Association of the *CDH1* Gene Variant (Promoter -160 C/A) with Breast Cancer and Its Linking with Prognostic and Clinical Features of Patients

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Abstract. Breast cancer (BC) is one of gynecological cancers worldwide. Because E-cadherin is essential protien for cells connection and maintains on original shape for epithelial tissue, this study supposed that *CDH1* -160 C/A SNP is linked with increased BC hazard and assess linking between this SNP genotypes and prognostic and clinical features of BC females. Subjects were divided into 75 BC cases and 50 healthy controls without family history to any cancer. This SNP variants was determined by PCR-RFLP technique. This study recorded a much increase in number of women up 45 years (p=0.0008). In addition, women who had BC family history had a significant increase (p=<0.00001) in BC incidence. These results found that cases with ductal carcinoma were significantly (p=<0.00001) more prevalent among BC patients. Also, distribution of BC appeared mostly in cases with grade II, tumor stage III that involved lymph node metastasis with significant differences (0.0003, 0.00005, <0.00001 respectively). This study explained that minor A allele of this SNP much risen in BC females compared to controls (p=0.005). Also, these results reported that C/A and A/A genotypes were correlated with increasing of patients ages as well as positive family history and ductal carcinoma with significant differences (p=0.03, p=0.0003, p=0.002). In sum, this study determined that the *CDH1* -160 C/A SNP was significantly increased BC hazard in Iraqi females. moreover, mutant A allele was significantly correlated with cases who had positive family history, ductal cancer and age over 45 years.

1 Introduction

The BC is now prevalent cancer worldwideand it exceeded lung cancer [1]. It is the more dominant cancer within females in the U.S. In 2023, the statistics recorded that 297,790 new cases of diffuse breast carcinoma in females [2]. In Iraq, BC is the more diffuse cancer within Iraqi females [3]. Etiology of BC includes dietary, lifestyle, hormonal risk factors and inheritance [4]. The BC is seen in advanced ages, and due to the patient's poor life expectancy and limited functional capacity at an advanced age, treatment options may be

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limited [5]. Tumor stage, lymph node metastasis and histological grade are essential indicators that determine to the advancing of the BC [6]. That were evaluated histopathologically in BC patients [7]. Metastasis and invasion of surrounding tissues are the first step of death in patients with BC that were correlated with upregulation of *CDH1* gene and absence of the cells adhering molecule, E-cadherin (E-cad) [8]. The E-cad encoded by the *CDH1* gene, tumor suppressor gene, situated on chromosome 16q22.1 [9,10]. The E-cad is the essential protein for conserving the stability of cellular-septum role [11]. Which modulates cell proliferation and polarity [12]. Down-regulation of the E-cad is an early event of the epithelial-mesenchymal transition (EMT) during cellular transformation [13]. The EMT is a process related in advancing and transformation of cancer [14]. The E-cad function is affected by genetic variations. Polymorphisms are the most common alteration in the DNA chain consisting a single nucleotide polymorphism (SNP) [15]. The more dominantly promoter SNPs were -347 G/GA, -160 C/A and 3'-UTR +54 C/T that had a direct influence on the *CDH1* gene function [16]. The -160 C/A SNP, which represents a common SNP of *CDH1* gene, linked with advancing of neoplasm via changing transcriptional activity when the normal C allele change to variant A allele by different mechanisms [17]. In last years, the linking of this SNP with the incidence of numerous cancers were achieved.

After research in previous studies, no study on BC has been oficiated to identify the genetic variations of *CDH1* gene, neither in Iraq nor in other Arabic countries. This study was orientated to explaining the major action of the *CDH1* -160 C/A SNP as a essential hazard indicator for BC in Iraqi females and understanding the connection between this SNP genotypes and demographic and clinicopathological features of BC females.

2 Methods

2.1 Study group

Seventy-five cases previously diagnosed with the BC were consisted in this study. The BC cases were recognized to the National Hospital of Oncology and Hematology in Al- Najaf province, Iraq. As the BC is proliferated mostly in females, the study population involved females only. The BC cases were diagnosed histologically by pathologists from the hospital. This study conducted between September, 2023 to June, 2024. This study included fifty randomly chosen age-gender matched healthy females as controls from the students and employees of the universities and also from the neighbors during the same period. The control subjects were non-proportional to the patients, destituting history or indicator to cancer or any other genetic diseases. During the collection of blood samples, the demographic and clinical data to all patients collected from data sheets in the hospital or by interviewing the patients directly.

