How to Cite:
Rafeef, F. K., & Al-Husseini, R. M. A.-H. (2022). Association of the genetic variants of XRCC1 DNA repair gene as genetic factors for breast cancer in Iraqi patients. *International Journal of Health Sciences*, *6*(S2), 14125–14136.
https://doi.org/10.53730/ijhs.v6nS2.8691

Association of the genetic variants of XRCC1 DNA repair gene as genetic factors for breast cancer in Iraqi patients

Fatima Kadhem Rafeef
Faculty of Science, University of Kufa, Iraq
Corresponding author email: fatimakadhemrafeef@gmail.com

Dr. Rand Muhammed Abdul-Hussein Al-Husseini
Professor, Faculty of Science, University of Kufa, Iraq
Email: rand.alhusseini@uokufa.edu.iq

***Abstract*---**Breast cancer (BC) is the second leading cause of cancer deaths worldwide and the most common type of cancer among women. This study was included blood of 75 Iraqi women with confirmed breast cancer and 25 samples with normal breast tissues were considered as control group. The restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) technique was performed the detection and genotyping for polymorphisms of *X-Ray Repair Cross Complementing* 1 (*XRCC* 1) gene Arg194Trp. The genotype distribution of the *XRCC 1* Arg194Trp showed significant difference (p<0.05) between controls (Arg/Arg: n= 25, 100%; Arg/Trp and Trp/Trp n=0) and breast cancer patients (Arg/Arg: n= 47, 62.67%; Arg/Trp: n=2, 2.67%; Trp /Trp: n=26, 34.67%). The result showed an increased in Trp/Trp genotype and Trp allele of the *XRCC1* Arg194Trp in breast cancer patients than control.

***Keywords*---**breast cancer, XRCC 1 Arg194Trp, polymorphism, Iraq.

Introduction

Breast cancer (BC) is the most common cancer among women worldwide and the leading cause of cancer deaths among Iraqi women and the incidence is still increasing [1]. It is a multifactorial disease and many factors including genetic, environmental, reproductive and lifestyle related factors effect forming of the disease [2]. Even though the mechanism underlying breast carcinogenesis is not fully understood, various risk factors are defined for the disease, such as induction of DNA damage by endogenous and exogenous agents [3]. Failure in repairing a damage in the chemical structure of DNA plays an important role in

cancer progression [4]. Detection and repair of DNA damage by DNA repair mechanisms, plays an important role in preventing carcinogenesis, maintaining genome integrity and protecting against mutations [3]. A common genetic variant in *XRCC* 1 (base C to T) in exon 6 result in an arginine (Arg) to tryptophn (Trp) db snp no.(rs1799782) substitution at codon 194 near the N-terminal domain these polymorphisms may change the DNA repair activity [5]. This study aimed to determinate the *XRCC1* Arg194Trp in breast cancer Iraqi patients and controls by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Methods

Study group

This study was carried out in in Euphrates Cancer Hospital and laboratory of molecular biology in the Department of Biology / Faculty of Science – University of Kufa, during the period from February 2021 through March 2022. This study was included 75 Iraqi women with confirmed breast cancer. These samples were collected from laboratory of Euphrates Cancer Hospital in Al- Najaf province. . Epidemiological information's about patients like age at diagnosis of cancer , body mass index (BMI), marital status, residence , education level, the work, family history was collected from patients questionnaire and data sheets from hospital . Twenty-five samples with normal breast tissues were considered as control group for this study.

DNA isolation and RFLP-PCR Technique

Genomic DNA was isolated using protocol from Genomic DNA Mini Kit was designed specifically for purifying DNA from blood. Amplification of the sequences of SNPs fragments PCR thermocycler (BioRad/ USA) programed for 34 cycles :denaturation at 94°C for 0.45 sec, annealing at. 62°C for 0.45 sec, extension at 72 °C for 1min and the PCR amplification has been completed by a final extension at 72°C for 5 min (Table 1). Primers sequences that used to amplify the *XRCC1* Arg194Trp polymorphism of gene *XRCC1* are listed in in Table 2 , were synthesized by macrogan/ china. All these primers were reached as lyophilized form with different concentration. Primer containers were first centrifuged at 13,000 rpm for 3 minutes, and then reconstituted with appropriate volume of TE buffer for each one (according to the manufacturer) in order to get 100 pmole/µl (stock solutions). Working solution with 10 pmole/µl, was prepared from stock solutions.

