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RESEARCH ARTICLE

Association of HER2 [ILe655Val] gene polymorphism and Breast Cancer Risk in Iraqi females Population

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Abstract

Introduction: Breast cancer is progressively more global problem. It is considered the first or second most common cancer and major cause of death in women globally. Human epidermal growth factor receptor (HER-2) is proto-oncogene, a transmembrane glycoprotein receptor (p185) with tyrosine kinase activity. Mutation and overexpression of HER-2 gene leads to tumorigenesis through the overexpression of HER-2 enhances and prolongs the signals that trigger the transformation of the cells.

Objective: we aimed to study and investigate the association of HER-2 (ILe655Val: rs 1136201) gene polymorphism with the risk of breast cancer.

Methods: The genotypes and allele frequencies of HER-2 (ILe655Val) gene polymorphism were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis in 300 breast cancer patients and 200 controls after extraction of DNA from blood samples of all participants.

Results: The results revealed that the HER-2 gene A→G (ILe 655 Val) SNP genotype frequencies of wild genotype AA**,** heterozygous genotype AG and homozygous genotype GG were 47 %, 41.7 % and 11.3 % in cases of breast cancer group and 65 %, 29 % and 6% in the control group respectively. The heterozygous genotype AG was found increased the risk of breast cancer by about two folds higher than those of the wild genotype AA (OR= 1.62, 95% C.I= 1.15–2.4, P = 0.022). In contrast, the homozygous genotype GG was significantly increased the risk of breast cancer by about two and half folds higher than that found in wild genotype AA (OR= 2.44, 95% C.I= 1.23–5.1, P = 0.014).

Conclusion: We concluded that the Val allele of HER-2 gene was increased the risk of breast cancer in patients of Iraqi population.

INTRODUCTION

Breast cancer is progressively more global problem. It is considered the first or second most common cancer and major cause of death in women globally. Breast cancer affects women who have ages between 35 and 70 years, but it can also affect women in all ages [(1)]. It accounts one third of incidence cases of cancer in females in Western industrialized societies, The incidence of breast cancer is increasing almost everywhere throughout the world, although the mortality rate from breast cancer is declining in many high income countries[(2)]. In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry [(3)]. Generally, breast cancer occurred when the genetic mutations were built-up in certain genes, those that control cell growth and cell division or even repair of damaged DNA may cause

tumor through uncontrolled growth and division of cells [4,5]. Acquired genetic single nucleotide polymorphisms (SNPs) may take place during the lifetime, Genomic distribution of SNPs is not homogenous, a genetic variant that occurred in less than one percent of a given population is referred to the rare variant or point mutation. Some people refer to SNPs as a variant that not causing disease while the mutation is a disease causing one [6].

More recently report of Genome Wide Association Studies (GWAS) was identified more than seventy single nucleotide polymorphisms (SNPs) that influence breast cancer risk [7]. Numerous studies showed that the significant association between HER-2 proto-oncogene a transmembrane glycoprotein receptor with a chromosomal location of 17q21, encodes a transmembrane glycoprotein (p185) with tyrosine kinase activity. This protein is a member of the epidermal growth factor receptors family that controls a variety of cellular functions, including cell proliferation. In human cancers, amplification and/or overexpression are the main mechanisms of the HER2 gene activation. The alterations of this gene are found in more than 20% of human breast cancers, indicating that this gene may have an important role in this common malignancy[8]. Clinically, HER2 (rs 1136201) gene polymorphism (ILe 655 Val) overexpressed and/or amplified in breast cancer tissue is associated with steroid hormone receptor-negative tumors, worse histopathological grades, high rate of proliferation, and reduced response to chemotherapy and hormonal therapy[8, 9]. An increasing evidence that indicate the gene encode growth factor receptors can be activated through mutations in their coding sequences that suggested to be an indicator of poor prognosis [9]. The present study compare HER-2 (ILe655Val) genotypes and allele distributions between patients with breast cancer and healthy individuals. The association of HER2 (ILe655Val) gene polymorphism with the risk of breast cancer was also investigated in Iraqi population.