2.2 Isolation of the DNA and PCR amplification

After collection 1 ml from patients and controls blood in sterile tubes with anticoagulant EDTA, the DNA isolated from whole blood and extracted by specific kit (Geneaid Biotech, Taiwan) according to the procedure from the manufacture company to isolating pure DNA from whole blood. Concentration and purity of extracted DNA samples were limitated by using Nanodrop spectrophotometer (BioDrop\USA). DNA samples displaying the 260/280 values between 1.8 and 2.0 were supposed as pure. Isolated DNA was conserved at -20˚C for future utilizing in genotyping. The primer sequences used for PCR amplification of this SNP were (forword) 5′-TGATCCCAGGTCTTAGTGAG-3′ and (reverse) 5′-

AGTCTGAACTGACTTCCGCA-3′ that yielded DNA piece (328 bp). Isolated DNA was used as PCR template. Each PCR reaction was 20 µL consisting 2 µL DNA, each primer 2 µL, half of the volume was PCR Master Mix (10 µL) and complete the volume by nuclease-free water (4 µL). Thermal cycler was used for PCR amplification and the procedure condition was as shown: first den. at 95 degree for 45 sec, followed by annealing at 63 degree for 45 sec, ex. at 72 degree for 30 sec and ultimate ex. at 72 degree for 5 min and refrigerating down to 4 degree. The size of the amplified PCR yielding was determined by electrophoresis on 1% agarose gel and added 5 µL DSRed Nucleic Acid Stain, the result appeared under ultraviolet light transilluminator.

2.3 The RFLP-PCR technique

In the case-control study, the RFLP-PCR technique was desgined for disclosing and genotyping of this SNP in samples. The PCR product (328 bp) digested by DNA cutting enzyme *BsteII* and incubated for 15 min at 37 degree. The variants were limitated by electrophoresis of DNA pieces appeared after cutting (two bands: 218 and 110 bp for C/C variants, one band: 328 bp for A/A variants and three bands: 328 bp, 218 bp and 110 bp for heterozygous C/A variants).

2.4 Statistical Analysis

The IBM SPSS Statistics (v23.0) was utilized to finding statistical result of all the values. For comparison of the allele and genotype frequencies and other value of results between groups, chi-square test was used with p value ($p \leq 0.05$).

3 Results

3.1 Association between the BC and the prognostic features

In this study, the prognostic features to the BC patients were summarized in table 1. The ages patients were divided to <45, 23 (30.6%) and >45, 52 (69.4%). The females ages started from 29 to 78 years (p=0.0008). Family history assessment for the BC cases revealed that 16 (21.3%) cases had family history of the cancer while of 59 (78.7%) cases without family history (p=<0.00001). Grading criteria of the presented cases revealing that grade I was reported in 20 (26.7%) cases, grade II in 41 (54.7%) cases and gradeIII in 14 (18.6%) (p=0.0003). The lymph node metastasis assessment of cases of the BC found that 61 (81.3%) cases had been positive LN while just 14 (18.7%) cases had been negative LN (p=<0.00001). Assessment of the staging of the BC cases according to the TNM staging system revealed that, there were 5 cases (6.7%) had been with stage I, 31cases (41.3%) had been with the stage II, 26 cases (34.7%) had been with the stage III and 13 females had been with the stage IV (p=0.00005). The results in the table explained the histologic type of the BC patients which showed that 65 (86.6%) cases had been ductal type, 5 (6.7%) cases had been lobular type and finally, 5 (6.7%) cases had been mixed (ductal and lobular) (p=<0.00001).