The restriction fragment length polymorphism -PCR technique was performed for detection and genotyping *XRCC1* Arg194Trp gene polymorphisms. The 491bp PCR product was digested with 10 unit of *MspI* restriction enzyme (10 units is sufficient, generally 1µl was used ([6],Source: An *Moraxella* sp at 37 °C (synthesized by New England Biolabs, Inc, Cat. No. R0106L). After the *MspI* digestion (it left to digest *for 35 min* and can also be used safely in overnight digestions), a 178 bp fragment resulting for each sample serves as an internal control for complete enzyme digestion and one of these results were yielded for each sample as following:

a) 292 and 21bp fragments for allele Arg (homozygous genotype patient, Arg/Arg).
b) 313, 292 and 21 bp fragments for both Arg and Trp allele (a heterozygous genotype patient, Arg/Trp).
c) 313 bp fragment for allele Trp (homozygous genotype patient, Trp/Trp).

Finally, the gel electrophoresis method, which included preparing the gel loading and running the gel, was done according to [7]The DNA Green Viewer stained gel was visualized under UV light and photographed .

Table 1: Reaction condition for *XRCC1* Arg194Trp gene

Gene	Initial Denaturation	Numbers of Cycles	The compositions of each cycle			Extension Step
			Denaturation	Annealing	Extension	
Arg194Trp (C>T) (rs1799782) polymorphism	95°C for 3 min	34cycles	94°C for 0.45 sec	62°C for 0.45 sec	72°C for 1min	72°C for 5 min

Table 2: Primers sequences for *XRCC1* Arg194Trp gene.

Gene	Primers sequences		PCR product	Ref.
*Arg*194Trp (C>T) (rs1799782) polymorphism	F	5′-GCCCCGTCCCAGGTA-3′′	491bp	(Zhang *et al.,* 2005)
	R	5′-AGCCCCAAGACCCTTTCACT-3′		

Bio Statistical Analysis

Statistical analyses of all results were carried out by the help of Statistical Package for the Social Sciences (SPSS) version 23 software statistical package using t-test and Chi-square test (with P value at level of significance less than 0.05) to compare value of results between groups. Result values were expressed as number of patients, or percentages.

Results and Discussion

Breast cancer is a polygenic disease. The polygenic model of breast cancer suggests that there are multiple low penetrance alleles, each or in combination, together with environmental interactions that have a small effect on breast cancer risk . The DNA repair is essential for maintaining genomic integrity. Deficiencies in the DNA repair pathway lead to genetic instability which in turn may lead to cancer development. Genetic polymorphisms in DNA repair genes may contribute to differential DNA repair capability between individuals [8] [9]. Assessment of age presentation of patients at diagnosis revealed that 16 (21.33%) were in age group 24-40, 40 (53.34%) were in age group 41-57, 16 (21.33%) were in age group 58-74, 3 (4%) were in age group 75-92 (figure 1). Their ages ranged from 24 to 82

years, with a mean age of 49.6 years, while the ages of the control groups were close to those of the BC patients, its ranged from 24 to70 years with a mean age of 46.68 years.

