Association of TP53 [Arg72Pro] Gene Polymorphism and Breast Cancer Risk in Iraqi female patients

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Abstract

Breast cancer is considered the first or second most common cancer and major cause of death in women globally. The TP53 tumor suppressor gene is one of the most commonly mutated genes in all types of human cancer including breast cancer. numerous studies showed that the significant association between Tumor suppressor protein TP53 gene polymorphism (Arg72Pro: rs 1042522) and the risk of breast cancer. We aimed to study and investigate the association of TP53 (Arg72Pro) gene polymorphism with the risk of breast cancer. The genotypes and allele frequencies of TP53 (Arg72Pro) 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. PCR product was digested by BstUI restriction enzyme and the DNA fragments resolved by electrophoresis in 2% analytical grade agarose gel. The results indicated that the Tumor protein TP53 gene G→C (Arg 72 Pro) SNP genotype frequencies of wild genotype (GG) , heterozygous genotype (GC) and homozygous genotype (CC) were 23.3 %, 50.3 % and 26.4 % in cases of breast cancer group and 55 %, 37.5 % and 7.5% in the control group respectively. The heterozygous genotype (GC) was found increased the risk of breast cancer by five folds higher than those of the wild (GG) genotype (OR= 4.77, 95% C.I= 3.12–7.29, P = 0.0001). In contrast, the homozygous genotype (CC) was recorded that statistically significant increased the risk of breast cancer by seven folds higher than that found in wild genotype (GG) after adjustment for age and BMI (OR= 6.98, 95% C.I = 3.95-12.4, P = 0.0001). We concluded from this study that the Pro allele of TP53 gene was increased the risk of breast cancer in patients of Iraqi population.

Key words: Breast cancer, TP53, SNP, Polymorphism, Iraq

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Introduction:

Breast cancer is one of the most common cancer types among women, it estimated about 23% of female cancer with increased in mortality rate in low resource countries in spite of substantial improvement in survival from breast cancer disease has been recorded in high-income countries such as in USA, the risk continues to increase and survival rates in middle and low income countries remain low [(1)]. Since the early 1990s breast cancer rates have been increased globally, although the survival rates have continued to rise at the past 20 years partially due to advances in breast cancer screening, early diagnosis and aggressive treatment modalities [(1, 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)]. Breast cancer is a heterogeneous disease regarding it's morphology, invasive behavior, metastatic capacity, hormone receptor expression and clinical outcome. There are many risk factors for breast cancer including genetic factors which account for 25-30% of the incidence, from this percentage only 15-30% heritable component of breast cancer is due to known familial highly penetrate genes such as BRCA1 and BRCA2 and the others are sporadic, the significant contribution of genetic factors remain undetermined[(4)].

Single nucleotide polymorphism is one of the most of genetic markers that used in the last years and have popularity for study of genetic phenotypes[(5,6)]. Genomic Wide Association Studies (GWAS) have identified more than 70 single nucleotide polymorphisms (SNPs) that influence on breast cancer risk and may have an impact on breast cancer prognosis[(7)]. Some genetic polymorphisms have functionally significant effect on the gene product which make them the most useful types of polymorphisms in disease association studies, other polymorphisms are simply useful as markers [(5)]. Numerous studies showed that the significant association between Tumor suppressor protein TP53 (rs 1042522) gene polymorphism in exon 4 (Arg 72 Pro) and breast cancer[(8-10)]. The TP53 tumor suppressor gene, is one of the most commonly mutated genes in all types of human cancer. It was located on chromosome 17p13.1 and the most involved genetic factor for breast cancer, the gene of tumor suppressor protein TP53 occupies a central role in mediating cellular responses to DNA damage. Its activation results either in cell growth arrest or in apoptosis. TP53 gene contains a variety of polymorphisms and mutations [(8)]. Mutations in this gene are associated with more than 50% of human cancers, and 90% of them affect TP53-

DNA interactions, and result in a partial or complete loss of transactivation functions, many common polymorphic variants in the germline also show functional effects related to cancer development[11, 12]. We designed our study to examine the relation between Arg 72 Pro polymorphism in TP53 gene and the risk of breast cancer and to compare TP53 (Arg 72 Pro) the genotypes and allele frequencies between patients with breast cancer and healthy individuals 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. Informed consent was obtained from all participating individuals and the study was carried out with approval of Medical ethics committee /Faculty of Medicine / Kufa University.