Table 1. Prognostic features of the BC patients

Variable	**n**	**Patients**	***p*-value**
Total	75		
Age (years):			0.0008*
<45	23	30.6%	

>45	52	69.4%	
Family history			
family history	16	21.3%	<0.00001*
without family history	59	78.7%	
Histological grade			
gradeI	20	26.7%	0.0003*
gradeII	41	54.7%	
gradeIII	14	18.6%	
Lymph node metastasis			
positive	61	81.3%	<0.00001*
negative	14	18.7%	
TNM stage			0.00005*
stageI	5	6.7%	
stageII	31	41.3%	
stageIII	26	34.7%	
stageIV	13	7.3%	
Cancer type			
ductal	65	86.6%	<0.00001*
lobular	5	6.7%	
mixed (ductal and lobular)	5	6.7%	

values were manifested as (n%). (*) Determines noticeable dismilarity ($p \leq 0.05$), (n= number, p= probability).

3.2 Genotyping and frequency of the *CDH1* SNP (-160 C/A) in the BC

The genotyping and allelic distribution in BC females and healthy females together and their linking with hazard of the BC were shown in Table 2. The homozygous -160 C/C genotype was more dominantly appeared in all samples (cases and controls) (61.33% and 84%, respectively). The C allele had dominant frequency and normal genotypes (C/C) in this SNP, any change to another alelle or genotypes (C/A, A/A), this is indicator to occurrence genetic variant in this SNP by different mechanisms. The genotypic distribution of the C/C (normal homozygous), the C/A (heterozygous) and the A/A (variant homozygous) genotypes were 61.33, 33.33 and 5.34% in cases, and 84, 16% and 0 in controls. Both homozygous (A/A) and heterozygous (C/A) frequencies were significantly increased in the BC cases (5.34% and 33.33% vs 0% and 16%, respectively in controls), and provided a significant ($p \leq 0.05$) association exhibiting of about 2.85-fold (OR=2.85, 95% (CI)= 1.16-7.01, p=0.02) increase in risk of the BC. The frequencies of prevalent C allele and A allele were 78% and 22% in cases, whereas in controls, the C allele and mutant A allele were 92% and 8%, respectively (Table 2). Statistical analysis showed that minor -160A allele was positively linked at highly significant differences with the BC as risk factor (OR=3.24, 95% CI=1.43-7.36, p=0.005).

Table 2. Genotypic distribution of -160 C/A SNP at the *CDH1* gene in the study samples (BC females and healthy females) and BC hazard

Genotype/allele	BC females, n=75	Healthy females, n=50	(odds ratio OR, (confidence interval 95% CI)	*p*-value
C/C	46 (61.33%)	42 (84%)	1.00 Ref	
C/A	25 (33.33%)	8 (16%)	2.85 (1.16-7.01)	0.02*
A/A	4 (5.34%)	0 (0%)	Not applicable	
C	117 (78%)	92 (92%)	1.00 Ref	
A	33 (22%)	8 (8%)	3.24 (1.43-7.36)	0.005*

Values were manifested as (n%). (*) Determines noticeable dismilarity ($p \leq 0.05$), (n= number, *p*= probability).

3.3 Association between the -160 C/A genotype and age

The association of -160 C/A SNP and the age of BC patients (Table 3), showed that: among the patients whose had age <45 (n=23); 19 (79.2%) were homozygous genotype (C/C), no patients found as homozygous genotype (A/A), and 4 (20.8%) were heterozygous genotype (C/A). In the patients whose had age >45 (n=52); 27 (50.9%) were C/C genotype, 21 (41.2%) were C/A genotype, and 4 (7.9%) were A/A genotype (*p*=0.03).

Table 3. Linking between the -160 C/A SNP genotype and age of BC females

Age groups (years)	Genotype frequency			*p*-value
	C/C	C/A	A/A	
<45 (n=23)	19 (79.2%)	4 (20.8%)	0	0.03*
>45 (n=52)	27 (50.9%)	21 (41.2%)	4 (7.9 %)	

Values were menifested as (n%). *p* value appears noticeable dismilarity ($p \leq 0.05$), (n= number, *p*= probability).

3.4 Association between the -160 C/A genotype and family history

Regarding to the frequency of family history, these results found that in females whose carcinoma that had positive family history (n=16): 7 (43.8%) were homozygous genotype (C/C), 5 (31.3%) were heterozygous genotype (C/A), and 4 (25%) were homozygous genotype (A/A). While the females whose carcinoma that do not have family history (n=59): 39 (66.1%) were homozygous (C/C), no patients found as homozygous genotype (A/A), and 20 (33.9%) were heterozygous genotype (C/A) (*p*=0.0003)

Table 4. Linking between the -160 C/A SNP genotype and family history of BC females.