Material and method:

This case-control study included two groups: the first group comprised of 300 females histopathologically diagnosed with breast cancer either familial or sporadic, their ages ranged between 22-77 years and mean±SD (49.6±10.9) year. They were selected from Oncology unit in AL-Sadder Medical City teaching hospital in Al-Najaf Province when they attended to the hospital for treatment or for check up and some of them admitted in the hospital, they are from middle and south regions of Iraq. Any subject suffered from the following health problems were excluded from the current study: diabetes mellitus, cardiac diseases, hypertension, patients with primary renal dysfunction, patients with other types of malignancies not related to the metastasis of breast cancer. The second group included 200 apparently healthy female individuals, their ages ranged between(22-78) years and mean ± SD (47.1 ± 14.9) year. They were selected from general population, women attending to the hospital who were patients relatives, visitors, medical staff and their relatives and friends. Only individuals free from symptoms and signs of any chronic diseases such as DM, cardiac diseases, hypertension, renal diseases or others were selected to involvement in this study.

DNA extraction and mutation analysis:

The DNA was extracted from whole blood samples of patients and control groups by using ReliaPrep™ Blood gDNA Miniprep kit (Promega, USA) according to the manufacturer's procedure. The extracted DNA was stored at -20c° until used for amplification. The SNP in HER-2 transmembrane segment involving codon 655 was analyzed by PCR-RFLP. The transmembrane segment that consisted of 148 bp was amplified in PCR (Biometra, Germany) using the gene specific primers forward 5′-AGA GCG CCA GCC CTC TGA CGT CC- 3′ and reverse 5′-TCC GTT TCC TGC AGC AGT CTC CGC-3′. All PCR amplification performed in a total volume of 25 µl: 5 µl of extracted DNA, 15 pmol/L of each primer forward and reverse, 12.5 µl of Hot start green Taq Master Mix containing 2.5 units of Hot start Taq DNA polymerase, 1x PCR buffer with 1.5 mmol/L $MgCl_2$, and 200 µmol/L of each dNTP (Promega, USA). Thermal cycling conditions for PCR are as follows: initial denaturation at 94c° for 4min then amplified for 35 cycles of 94c° for 30 sec, 62c° for 30sec, 72c° for 30sec and final extension of 72c°for 7 min. PCR products were subjected to electrophoresis on the 2% ethidium bromide stained agarose gel and visualized under UV light. For RFLP analysis, 10 µl of each PCR product was digested with endonuclease BsmAI (New England Biolabs,UK) at 55c° for 15min. BsmAI enzyme digestion gave single fragment 148bp for the ILe allele and two fragments of 116 bp and 32 bp for Val allele. Fragments digested with BsmAI were subjected to electrophoresis on 3% analytical grade agarose gel (Promega, USA), stained with ethidium bromide and visualized under UV light.

Statistical analysis:

The output data of age and BMI distribution of breast cancer patients and healthy control groups expressed as interactive chi-square and Yates correct p-value that calculated by using the online software (http://quantspy.org). for genotypes, the Hardy-Weinberg equilibrium test was analyzed first by using the online software web. ASSO test (WWW.ekstoem.com). The associations between disease and genotypes were assessed by calculating OR and 95%

C.I. The statistical Package for the Social Sciences software version 20.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses and p-value <0.05 was considered statistically significant, the genetic power, which represents the power to detect the significant difference at level of 0.05 with the optimal level (80% or more), was calculated by online software OSSE.

