Association Between HER2Ile655Val Single Nucleotide Polymorphism and the Risk of Breast Cancer in the Kurdish Population

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Abstract

Introduction: The human epidermal growth factor receptor-2 (Her2) is a transmembrane protein responsible to regulate the growth and proliferation of breast cell with tyrosine kinase activity. Researchers have looked at the possibility that HER2Ile655Val has a role in generating of breast cancer. The genetic variants of HER2 and single nucleotide polymorphism have been explored as being connected with breast tissue neoplasia; however, research of this sort has not been carried out on the Kurdish community. The present study was aimed to investigate the role of HER2 gene polymorphism in the risk of breast cancer development in Kurdish population in Iraq and it is the first of its kind in the study area. *Methods:* We analyzed the distribution and frequency of Val/Val and Ile/Val genotype from blood of 151 patients who have cancer in breast tissue and 121 healthy individual controls using the combination of PCR and Restriction fragment length polymorphism, PCR-RFLP. *Results:* The result of this study found that there is twofold increase in the risk of malignant breast tumor in Kurdish women who carries $HER2^{Ile655Val}$ genotypes, Val/Val and Ile/Val ($P<0.005$). The rate of $HER2^{Ile655Val}$ polymorphism was 63.3% among breast cancer patients and this is the highest rate globally after Greek. Older ages, >45, recorded the highest rate of polymorphism ($P<0.005$) among patients, 62.5%. *Conclusion:* This result concluded that there is an association between HER2 codon 655 polymorphisms with the risk of breast cancer among Kurdish population.

Keywords: HER2, Breast cancer, PCR-RFLP, single nucleotide polymorphism, Genetic variations

1. Introduction

Breast cancer is counted as one of the most common types of cancer among women and the number of breast-cancer patients is still increasing in some countries which creates a public health issue [1,2]. Breast cancer is counted as the most frequent cause of cancer death among women worldwide [3]. In 2000, the breast cancer was the most common malignancy among women with the rate of 35.7 per 100, 000 [4]. In 2012, the occurrence of breast cancer was about 1.7 million [5] and 2018, two million of cases were diagnosed [3,6]. It caused to large number of deaths (512,900) in 2012 worldwide [5] and it caused 42,000 deaths in 2017 only in USA [7]. In general, 60% of cancer deaths can be seen in developing countries such as India [5].

It has been shown in several articles in China, Australia, and Taiwan that there is association between HER2Ile655Val single nucleotide polymorphism and rising the risk of malignant cancer of breast in women [8–10]. Human epidermal growth factor receptor 2 (HER2) is an integrated plasma-membrane glycoprotein receptor that has a mass of 185 kilodaltons, and the gene that encodes it can be found on human chromosome 17q21. HER2 has as intrinsic tyrosine kinase activity which belongs to the growth factor family, ErbB [11][12]. The overexpression of HER2 has been found in many types of cancer, stomach, breast and ovarian cancer [13] and this overexpression is recorded in breast tissue cancer at the rate of 20-30% [14,15].

A single nucleotide polymorphism (SNP) was identified for first time by Papewalis and his colleagues at the codon number 655 of the gene, HER2. This mutation is a missense mutation that changes isoleucine (ATC) to valine (GTC) [16]. There are different records regarding the rate and incidence of breast cancer linked to HER2 single nucleoid polymorphism in different geographical regions and among different ethnicity [8,17–19]. However, this role of SNP in the appearing of breast cancer has not been identified in Kurdish populations of Iraq until now.

In Asia, Europe and middle east there are several studies on HER2 SNP and its role in developing breast cancer among women. In addition, there are studies in the countries bordered with Kurdish region include Iran, Turkey, Arab ethnicity of Iraq, but there is no study in Kurdistan region of Iraq. Therefore, the main goal of the present study was to find the link

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between HER2Ile655Val (rs1136201) gene single nucleotide polymorphism with the risk of breast cancer among Kurdish women and this study is the first of its kind among Kurdish population of Iraq.