The estimated incidence of breast cancer increased in the second age group (41-57), with significant difference ($p< 0.05$) between groups. These results showed that the women with age between 41 to 57 years or older are more at risk than younger patients for breast cancer, These results are explained by several known and suspected causes of an age-dependent susceptibility to cancer as, mutations increase with age, aging tissue and cellular microenvironment, a tumor suppressor lifetime carcinogenic exposure, decreased ability to repair DNA, oncogene activation, and amplification, decrease tumor suppressor gene activity, microenvironment alteration, including hormonal alterations or exposures and decreases immune surveillance due to immune senescence [10, 11]

These results agreed with Al-naqqash *et al.* (2019) study that showed the Iraqi breast women study includes 1349 women diagnosed with breast cancer of young age, of whom 1147 (85%) were over 40 years old, 106 (7.9%) were 36-40, 65 (4.8%) were 31-35 years old, 28 (2.1%) were 26-30 years of age and three (0.2%) were between the ages of 20-25 years. These results also agreed with Alwan *et al.* (2019) study that documented in Iraq which recorded that among 1172 women with a breast cancer The highest frequency was observed between ages (35–49) and (60–64) years while Before 35 and 64 years of age, only 4.4 percent and 11 percent had been diagnosed. A study involving 100 cases in Iraq found that the age group 40-49 years (25%) represent the highest percentage [12]. The results were close to the results of this study. Wong *et al.* (2018) study showed that the patient in age group 41-50 years(38.2%) represented the highest rate among study group and the 51-60 years (29.6%) the second while >80 years (0.2) were the smallest .

These findings were consistent with those of AL-Nuaimee *et al.* (2020) which showed that the most common age group of incidence was above 45 years old, which accounts for 80% of women, and showed significant difference ($P<0.05$) compared to other age groups and followed by the age group of 18-45 years which account for 15% of women while the age group of below 18 years accounts for 5% of women only.

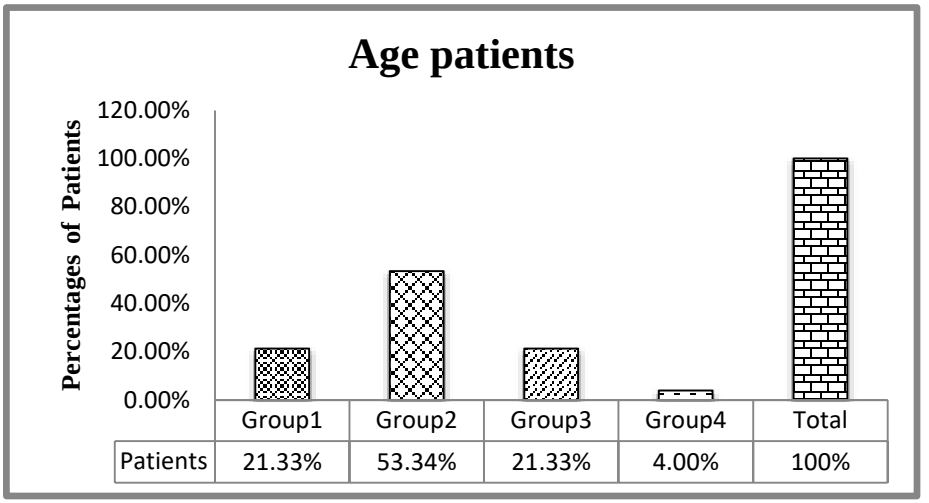

Figure 1: Age distribution of patients presented with breast cancer. (Group1: 24-40, Group2: 41-57, Group3: 58-74, Group4: 75-92).

The results in Table 3 described the characteristics of the BMI in breast cancer patients and controls that shows increased BMI in breast cancer patients, half of patients were presented with obesity .

Table 3: BMI in breast cancer patients and controls

BMI (range)	Breast Cancer Patients (n=75)	Controls (n=25)	*p*-values
Normal weight (18.5-24.9 kg/m^2)	19 (25.3%)	12 (48 %)	0.209
Underweight (below 18.5)	1(1.3%)	0 (%)	0.911
Overweight (25-29.9 kg/m^2)	18 (24 %)	10(40 %)	0.131
Obese (>30 kg/m^2)	37 (49.4%)	3 (12 %)	0.0001**

BMI: body mass index, results values were expressed as number of patients (results also represented as a percentage (%)), *: $p< 0.05$ or significant differences between percentages.