DNA extraction and mutation analysis:

Whole blood samples of patients and control groups were used to extract DNA. The extraction of DNA was done 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 single nucleotide polymorphism at codon 72 is located in exon 4 of TP53 gene (Arg 72 Pro) in the prolin rich region of the protein was analyzed by PCR-RFLP. The segment that consisted of 396 bp was amplified in PCR (Biometra, Germany) using the gene specific primers forward 5′-CTG GTA AGG ACA AGG GTT GG - 3′ and reverse 5′- ACT GAC CGT GCA AGT CAC AG -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 5 min then amplified for 35 cycles of 94c° for 30 sec, 55c° 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 BstUI (New England Biolabs,UK) at 60c° for 15 min. BstUI enzyme digestion gave single fragment 396 bp for the Arg allele and two fragments of 231 bp and 165 bp for Pro allele. Fragments digested with BstUI were subjected to electrophoresis on 2% 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 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 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 pres vented 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	%	controls= 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

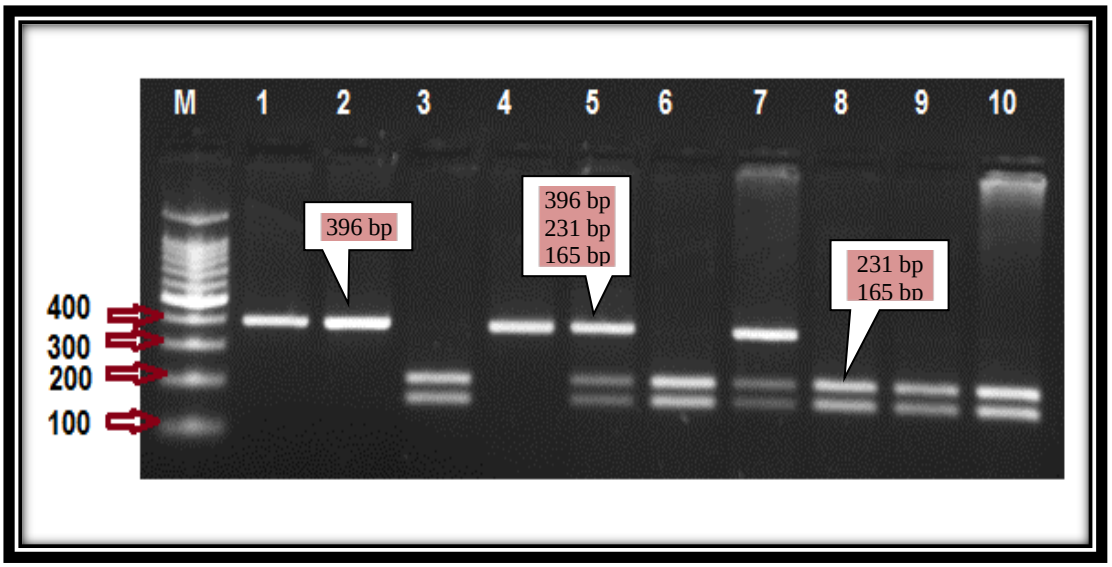

Figure 1: Restriction digestion of PCR product for TP53 gene G→C(Arg 72 Pro) SNP. Lane M: DNA Ladder, Lane 1, 2, 4 and 10 wild genotype (GG), Lane 5, and 7 heterozygous genotype (GC), Lane 3, 6, 8, 9 and 10 homozygous genotype (CC).

Table 2: Genotype and allele frequencies of Arg 72 Pro polymorphism for TP53 gene in patients and control groups

Genotype Arg72Pro G→ C	Cases no.	%	Control no.	%	Crude model OR[a]	(95% C.I)	Adjusted model OR[b]	(95% C.I)	P-value
	300		200						
GG	70	23.3	110	55	1.00				
GC	151	50.3	75	37.5	4.76	(3.1-7.3)	4.77	(3.12-7.29)	0.0001
CC	79	26.4	15	7.5	6.99	(3.85-12.3)	6.98	(3.95-12.4)	0.0001
Allele frequency									
G	291	48.5%	295	73.75%	1.00				
C	309	51.5%	105	26.25%	2.96	(2.24-3.90)	2.98	(2.26-3.92)	<0.0001

[a] Multinomial logistic regression. [b] Multinomial logistic regression stratified 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 Tumor protein TP53 gene G→C. It is found to be (100 %), it seemed to be within the optimal level (80% or more). Genotyping frequencies

of TP53 (Arg 72 Pro) gene was consistent with Hardy Weinberg Equilibrium in breast cancer group (P= 0.53) and in control group(P= 0.65).