Results:

This case-control study consisted of 300 female patients group diagnosed with breast cancer and age matched 200 healthy control group, the study groups were classified into age intervals due to high range of ages between twenties and seventies in patients group and control group (22-77) and (22-78) respectively. Table (1) represented the demographic characteristics for the two groups of study. Accordingly, the high frequency of breast cancer patients increased with age intervals 40-49 and 50-59 then it started to decline. More than one third of the patients (36%) were in the premenopausal age (40-49) and 17.3% were under 40 years and the decline occurred in their sixties of their age. Also this table appeared significantly increased of BMI in breast cancer patients when compared with those of healthy control (P< 0.05), more than half of patients (158, 52.7 %) were presented with obesity (BMI ≥ 30 kg/m²). On the other hand, more than half of control group (123, 61.5 %) were presented with overweight (BMI= 25-29.9 kg/m²). When the studied individuals classified according to the educational level, 195 (65%) of patients were presented with low level of education (primary school or less); 75 (25 %) of patients with middle level of education (secondary school) but the lowest number 30 (10%) of patients were with the high level of education (university or above), there were significant differences between patients and control group (P<0.05). Also the study individuals were divided according to the parity, the large number of patients 136 (45.3%) were presented to have four or more children, 85 (28.3%) presented to have two or three children, 53 (17.7%) have one child while the small group number presented with nullparous 26 (8.7%), there were significantly different from that of healthy control (P<0.05). There was no significant differences between patients and control individuals in marital status and residence (p>0.05).

Table 1: General Characteristics of breast cancer patients and healthy control subjects.

Characteristics	Patients (300)	%	Control (200)	%	P- value
Age (years)					
20-29	7	2.3%	8	4%	0.8
30-39	45	15%	35	17.5%	0.26
40-49	108	36%	75	37.5%	0.015
50-59	90	30%	40	20%	0.000
60-69	40	13.3%	30	15%	0.79
70-79	10	3.4%	12	6%	0.66
BMI (kg/m²)					
18.5-24.9	39	13%	49	24.5%	0.28
25-29.9	103	34.3%	123	61.5%	0.18
≥ 30	158	52.7%	28	14%	0.0001
Marital status					
married	196	65.4 %	125	62.5 %	
unmarried	28	9.3 %	10	5 %	
widow/divorced	76	25.3 %	65	32.5 %	0.07
Residence					
Urban	138	46 %	90	45 %	
Rural	162	54 %	110	55 %	0.82
Educational level					
Low	195	65 %	144	72 %	
Middle	75	25 %	36	18 %	
High	30	10 %	20	10 %	0.016
Parity					
Nullparous	26	8.7 %	14	7 %	
1	53	17.7 %	27	13.5 %	
2 – 3	85	28.3 %	40	20 %	
≥ 4	136	45.3 %	119	59.5 %	0.03

interactive chi-square test, BMI: body mass index, P< 0.05 : statistically significant