Family history status	Genotype frequency			*p*-value
	C/C	C/A	A/A	
positive family history (n=16)	7 (43.8%)	5 (31.3%)	4 (25%)	0.0003*
without family history (n=59)	39 (66.1%)	20 (33.9%)	0	

Values were menifested as (n%). *p* value appears significant difference ($p \leq 0.05$), (n= number, *p*= probability).

3.5 Linking between the *CDH1* -160 C/A SNP and clinical features in the BC females

The genotypic circulating of this SNP was interacted with the clinical features in BC females like cancer type, grade, stage and lymph node metastasis (Table 5). These results showed that: within the females whose had ductal type (n=65) cancer cases; 40 (61.5%) were C/C genotype, 23 (35.4%) were C/A genotype, 2 (3.1%) were A/A genotype. In females whose had lobular type (n=5); 5 (100%) were C/C genotype, no patients found as C/A genotype, and no patients found as homozygous genotype (A/A). Recently, In females whose had mixed (ductal & lobular) (n=5); 1 (20%) were homozygous (C/C), 2 (40%) were C/A genotype, and 2 (40%) were A/A genotype (p=0.002) (Table 5). The results of this study showed that: within females whose had grade I (n=20) cancer cases; 13 (65%) were C/C genotype, no patients found as A/A genotype, and 7 (35%) females whose C/A genotype. In females whose carcinoma were grade II (n=41); 25 (61%) were C/C genotype, 13(31.7%) were C/A genotype, and 3 (7.3%) were A/A genotype. Recently, In females whose carcinoma were grade III (n=14); 8 (57.1%) were homozygous (C/C), 5 (35.8%) were C/A genotype, and 1(7.1%) were A/A genotype (p=0.8) (Table 5). The association of -160 C/A polymorphism and TNM staging of the BC found that: within the females whose carcinoma were in stage I (n=5); 3 (60%) were C/C genotype, no patients found as A/A genotype, and 2 (40%) were C/A genotype. In the females whose carcinoma were in stage II (n=31); 20 (64.5%) were homozygous genotype (C/C), 10 (32.2%) were C/A genotype, and 1 (3.3%) were A/A genotype. In the females whose carcinoma were in stage III (n=26); 15 (57.7%) were C/C genotype, 8 (30.8%) were C/A genotype, and 3 (11.5%) were A/A genotype. Recently, in the females whose carcinoma were in stage IV (n=13); 8 (61.5%) were homozygous (C/C), 5 (38.5%) were C/A genotype (), and no patients found as A/A genotype (p=0.75) (Table 5). Regarding to the state of lymph node metastases, these results found that in females whose carcinoma had positive lymph node metastasis (n=61): 37 (60.6%) were C/C genotype, 20 (32.8%) were C/A genotype, and 4 (6.6%) were A/A genotype. While cases with negative lymph node metastasis (n=14): 9 (64.3%) were homozygous (C/C), no patients found as A/A genotype, and 5 (35.7%) were C/A genotype (p=0.61) (Table 5).

Table 5. Linking between the CDH1 -160 C/A SNP in the BC females

Subjects		Genotype Distribution Results			*p*-value
		C/C	C/A	A/A	
Cancer type	Ductal type (n=65)	40 (61.5%)	23 (35.4%)	2 (3.1%)	0.002*
	Lobular type (n=5)	5 (100%)	0	0	
	Mixed type (n=5)	1 (20%)	2 (40%)	2 (40%)	
grade	I (n=20)	13 (65%)	7(35%)	0	0.8
	II (n=41)	25 (61%)	13 (31.7 %)	3 (7.3 %)	
	III (n=14)	8 (57.1%)	5 (35.8%)	1 (7.1%)	
TNM stage	I (n=5)	3 (60%)	2 (40%)	0	0.75
	II (n=31)	20 (64.5%)	10 (32.2%)	1 (3.3%)	
	III (n=26)	15 (57.7%)	8 (30.8%)	3 (11.5%)	
	IV (n=13)	8 (61.5%)	5 (38.5%)	0	
Lymph node metastases	positive (n=61)	37 (60.6%)	20 (32.8%)	4 (6.6%)	0.61
	negative (n=14)	9 (64.3%)	5 (35.7%)	0	

Values were menifested as (n%). p value appears noticeable dismilarity ($p \leq 0.05$), (n= number, p= probability).