The obesity and excess body weight is generally recognized as a significant risk factor for many common cancers included BC [13]. On the other hand these results agree with other study that found higher BMI is associated with increased risk of breast cancer [14],while other study suggested the significant increase in incidence of IDC type of BC compare to ILC in women with BMI ≥ 25 kg/m^2 and the IDC is the most type of BC in the world, This results may be explained to increase fats and muscles mass in chest of women lead to pressing on milk ducts in breast tissue leading to increased risk of IDC type of BC [15]**.**

Demographic and clinico-pathological characteristics of breast cancer patient demonstrated in Table 4 . The results described the characteristics of the marital status among breast cancer patients which showed that the majority (71, 94.66%) of patients were married while 4 (5.34%) were unmarried .The characteristics of the residency in breast cancer patients revealed that the

majority 67, (89.34%) of patients were living in the urban area while 8 (10.66%) were living in the rural area. The results of the educational level in breast cancer women revealed that, 34 (45.33%) of patients were presented with illiterate education ; 24 (32 %) of patients with primary school , 10 (13.34%) with secondary school but the lower number 7 (9.33%) of patients were presented with high level of education (university or above). The results also described the occupations of patients , 67(89.33%) were housewives (not working), while worker women were 8(10.66%) . The results in Table 4 described the characteristics of the family history in breast cancer patients found that without family history were 35(46.66%), with family history with BC were 18(24%) and family history any cancer were 22(29.34%). Depending on the site from which the tumor originated the tumors with highest frequency of occurrence were ductal carcinomas 69(92 %) followed by lobular carcinomas 6(8%) (Table 4) .

Table 4: Demographic characteristics of Breast Cancer Patients

Characteristics	Sub-groups	Breast Cancer Patients (n=75)
Marital Status	Married	71 (94.66%)
	Unmarried	4 (5.34%)
Residency	Rural	8(10.66%)
	Urban	67(89.34%)
Levels of Education	Illiterate	34 (45.33%)
	Primary	24(32%)
	Secondary	10(13.34%)
	University	7(9.33%)
The Work	Housewives	67(89.34%)
	Worker	8(10.66%)
Family History	Yes	40(53.34%)
	No	35(46.66)
Type of Breast Cancer	IDC	69(92 %)
	LDC	6(8%)

Table 4.1: Data were expressed as number of patients (results values also represented as a percentage (%)).Abbreviations: IDC:invasive ductal carcinoma; LDC: invasive lobular carcinoma.

Among the 25 healthy subjects; 25 (100%) had found as homozygous (Arg/Arg) alleles, no one found as heterozygous genotype (with the Arg and Trp alleles (Arg/Trp), and no healthy found as homozygous genotype (Trp /Trp) alleles; (Arg/Arg: n= 25, 100%; Arg/Trp and Trp/Trp: n=0) (Table 5 & figure 3).

Table 5: The results of genotypic frequencies of *XRCC1* Arg194Trp gene in patients and controls

Genotypes	Healthy controls (N=25)	Breast cancer patients (N=75)
Arg/Arg	25 (100%)	47 (62.67%)
Arg/Trp	0 (0%)	2 (2.67%)

Trp /Trp	0 (0%)	26 (34.67%)
p -value	0.002**	
Alleles frequency	N(%)	N(%)
Arg allele	50 (100%)	96 (64%)
Trp allele	0 (0%)	54 (36%)
X^2 *p*-value OR (95%CI)	24.658 0.0001** 1.563 (1.386-1.762)	

Data were expressed as number and a percentage (N%). **p* <0.05 significant. Abbreviations: X^2= chi-square, OR= odds ratio, CI= confidence interval.

Among the 75 of breast cancer patients; 47 (62.67%) had found as homozygous (Arg/Arg) alleles, 2 (2.67%) found as heterozygous genotype (with the Arg and Trp alleles (Arg/Trp)), and 26 (34.67%) had found as homozygous genotype (Trp /Trp) alleles; (Arg/Arg: n= 47, 62.67%; Arg/Trp: n=2, 2.67%; Trp /Trp: n=26, 34.67%) (Table 5& figure 3).