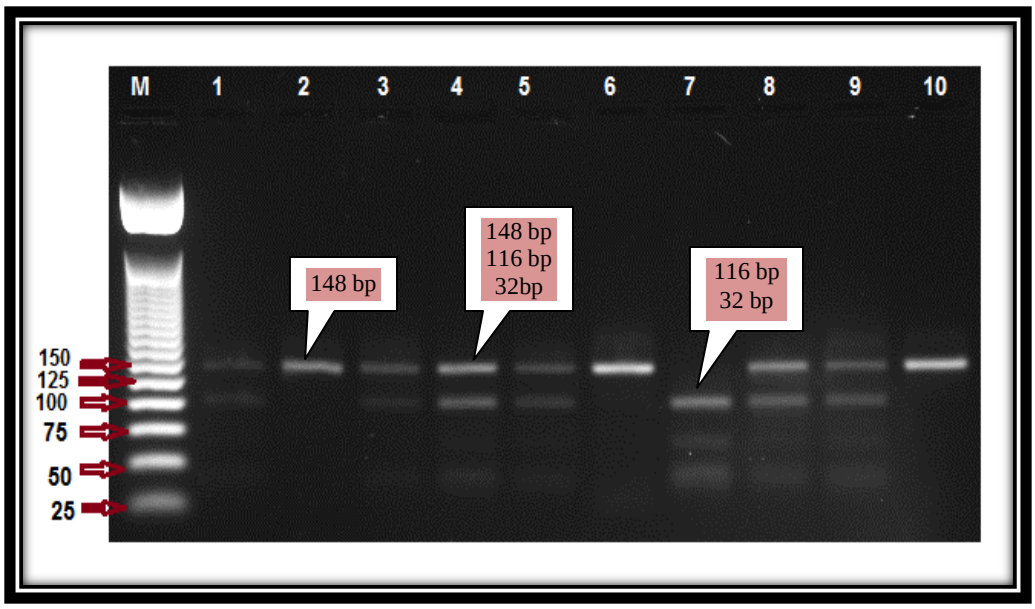

Figure 1: Restriction digestion of PCR product for HER2 gene A→G(ILe 655 Val) SNP. Lane M: DNA Ladder, Lane 2, 6 and 10 wild genotype (AA), Lane 1, 3, 4, 5, 8 and 9 heterozygous genotype (AG), Lane 7 homozygous genotype (GG).

Table 2: Genotype and allele frequencies of ILe 655 Val polymorphism for HER-2 gene in patients and control groups.

Genotype ILe655Val A →G	**Case no.**	**%**	**Control no.**	**%**	**Crude model OR[a]**	**95%C.I**	**Adjusted model OR[b]**	**95% C.I**	**P-value**
	300		200						
AA	141	47	120	60	1.00				
AG	125	41.7	68	34	1.6	(1.1-2.3)	1.62	(1.15-2.4)	0.022
GG	34	11.3	12	6	2.4	(1.2-4.9)	2.44	(1.23-5.1)	0.014
Allele frequency									
A	407	67.8	308	77	1.00				
G	193	32.2	92	23	1.61	(1.19-2.12)	1.6	(1.2-2.1)	0.0017

[a] Multinomial logistic regression; [b] Multinomial logistic regression adjusted for age and BMI; n : number; C.I, confidence interval; OR, odds ratio.

The genetic power was calculated, it represents the power to detect a significant difference at level of 0.05 for HER-2 gene A→G . It is found to be (99.9 %). It seemed to be within the optimal level (80% or more). Genotyping frequencies of HER-2 (ILe 655 Val) gene was consistent with Hardy Weinberg Equilibrium in both control group (P=0.57) and breast cancer group (P=0.43). The analysis of results indicated that the HER-2 gene A→G (ILe 655 Val) SNP genotype frequencies of wild genotype (AA), heterozygous genotype (AG) and homozygous genotype (GG) were 47 %, 41.7 % and 11.3 % in cases of breast cancer females group and 60 %, 34 % and 6% in control group respectively. The heterozygous genotype (AG) was increased the risk of breast cancer by about two folds higher than those of wild genotype (AA) (OR= 1.62, 95% C.I= 1.15–2.4, P = 0.022). On the other hand, the homozygous genotype (GG) was statistically significant increased the risk of breast cancer by about two and half folds higher than that found in wild genotype (AA) after adjustment for age and BMI (

OR= 2.44, 95% C.I= 1.23–5.1, P = 0.014). No significant variations were obtained when the analysis was carried out without adjustment. The allele frequency of A was found to be 67.8 % in breast cancer patients and 77 % in control group, while the frequency of G allele in breast cancer cases 32.2 % which is higher than G allele frequency in control group that equal to 23 % , the G allele frequency increased the risk of breast cancer by about two times when compared with those of A allele (OR= 1.6, 95% C.I= 1.2–2.1, P = 0.0017) as presented in table (2).