4 Discussion

This study observed that when the age increase, the incidence of BC increase because the changes in reproductive patterns to females and the use of postmenopausal hormone therapy [18]. Also, women who had family history to the disease were more susceptible to the hazard of the BC. In addition, these results found that cases with ductal carcinoma were more dominant among breast cancer patients. Moreover, the frequency of the BC increased mostly in cases with grade II, tumor stage III that involved lymph node metastasis. The action of E-cad in the advancing of cancers, the E-cad was a neoplasm inhibitor and a contributor of neoplasm formation [19], down-regulation of the E-cad expression has been shown in many carcinomas such as diffuse gastric carcinoma [20], colon and rectum carcinoma [21] and bladder carcinoma [22] and hereditary lobular breast carcinoma [23]. Individual differences are mostly caused by the SNPs. Moreover, these genetic markers are becoming significant indicators in clinical diagnosis and therapy. Single-nucleotide variations are determined with a range of harmful diseases, including autoimmune diseases, cardiovascular disease, malignancies, and inherited metabolic diseases [24]. The −160 C/A SNP is the more dominantly studied the *CDH1*-SNPs in cancers linked studies. The variant allele (A) was previously shown to decreased the E-cad expression [25]. These results suggested that homozygous A/A genotype and heterozygous C/A genotype present an improved risk of the BC. Also, this study found that the distribution of the A allele in the cases was significantly risen than controls. These results approved with results of the study by Vishteh *et al*. [26] registered that the rate of C/A variant in diffuse gastric carcinoma was much risen than the controls. These results agreed with another study explained that the -160 A/A variant was linked with an risen hazad of the gastric cancer in Oman [27]. Also, for asthma, a study was done by Wang *et al.* [28] reported that a presence of the *CDH1* AA/CA genotypes were linked with childhood asthma comparsion to children have the *CDH1* C/C genotype. The present study reported that the mutant A allele is linked with the BC hazard (p-value=0.005 OR=3.24 (95%CI) =1.43-7.36). In addition, heterozygous and homozygous (C/A+A/A) genotypes also demonstrated positive connection with (p-value=0.02, OR=2.85 (95%CI)=1.16-7.01) higher susceptibility to breast cancer development. This study were in approving with another study which found this SNP was significantly enhanced association with the advancing of PC in the Bangladeshi population [29]. Finally, this study explained the interaction of the *CDH1* -160 genotypes with some prognostic and clinical features such as age, family history, cancer type, grades, stages of cancer and lymph node metastasis within the BC patient group. From the comparison, those data showed that the mutant A allele was much correlated with hazard of the BC in older women that who had positive family history and ductal cancer type. These results agreed with results of a study found that the state of a family history in ESCC and GCC cases was much risen than that in controls (p=<0.0001) [30]. Also, these results approved with a study evaluated that IDC cases with the A allele of this SNP was risen with ILC cases (p= 0.04) [31]. On other hand, these results were not in agreement with a study's data explained that no linking was recorded with the -160 C/A SNP and ages of the BC patients [15]. In addition, this study recorded that the grade II, stage II and positive lymph node metastasis were more likely to be linked with BC hazard in this study samples. These findings were similar to some other studies who explain the connection between grade, stage and lymph node metastasis and the threat to BC incidence [23, 31, 15]. In sum, this study reported that homozygous (A/A) and heterozygous (C/A) genotypes of the *CDH1* -160 C/A SNP may participate an improved threat to the distribution of the BC within Iraqi females. In addition, women carrying these genotypes along with positive family history and ductal type as well as increase age were more vulnerable. Also, this study observed that the frequency of the C/A genotypes were higher in patients with grade II, stage II and positive

lymph node metastasis comparsion with the grade III, stage III and negative lymph node metastasis group and there were more susceptible to BC development compared to another group.

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