</b>	<b>ER<sup>+ve</sup> PR<sup>+ve</sup> Her2<sup>-ve</sup> 64 cases</b>	<b>Triple –ve 10 cases</b>		
<b>II</b>	3 (4.7)	0 (0)	1	
<b>ID</b>	52 (81.2)	8 (80)	1.15 (1.04- 1.27)	0.66
<b>DD</b>	9 (14.1)	2 (20)	1.22 (0.92- 1.61)	0.6
<b>Dominant</b>	II vs ID+ DD		1.16 (1.06- 1.28)	0.64
<b>Recessive</b>	II+ ID vs DD		1.43 (0.35- 5.87)	0.46
<b>Over-dominant</b>	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<sup>+</sup> PR<sup>+</sup> Her2<sup>+</sup>) of hormonal status vs good prognostic hormonal status luminal A model (ER<sup>+</sup> PR<sup>+</sup> Her2<sup>-</sup>) in BC group.**

Model	Genotype # (%)		OR (95% CI)	Sig. (P)
Co-dominant	ER <sup>+</sup> PR <sup>+</sup> Her2 <sup>-</sup> 64 cases	ER <sup>+</sup> PR <sup>+</sup> Her2 <sup>+</sup> 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<sup>-</sup> PR<sup>-</sup> Her2<sup>+</sup>) 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	.746
	positive	10	5.0200	.27195	
ID	negative	23	4.5861	.17868	.539
	positive	106	4.7026	.07922	
DD	negative	6	4.3833	.36553	.668
	positive	14	4.5429	.18299	

**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	.465
	positive	9	4.9556	.29539	
ID	negative	30	4.6933	.17200	.931
	positive	99	4.6784	.07898	
DD	negative	4	4.7750	.37500	.408
	positive	16	4.4250	.18405	

**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. <sup>a</sup>
MPI	II	9	4.6444	.20556	.369
	ID	95	4.4815	.05216	
	DD	17	4.3941	.13380	
PPI	II	5	5.8000	.12247	.682
	ID	26	5.8808	.04804	
	DD	2	4.700	13.000	
					.883 <sup>b</sup>

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