2. Methods

Sample collection

A total of 196 samples (151 cases and 121 controls) were collected from Hiwa cancer hospital in Sulaimani/Iraq from July 2021 to late 2022. The case samples were taken from females who were diagnosed with invasive breast cancer and the controls were healthy women. The mean age of the participants of this study was 50.7 years old. Women of this study (cases and controls) were informed about the object of this study and about the procedure of the work. Ethical approval was granted from the College of health and medical technology/SPU by the ethical review committee. The clinicopathological characteristics and history of the patients were recorded in a prescribed questionnaire.

Genotyping

Blood was taken from 272 females who were participated in this study (151 cancer patients and 121 healthy women). In general, blood samples (5 ml) were taken from every participant and put into a collected tube containing EDTA and kept at 4 ºC before transportation to the laboratory for molecular biological study. DNA extraction was carried out by DNA extraction kit according to the manufacturer protocol (GeNet Bio Co., Daejeon, Korea).

Analysis of HER2 polymorphism and Restriction Fragment Length Polymorphism (RFLP)

A part of the gene of interest containing SNS, HER2 was amplified by polymerase chain reaction (PCR) using HER2 specific set of primers [8] to amplify 148 base pairs (bp).

Forward primer: AGAGCGCCAGCCCTCTGACGTCCA

Reverse primer: TCCGTTTCCTGCAGCAGTCTCCGC

The volume of 50 PCR reaction was prepared containing 25 ul 2X Taq polymerase mastermix, 5 ul of human DNA, 2 ul of 5 picomole of each primer. Then the PCR reaction was carried out as follw: Initial heating at 95 ºC for 5 minutes, them 35 cycles of the PCR was followed

1. 95ºC for 30 seconds
2. 62ºC for 30 seconds
3. 72ºC for 30 seconds)

the reaction was ended with 72ºC for 7 minute for further extension of the DNA fragment.

RFLP was performed according to the procedure described by Xie et al. (2000) [8]. 50 ul of the PCR product (148 bp) was directed to enzymatic digestion, 1U BsmAI (New England Biolabs (NEB), Boston, MA, USA) to identify mutation (single nucleotide polymorphism). The digestion gives two DNA bands of 32 bp and 116 bp when the DNA contain the recognition site of the enzyme, GTC. The products of the reaction were then fractionated on 4% of agarose gel stained with DNA stain.

Statistical analysis

All required statistical analysis was done using statistical method (SPSS 24). The statistical protocol that was applied in this study to determine the results of the current data included:

1- Alpha-cronbach has been used for testing the reliability of the questionnaire.

2- Descriptive statistical data analysi (Frequency, Percentage,Mean & Stander deviation)

3-Inferential data analysis: Chi square & Independent sample T-test at P-value < 0.05. B.

The significance level of all statistical procedures was determined at (F test), P<0.000. There are criteria of the probability level of determining the significance of the test: P value as:

1. High significant (P< 0.001)
2. Significant (P< 0.05)
3. Nonsignificant (P> 0.05)
4. Very highly significant (P< 0.000)

3. Results

This study was done for the first in the region time to investigate the HER2 genotype among cancer patients of Kurdish ethnic in Kurdistan region of Iraq to indicate the risk of increasing breast cancer as mentioned previously in different studies in different populations. The result of HER2 genotype distribution and polymorphism among Kurdish cancer patients was clarified in Table 1. Three genotypes of HER2 were identified after enzymatic digestion of the PCR product: Healthy homozygous (Ile/Ile), carrier heterozygous (Ile/Val), and risky homozygous (Val/Val) (Figure 1). The study was caried out on blood DNA of 272 participants (151 malignant cases and 121 normal control women (Healthy individuals).

The result showed that the distribution of Val/Val and Ile/Val genotype was significantly higher in breast cancer patient (cases), 63.6% than healthy women (controls), 32.21%. The distribution of Val/Val and Ile/Val genotype among cases is two times higher than control which means it increases the risk of breast cancer by two folds (P<0.05).

Among breast cancer cases, Val/Val and Ile/Val genotype are higher than Ile/Ile by relatively two times which means the risk of breast cancer is two folds higher among women cases who have Val/Val and Ile/Val genotype among Kurdish population (P<0.05). In addition, the rate of heterozygous Ile/Val (47.7%) was higher than homozygous Val/Val (15.9%) by three folds, and the same result was seen with Ile/Val genotype in control (17.8% and 0%, respectively) p<0.005.