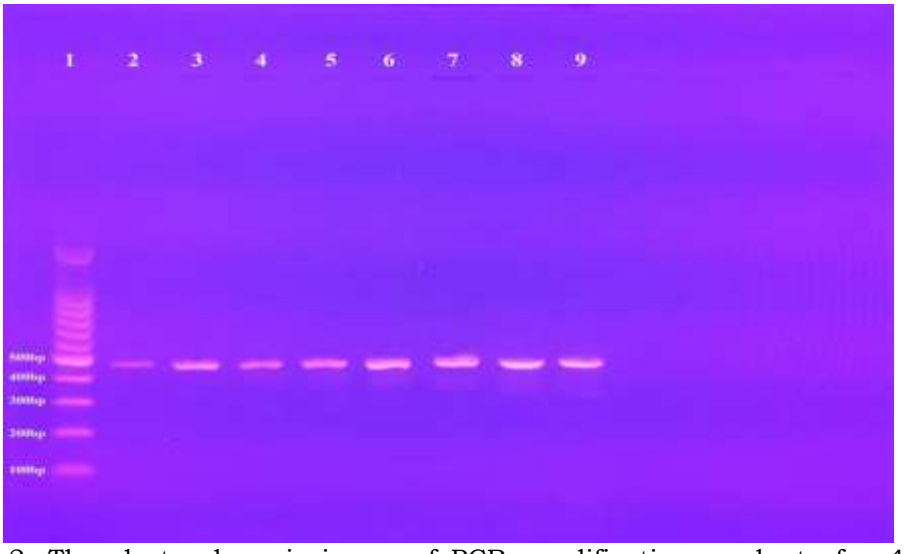

Figure 2: The electrophoresis image of PCR amplification product of a 491 bp fragment including the position of *Arg194Trp* SNP in the *XRCC1* gene (Lane 1: 100 bp DNA Ladder; Lane 2 to 9: the 491bp PCR product.

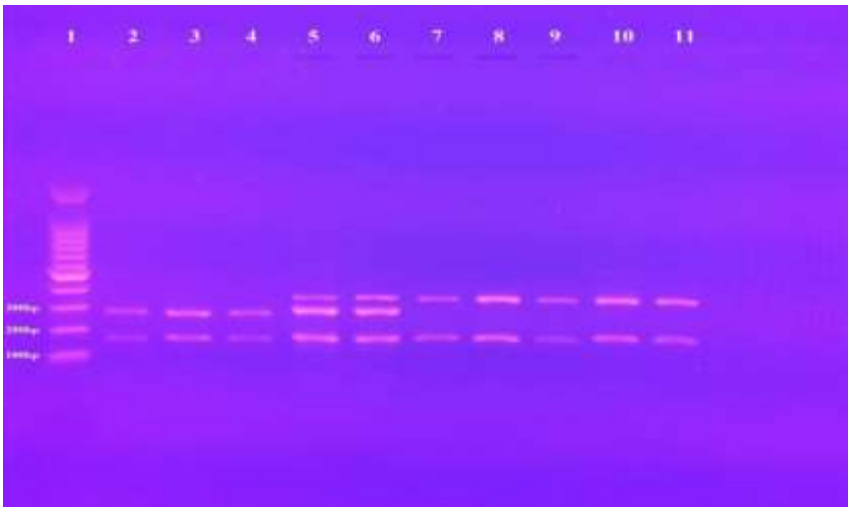

Figure 3: The electrophoresis image of RFLP-PCR analysis of XRCC1 Arg194Trp gene SNP. Lane 1: 100 bp DNA Ladder; Lane 2,3, and 4: homozygous genotype, Arg/Arg (292,178, and 21bp which undetectable on gel because of small size); Lane 5, and 6: heterozygous genotype, Arg/Trp (313, 292,178 and 21 bp fragments); Lane 7, 8,9,10 and 11: homozygous genotype, Trp/Trp (313, and 178 bp).