The results indicated that the Tumor protein TP53 gene G→C (Arg 72 Pro) SNP genotype frequencies of wild genotype (GG) **,** heterozygous genotype (GC) and homozygous genotype (CC) were 23.3 %, 50.3 % and 26.4 % in cases of breast cancer females group and 55 %, 37.5 % and 7.5% in the control females group respectively. The heterozygous genotype (GC) was found increased the risk of breast cancer by five folds higher than those of the wild (GG) genotype (OR= 4.77, 95% C.I= 3.12–7.29, P = 0.0001). In contrast, the homozygous genotype (CC) was statistically significant increased the risk of breast cancer by seven folds higher than that found in wild genotype (GG) after adjustment for age and BMI (OR= 6.98, 95% C.I = 3.95–12.4, P = 0.0001). No significant variations were obtained when the analysis was carried out without adjustment. The allele frequency of G was found to be 48.5 % in breast cancer patients and 73.75 % in control group, while the frequency of C allele in breast cancer cases 51.5 % which is higher than C allele frequency in control group that equal to 26.25 %. The C allele frequency increased the risk of breast cancer by three times when compared with those of G allele (OR= 2.98, 95% C.I= 2.26–3.92, P < 0.0001) as presented in table (2).

Discussion:

Breast cancer has been associated with variety of risk factors of both genetic and epigenetic changes[13]. Common genetic polymorphisms could be affect the protein expression or functions within genes that involved main fundamental cellular pathways for example maintenance of cell cycle, DNA repair, cell proliferation and carcinogen degradation which might predispose the persons to cancer [14], included breast cancer[14,15]. TP53 is the essential tumor suppressor gene which plays major role in preserved integrity of genome, Arg 72 Pro (rs 1042522) gene polymorphism is the common SNP occurs in this gene[16].

Among three hundreds female breast cancer cases that genotyped in this study, there were 70 (23.3%) patients carrying TP53 Arg/Arg (GG) wild genotype, 151 (50.3 %) patients carrying TP53 Arg/Pro (GC) heterozygous genotype and 79 (26.4 %) patients carrying TP53 Pro/Pro (CC) homozygous genotype respectively. As well as the results showed a predominance of the C allele with frequency of 51.5 % in breast cancer patients when compared with 26.25% in control group as appeared in table (2). The results of the present study consistent with several case control studies that

suggested TP53 codon 72 retained in GC heterozygous and C allele genotype frequency was higher risk of breast cancer as recorded in Germany female breast cancer patients by Wang-Gohrke et.al[17], in English by Good et.al[18], in Russian by Suspitsin et.al[19], in Japanese by Noma et.al[20], in Swedish by Sjalander et.al[21], in Slovakian by Franekova et.al[22], in North Indian by Singh et.al[23], in Taiwanese by Fang-Ming Chen et.al[24], in Iranian by Hojjat Rouhi et.al[25]. In contrast, the present study disagree with other researchers who showed there was no association between TP53 codon 72 polymorphism and female breast cancer risk as reported in one Arab population, in Tunnisian women by Mabrouk et.al [26], and also disagree with other populations as Pakistanian by Khaliq et.al [27], in Finnishian by Tommiska et.al [28] and Iranian by Khadang et.al [29]. We concluded from the present study that significantly enhanced breast cancer risk associated with proline allele in TP53 (Arg72Pro) gene polymorphism. Heterozygous patients of Arg/Pro carriers at five folds risk while homozygous patients of Pro/Pro carriers were at higher risk of breast cancer by seven folds when compared with wild Arg/Arg genotype patients, suggesting that Pro allele can use as an indicator of genetic susceptibility to the breast cancer.

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