Table 1. The distribution of HER2 genotype of breast caner cases and controls among Kurdish ethnicity.				
		HER2 genotypes		Total
		Ile/Ile	Val/Val and Ile/Val	
Control	Frequency	82	39	121
	% Within row	67.8%	32.21%	100.0%
Case	Frequency	55	96	151
	% Within row	36.4%	63.6%	100.0%
Total	Frequency	137	135	272
	% Within row	50.4%	49.6%	100.0%

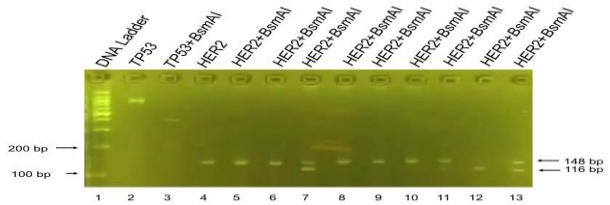

Figure 1. HER2 genotypes among Kurdish population. The PCR product of HER2 (Lane4) was digested using BsmA1 (lane 5-13) to find different genotypes, Lane 7 and 13: Ile/Val with (148+116 bp) Lane 12: Val/Val (116 bp), Lane 5, 6, 8, 9, 10, 11: Ile/Ile (148 bp). L1: M-100 bp, L2: TP53 gene containing restriction site to BsmA1and L3 is TP53 after enzymatic digestion used as a control to observe the activity of the enzyme.

Demographic characteristic of the patients was analyzed among women who carry valein allele. Different age groups and marital states were investigated among patients (Val allele carriers). The highest rate of the HER2Ile655Val genotype was found at age group older than 45 years old and the rate is higher in married than single. In addition, most of the breast cancer patients who carry valine allele were among uneducated and house wives (nonemployees) (Table 2).

Table2. Association between the cases in relation to Socio- Demographic characteristics among carrier women Val/Val and Ile/Val genotypes.							
Variables	Items	Ile/Val + Val/Val (N=151)		Ile/Ile (N=45)		Total	Significance test
		Fr.	%	Fr.	%		
Age (Years)	< 25	8	8.3	2	3.6	10	χ^2 =8.839 p= 0.032
	25 – 35	5	5.2	3	5.5	8	
	36 – 45	23	24	4	7.3	27	
	> 45	60	62.5	46	83.6	106	
	Mean ± S.D	48.89±13.73		53.93±12.09		T-test =-2.266 p=0.025	
Education	Bachelor	4	4.17	4	7.27	8	χ^2 =12.311 p= 0.015
	Diploma	8	8.33	1	1.82	9	
	High School	7	7.29	1	1.82	8	
	None	54	56.25	44	80.00	98	
	Primary school	23	23.96	5	9.09	28	
Occupation	Employee	15	15.63	5	9.09	20	χ^2 =1.299 p= 0.254
	Unemployed	81	84.38	50	90.91	131	
Marital Status	Married	90	93.75	47	85.45	137	χ^2 =2.861 p= 0.091
	Single	6	6.25	8	14.55	14	
	Total	96	100.00	55	100.00	151	

4. Discussion

In the last two decades, many researches have been published on HER2 Il655AA single nucleotide polymorphism and the frequency of distribution of the polymorphism was found in different geographical locations among different ethnic groups. In addition, many studies have concluded that there is association between of Ile655Val SNP for HER2 and the risk of breast tissue malignancy [20–22] and on the other hands, it remains controversial [14,15,23,24]. HER2 has a role in regulation and control of cell growth and proliferation in breast cancer cells and it controls the normal growth of breast cells [25].

According to the previous studies HER2Ile655Val single nucleotide polymorphism may have a role in appearing of breast cancer and its very common among Caucasian population [20–22]. Among Caucasian women, it's found that the Val allele contribute to increase the risk of women breast cancer, but the thing is different in Africa where homozygous allele Val/Val has been found to have a potential link with breast cancer [20,22]. In this study, we evaluated the potential role of HER2 gene polymorphism in Kurdish population for the first time and its association with breast cancer risks among Kurdish of women of Iraq.