That means the frequencies of Arg194Trp SNP in the *XRCC1* gene in the 75 Iraqi breast cancer patients in Al-Najaf province were with significant differences decrease with that of the 25 healthy controls group (p<0.05). The result showed an increased in Trp/Trp genotype and Trp allele of the *XRCC1* Arg194Trp in breast cancer patients than controls, and they were significantly increase more likely than controls to have the mutant allele (OR=1.563, 95%CI= 1.386-1.762, p=0.0001**). This conclusion indicates a possible role for the Arg194Trp genotype in breast cancer.

Breast cancer is a polygenic disease. The polygenic model of breast cancer suggests that there are multiple low penetrance alleles, each or in combination, together with environmental interactions that have a small effect on breast cancer risk . The DNA repair is essential for maintaining genomic integrity. Deficiencies in the DNA repair pathway lead to genetic instability which in turn may lead to cancer development. Genetic polymorphisms in DNA repair genes may contribute to differential DNA repair capability between individuals [8,9]

The previous studies demonstrated the potential role of Trp variant of *XRCC1* Arg194Trp gene as a potential risk factor of breast cancer [16, 17]. There are some studies in regards to role of its in breast cancer for example, in a Polish population Trp allele has associated with breast cancer [16]. And these results agree with results of contemporary study .This may be because *XRCC1* Arg194Trp was located on the highly conserved region, This region is a protein interacting domain and has an important role in base excision DNA repair mechanism. Therefore, amino acid substitution in this region could greatly alter the *XRCC1* capability to interact with other DNA repair protein components of BER complex [18].

The polymorphism at the *XRCC1* Arg194Trp had been studied in 202 of Iraqi population, distributed in two groups: 106 Arab and 96 Kurdish. It's found that 94 (88.6%), 12(11.4%) and 0% represented Arab individuals carrying the Arg/Arg, Arg/Trp, and no one Trp/Trp genotype respectively but in Kurdish group 94(97.9%), 2(2.1%) represented individuals had the Arg/Arg, Arg /Trp and no one had the Trp/Trp genotype respectively [19] .and this agreement with recent study. This study is consistent with Jalali and collegous (2016) Study on Iranian Kurdish population they found that allele frequencies for Iranian Kurdish healthy women were 45% , 40% and 15% for Arg/Arg, Arg/Trp and Trp/Trp respectively, comparing with BC patients which was much higher and associated of Trp allele with breast censer higher risk [Trp/Trp (55%) ,Arg/Arg (29%) and Arg/Trp (16 %)] .

This study is in contrast to another study on breast cancer in Arab Saudi population, they found that the allele frequencies for the healthy controls were 96 % found as Arg/Arg, 4% found as Arg/Trp and no one found as Trp/Trp , whereas as in in BC cases were 84% found as Arg/Arg, 16 % found as Arg/Trp, and no one found as Trp/Trp .so Arg/Trp showed significantly higher risk in breast cancer patients (p<0.05) when compared with controls [20] . It has been found that the distribution of *XRCC1* Arg194Trp polymorphism is significantly influenced by ethnicity [18]. In Arab of Iraq individuals the Arg allele frequency was 94% and 5% for the Trp allele , while in Kurdish of Iraq individuals the frequency of Arg allele was 99% and Trp allele was 1% .These results may indicates that the Arg allele is the most common allele in Iraqi population in both Arab and Kurdish, and there is a slightly high allele frequency were observed in Kurdish may due intermarriage leading to reduce the recessive allele in the population [19], This study is in agreement with them but in other study discovered that the frequency of the Trp allele of *XRCC1* Arg194Trp was higher in Asian populations than in African and Caucasian populations. African and Caucasian populations showed higher Arg allele frequencies [18].

The rate of Arg and Trp alleles of *XRCC1* Arg194Trp gene in the breast cancer patients and controls were 37% and 65 % for Arg allele, and 63% and 35 % for Trp allele, respectively (Jalali *et al* ., 2016). The results of this study are in agreement with their findings. The results of this study are in correspondence with other study results that showed control Arg allele was 92.31% and for breast cancer patient was 90.42% while allele Trp for control were 7.69% and for breast cancer patient was 9.58% [21]. Meta analysis study done by Moghaddam *et al.* (2016) comprise of a total of 14,793 breast cancer cases and 15,409 controls were included in assessment of *XRCC1* Arg194Trp. Four studies showed significant association and one study showed protective effect of *XRCC1* Arg194Trp and BC [22].