Discussion:

Breast cancer is the most common type of neoplasm among Iraqi females population[3]. The later report of Genome Wide Association Studies (GWAS) were identified more than seventy single nucleotide polymorphisms (SNPs) that influence breast cancer risk[7]. Mutation in the genes has been contributed to the susceptibility of transformation of carcinoma including breast cancer malignant tumor, the human epidermal growth factor receptor HER-2 is a proto-oncogene. Which is a member of EGFR family that plays important role in the cell growth regulation, differentiation and survival that involved in the development and growth of normal breast[10]. The human epidermal growth factor receptor2 is the main factor for treatment and prognosis in breast cancer, over expression of Her-2 gene was seen in about 15-25% of breast carcinomas and linked with metastatic, poor progression , and unresponsiveness of hormonal therapy[10-12]. The current case-control study investigated the relevance between Ile 655 Val SNP of Her-2 gene and the risk of breast cancer in 500 female individuals among them 300 breast cancer patients group and 200 age matched genotyped in this study. Among female breast cancer cases, there were 141(47%) patients carrying HER-2 ILe/ ILe (AA) wild genotype, 125 (41.7 %) patients carrying HER-2 ILe/Val (AG) heterozygous genotype and 34 (11.3%) patients carrying HER-2 Val/Val (GG) homozygous genotype and allele distribution in control group were 120(60%) females carrying HER-2 ILe/ ILe (AA) wild genotype, 68 (34%) females carrying HER-2 ILe/Val (AG) heterozygous genotype and 12 (6%) females carrying HER-2 Val/Val (GG) homozygous genotype respectively, from these results we appeared that increased the risk of breast cancer by about two folds in heterozygous AG allele genotype and approximately two and half folds in homozygous GG allele genotype when compared with the reference genotype AA allele as in table (2), the results appeared the distribution of heterozygous and homozygous was specific for the females population in Iraq. Our results agreement with many reports of different populations in the world that reported (ILe 655 Val) SNP was strong candidate in the susceptibility of female breast cancer, Xie et.al [13] was the first researcher who reported the association of ILe 655 Val in HER-2 gene with increased the risk of breast cancer among polymorphisms of Chinese population. Other studies demonstrated an equal importance of ILe 655 Val gene polymorphism in development of breast cancer in Slovak by Zubor et.al[14], Portguese by Pino et.al[15], Ashkenazi Jewish by Rutter et.al[12], Australian by Montogomry et.al[16], African-American and white women in north Carolina in America by Millikan et.al[11] populations. In contrast, the outcomes of the current study disagree with many studies among other populations[17, 18-21], these conflicting in reports may be attributed with small sample size of subjects in these reports that leads to decrease statistical power to detect the association between (ILe 655 Val) SNP and risk of cancer, another factor could be a variable influence of environmental factors with respect of etiology of cancer in different populations[22].

The current study analyzed the frequency of HER-2/neu gene polymorphism in control healthy individuals and patients of breast cancer, the frequency of val (G) allele in our population was relatively high and varies from 23 % in healthy individuals to 32.2 % in breast cancer women as shown in table (2), the distribution frequency of val allele in healthy women was similar to that present in population of Slovakia [14] , Japanese[23], Chinese women[13] and Afro-American[11] which were 15.84%, 14.9%, 11.1% and 24% respectively. Also the results revealed significant increased in val allele variant in breast cancer patients in comparison to control group with increased the risk of disease by approximately two times when compared with ILe (A) allele variant, the results were agreement with of earlier reports that revealed the presence of Val allele genotype is associated with higher risk of breast cancer including two meta analysis one of them performed by Tao et.al[24], who showed higher frequency of Val allele in breast cancer and the another meta analysis conducted by Lu et.al[25] who found significant association among African and Asian female breast cancer patients who carried out Val allele. In contrast, many studies were found no risk associated with HER-2/neu gene polymorphism and breast cancer such as study of Kara et al[26], Dahabreh and Murray[27], and Ma et al[28] these studies subjected to the same polymorphisms have different somatic variations on different ethnic groups. Finally, we concluded from this study that genetic polymorphism of (ILe655Val) in the HER-2 gene was associated with an increased the risk of breast cancer. Homozygous women for HER-2 Val allele in codon 655 were significantly increased the risk of breast cancer, suggesting that Val allele an indicator of genetic susceptibility to breast cancer.

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