In this study, it is identified that there is two folds increase in the risk of developing breast cancer in Kurdish population among women who bears two genotypes of HER2 gene, Val/Val and Ile/Val. The rate of Val/Val and Ile/Val genotype among breast cancer patients was 63.6% and it was 32.21% in control which is significant ($P < 0.05$) (Table 1). The rate of HER2Ile655Val polymorphism among Kurdish breast cancer patients, 63.6% is the second highest rate ever recorded after Greek at the rate of 73.2% [26,27]. This conclude that the Kurdish women who carries HER2Ile655Val genotypes are at risk of breast

tissue cancer more than all other countries worldwide after Greek and this is very concerning for health authorities and governors.

The rate of HER2 single nucleotide polymorphism in Kurdistan region of Iraq is higher than in population of the other countries neighbored or near to the Kurdish territory of Iraq. 63.6% of the polymorphism among Kurdish population is higher than Arab Ethnics who lives in the same country, Iraq (53%). This means that both geographical location and ethnicity may have an influence on the frequency and distribution of Val/Val and Ile/Val genotypes. The rate is also higher than: Iran 28.9%, Turkish 19%, and Egypt (39%) [28–30]. Therefore, the risk of breast tissue cancer among Kurdish women who carries HER2 Val/Val and Ile/Val SNP is higher than populations who lives in neighbor countries of Kurdistan region of Iraq.

The association was also investigated in different age groups (Table 2) of the patients. The highest rate of polymorphism was seen in age group, >45 at the rate of 62.5%. This means that HER2Ile655Val single nucleotide polymorphism risk is the highest among older ages (older than 45). This result is consistent with other studies in Portugal, Egypt and Australia [9,30,31]. The rates were also higher among married women and the reason may be linked to the ages of married women because usually older ages are married. Were as educated and employee has an effect on appearing cancer among carrier women. This may be due to the lack of knowledge of house wives and non-educated women to protect themselves and avoiding from cancer protection health authority guides in comparison to educated and employees.

The role of Her2 polymorphism to increase the risk of breast tissue cancer among females may depend on many factors in addition to the HER2 genotypes. One of the possibilities could be ethnicity. The rate is different in different ethnic groups, for example the rate of polymorphism is higher in Kurdish ethnic than Arab population of the same country. The second possibility may be related to the location, geographical region. In Iraq in general, the rate is higher than most of the countries worldwide [27]. Another reason may contribute to geographical region is the diet, environment and style of life. Therefore, detailed study is necessary to investigate the reasons of gene polymorphism among different population on the molecular level and their link to cancer risk.

5. Conclusion

In our study, we found a link between HER2 codon 655 polymorphisms with the risk of breast cancer. In Kurdish population, the rate of Val/Val and Ile/Val genotype is significantly higher in breast tissue cancer women than healthy women and the rate of HER2Ile655Val polymorphism among breast cancer cases is the highest worldwide after Greek. The cases are more common among older and married women. This study is the first of its kind in the study area; therefore further study is required on the base of molecular level to understand the association between ethnics, geographical regions with gene polymorphism and the risk of cancer.

Data Availability

All data available in the manuscript

6. Ethical Approval

The ethics form was approved by the ethical committee of the College of Health and medical technology, Sulaimani Polytechnic University

Conflicts of interest

There are no competing interests for any authors

Author's contribution

Israa Saadaldin Shmasaldin: She is master student and she has done almost all of the works from the project design to the writing. Muhammed Babakir-Mina: He is the first supervisor of the student and he designed the project for the student and supervising it. He helped in writing and reviewing. Taib Ahmed Hama Soor: He is the second supervisor of the student and he designed the project for the student and supervising it. He helped in writing and reviewing. Solhan Mustafa Ahmadissa and Payam Omer Radha:They are Lab technician and they are expert in molecular biology works. They helped the first author how to do the work and guided ger via their knowledge. And contributed to data collection and result analysis

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