On other hand , Smith *et al.* (2003) reported a weak association of the Trp allele with a risk of breast cancer occurrence in white women [23]. Furthermore study conducted in Egypt showed no association was detected between the *XRCC1* Arg194Trp polymorphisms and BC risk in all genetic models [24] and this agree with other studies For *XRCC1* Arg194Trp, that was not found to be associated with risk of breast cancer [21, 25]. This is in contrast to the results of the current

study. Taking together, these findings reveal that there is not an overall agreement to the effect of *XRCC1* Arg194Trp polymorphism on breast cancer .

Three studies found that risk of bladder cancer decrease by this polymorphism, they explained their observation by alternative hypotheses to interpret these findings: A first, hypothesis could be that these variants diminish the efficiency of the XRCC1 protein but still provide decreased risk from cancer. Under this scenario, cells with excessive oxidative damage that carry such variants would have decreased ability to repair DNA damage and might be more likely to undergo apoptosis or senescence. Such decreased efficiency could be an "advantage" if it avoided the transmission and clonal expansion of mutations. A second hypothesis is that another polymorphic gene might be in linkage disequilibrium with *XRCC1*. Interestingly, the Excision repair cross-complementation group 2 *(ERCC2) (XPD)* gene also maps to chromosome 19q13.2 adjacent to *XRCC1*, which maps to 19q13.2–13.3. *ERCC2* is involved in nucleotide excision repair of bulky adducts, such as those induced by many of the carcinogens in cigarette smoke [26-30].

Conclusion

The genotype distribution results of the Arg194Trp SNP of *XRCC1* gene showed a significant increased ($p<0.05$) in Trp/Trp genotype and Trp allele in breast cancer patients than controls. This conclusion indicates a possible role for this genotype in a risk of breast cancer occurrence in Iraqi women.

Acknowledgments

The authors is grateful to the authorities of University of Kufa, Faculty of Science for according permission to carry out this research work in the laboratory of molecular biology in the Department of biology

References

1. Baiee, H.A. and Z.F. Kizar, *Potential Risk Factors of Breast Cancer among Women Attending Teaching Hospitals in Babylon Province.* Medico Legal Update, 2020. 20(1): p. 971-977.
2. Patrono, C., et al., *Polymorphisms in base excision repair genes: Breast cancer risk and individual radiosensitivity.* World journal of clinical oncology, 2014. 5(5): p. 874.
3. Shadrina, A.S., et al., *Polymorphisms in DNA repair genes and breast cancer risk in Russian population: a case–control study.* Clinical and experimental medicine, 2016. 16(1): p. 21-28.
4. AlMutairi, F., et al., *Association of DNA repair gene APE1 Asp148Glu polymorphism with breast cancer risk.* Disease markers, 2015. 2015: p. 1-11.
5. Sterpone, S. and R. Cozzi, *Influence of XRCC1 genetic polymorphisms on ionizing radiation-induced DNA damage and repair.* Journal of nucleic acids, 2010. 2010.
6. Zhang, Z., et al., *Genetic polymorphisms in XRCC1, APE1, ADPRT, XRCC2, and XRCC3 and risk of chronic benzene poisoning in a Chinese occupational population.* Cancer Epidemiology and Prevention Biomarkers, 2005. 14(11): p. 2614-2619.

7. Sambrook, J. and D. Russell, *Molecular cloning: a laboratory manual 3rd edition.* Cold Spring Harbor Laboratory press. New York, USA. pp. 2275.
8. Mohrenweiser, H.W. and I.M. Jones, *Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation?* Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 1998. 400(1-2): p. 15-24.
9. Pharoah, P.D., et al., *Polygenic susceptibility to breast cancer and implications for prevention.* Nature genetics, 2002. 31(1): p. 33-36.
10. Mahouri, K., M. Dehghani Zahedani, and S. Zare, *Breast cancer risk factors in south of Islamic Republic of Iran: a case-control study.* EMHJ-Eastern Mediterranean Health Journal, 13 (6), 1265-1273, 2007, 2007.
11. AL-Nuaimee, N.M.A., A.A. Muhammad, and N.K. Fakree, *A Study On The Effects Of Risk Factors On The Pathology And The Development Of Breast Cancer In Iraqi Women.* Organization (WHO), 2020. 10: p. 18.
12. Al-Kazazz, Z.K. and Z.N. Nabat, *Study on Breast Cancer Patients and Some Variables in Babylon Province.* Indian Journal of Forensic Medicine & Toxicology, 2020. 14(2):p.884-887.
13. Renehan, A.G., et al., *Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies.* The lancet, 2008. 371(9612): p. 569-578.
14. Oyamienlen, C.S., et al., *Body Mass Index and Breast Cancer Risks Among Igbo Women in Imo and Abia States, Nigeria: A Case Control Study.* International Journal of Translational Medical Research and Public Health, 2019. 3(1): p. 31-37.
15. Kzar, H.H., M.E. Al-Gazally, and M.A. Wtwt, *Association of Body Mass Index and Age with Positive Receptors Expression and Metastasis Status Subtypes in Iraqi Women with Breast Cancer.* International Journal of Psychosocial Rehabilitation, 2020. 24(01).
16. Ginsberg, G., et al., *Polymorphism in the DNA repair enzyme XRCC1: utility of current database and implications for human health risk assessment.* Mutation Research/Reviews in Mutation Research, 2011. 727(1-2): p. 1-15.
17. Feng, Y.-Z., et al., *Association between the XRCC1 Arg194Trp polymorphism and risk of cancer: evidence from 201 case–control studies.* Tumor Biology, 2014. 35(11): p. 10677-10697.
18. Takeshita, H., et al., *Worldwide Distribution of Four SNPs in X-Ray and Repair and Cross-Complementing Group 1 (XRCC1).* Clinical and Translational Science, 2015. 8(4): p. 347-350.
19. 19. Al Obaidy, L.A., *XRCC1 codon 194 polymorphism in Iraqi population.* Iraqi journal of biotechnology, 2017. 16(3).p: 194-199.
20. Al Mutairi, F.M., et al., *Association of XRCC1 gene polymorphisms with breast cancer susceptibility in Saudi patients.* Asian Pacific Journal of Cancer Prevention, 2013. 14(6): p. 3809-3813.
21. Singh, P.K., et al., *Association of damaging nsSNPs of XRCC1 with breast cancer.* Meta Gene, 2017. 14: p. 147-151.
22. Moghaddam, A.S., et al., *XRCC1 and OGG1 gene polymorphisms and breast cancer: a systematic review of literature.* Iranian journal of cancer prevention, 2016. 9(1).
23. Smith, T.R., et al., *Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer.* Cancer letters, 2003. 190(2): p. 183-190.

24. Abdel Ghafar, M.T., et al., *Impact of XRCC1 genetic variants on its tissue expression and breast cancer risk: A case–control study.* Environmental and Molecular Mutagenesis, 2021. 62(7): p. 399-408.
25. Duell, E.J., et al., *Polymorphisms in the DNA repair gene XRCC1 and breast cancer.* Cancer Epidemiology and Prevention Biomarkers, 2001. 10(3): p. 217-222.
26. Thompson, L.H., et al., *Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: a third repair gene on human chromosome 19.* Genomics, 1989. 5(4): p. 670-679.
27. Smeets, H., et al., *A long-range restriction map of the human chromosome 19q13 region: close physical linkage between CKMM and the ERCC1 and ERCC2 genes.* American journal of human genetics, 1990. 46(3): p. 492.
28. Wang, Z. and N. Wu, *Association between XRCC1 and ERCC2 gene polymorphisms and*
29. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938
30. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864