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# Osteoprotegerin and DNA repair gene (*OGG1* and *XRCC1*) in females with breast cancer

*Original Article*

Wafaa Raji Alfatlawi
Applied Chemistry Department, College of Applied Science, University of Technology, Iraq
ORCID: 0000-0002-7266-1233
*wafaa.r.mohammed@uotechnology.edu.iq*
*(corresponding author)*

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*Abstract:*

Breast cancer is one of the most common cancers globally and is the second leading cause of death among women. Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily. The *OGG1* gene encodes an 8-oxoguanine DNA glycosylase, a crucial enzyme in the base excision repair pathway that removes oxidative DNA damage to prevent mutations. The *XRCC1* gene encodes a scaffold protein essential for the repair of DNA damage. The aim of this study was to investigate the associations between breast cancer risk and OPG as a diagnostic marker in Iraqi females. Additionally, we investigated whether breast cancer risk is associated with four amino acid substitution variants in DNA repair genes, including *XRCC1* Arg194Trp and *OGG1* Ser326Cys, which are involved in nucleotide excision repair. One hundred thirty-two samples were collected from the Baghdad Teaching Hospital in Baghdad, Iraq. Results show significant differences in OPG levels with a *p*-value of ≤ 0.05. In the *OGG1*, the allele C/C showed a high odds ratio (OR=3.5) in newly diagnosed females with breast cancer. In the chemotherapy patient group, the allele G/G shows a high OR of 1.8. The *XRCC1* gene analysis revealed that allele A/G shows a high OR of 15.2 in newly diagnosed females with breast cancer, while in chemotherapy patients, the allele A/G shows a high OR of 2.79. Gene polymorphism Ser326Cys (rs1052133) is linked to increased risk of breast cancer. The *XRCC1* Arg399Gln polymorphism has been related in a significant way to increased breast cancer risk, particularly in newly diagnosed and chemotherapy-treated females.

*Key words:*
breast cancer, DNA repair, osteoprotegerin, *XRCC1*, *OGG1*

*Apstrakt:*

**Osteoprotegerin i geni za popravku DNK (*OGG1* i *XRCC1*) kod žena sa karcinomom dojke**

Karcinom dojke je jedan od najčešćih kancera globalno i predstavlja drugi vodeći uzrok smrti kod žena. Osteoprotegrin (OPG) je član superfamilije receptora tumor nekroznih faktora. Gen *OGG1* kodira 8-oksoguanin DNK glikozilazu, ključni enzim u procesu popravke putem isecanja baza, kojim se uklanjaju oksidativna oštećenja na DNK. Gen *XRCC1* kodira uvijen protein, koji je takođe ključan za popravku DNK oštećenja. Cilj ove studije bio je da se istraži povezanost između rizika za karcinom dojke i OPG-a kao dijagnostičkog markera kod Iračanki. Pored ovoga, istraživali smo i da li je rizik za karcinom dojke povezan sa četiri varijanti substitucije aminokiselina kod gena za popravku DNK, uključujući *XRCC1* Arg194Trp i *OGG1* Ser326Cys, koji imaju ulogu u popravci DNK isecanjem nukleotida. Sto trideset i dva uzorka sakupljena su u Edukativnoj bolnici u Bagdadu, Irak. Rezultati su pokazali značajne razlike u nivoima OPG sa *p*-vrednostima ≤ 0,05. Kod *OGG1*, alel C/C pokazao je veliki odnos šansi (OR=3,5) kod novodijagnostikovanih žena sa karcinomom dojke. U grupi pacijentkinja koje su primale hemoterapiju, alel G/G pokazao je visok OR od 1,8. Analiza gena *XRCC1* pokazala je da alel A/G pokazuje visok OR od 15,2 kod novodijagnostikovanih žena sa karcinomom dojke, dok je alel A/G imao OD od 2,79. Genski polimorfizam Ser326Cys (rs1052133) se povezuje sa povećanim rizikom od karcinoma dojke. Polimorfizam Arg399Gln *XRCC1* gena je značajno povezan sa povećanim rizikom od kancera dojke, posebno kod novodijagnostikovanih pacijentkinja i onih tretiranih hemoterapijom.

*Ključne reči:*
karcinom dojke, popravka DNK, osteoprotegerin, *XRCC1*, *OGG1*

## Introduction

Breast cancer is one of the most common cancers in women worldwide, accounting for approximately 570,000 deaths in 2015. Over 1.5 million women (25% of all women with cancer) are diagnosed with breast cancer every year throughout the world. The cancer rates have increased in Iraq in recent years. This might be explained by increasing life expectancy coupled with changing patterns of behavioral risk factors associated with higher non-communicable disease risk, such as tobacco and alcohol use, obesity, physical inactivity, and an unhealthy diet. This ratio is expected to increase across all cancer types (Salih et al., 2025).

Breast cancer is a metastatic cancer and can commonly transfer to distant organs such as the liver, bones, lungs, and brain, which mainly accounts for its incurability. Early diagnosis of the disease can lead to a good prognosis and a high survival rate (Siegel et al., 2018).

Osteoprotegerin (OPG) is a glycoprotein that belongs to the tumor necrosis factor receptor superfamily (TNFRSF11B) and forms homodimers to function as a secreted decoy receptor for ligands like RANKL and TRAIL. Its primary role is to regulate bone turnover by inhibiting osteoclast genesis and other biological processes, including immune responses, vascular health, and cancer progression (Wang et al., 2022). Elevated OPG levels have been linked to poorer outcomes in some cancers, such as pancreatic cancer and hepatocellular carcinoma, suggesting its value as a prognostic marker. Therapeutically, OPG can reduce tumor burden in bone metastasis models by inhibiting osteoclast activity, and its ability to block TRAIL offers a potential avenue for enhancing apoptosis in tumor cells (Geerts et al., 2020). Elfar et al. (2017) tested blood OPG and RANKL levels and found that serum OPG had a sensitivity of 59% and a specificity of 92% in identifying women with breast cancer, while the corresponding values for the RANKL/OPG ratio were 73% and 72%, respectively. OPG modulates mammary epithelial cell proliferation. Thus, the development of breast cancer is affected by abnormalities in the RANK–RANKL pathway, and the progression of breast cancer is influenced by RANK–RANKL expression, which is directly linked to the survival and proliferation of mammary epithelial cells (Wu et al., 2020). Chemotherapy drugs are designed to create free radicals within tumor cells, causing lethal damage to DNA and other crucial cellular components, leading to the death of the cell and cancer growth inhibition. The resulting oxidative stress can also lead to serious side effects related to chemotherapy (Tas et al., 2005). Numerous studies have investigated the role of vitamin D in reducing the risk of breast cancer (Nsaif, 2019; Nechuta et al., 2011). Vitamin D and calcium are metabolically highly correlated dietary factors that exhibit anticarcinogenic effects by regulating cell proliferation, differentiation, and apoptosis in both normal and malignant breast cells (Momivand et al., 2024). *BRCA1/2* mutations significantly increase breast cancer risk by impairing DNA repair, while variants in *XRCC1* and *OGG1*, which are also involved in DNA repair, can modify this risk. *XRCC1* acts as a scaffold protein in base excision repair (BER), while *OGG1* repairs oxidative DNA damage and coordinates other repair enzymes like DNA polymerase β and DNA ligase III. Mutations in *OGG1* and *XRCC1* do not cause hereditary breast cancer like *BRCA1/2*, but polymorphisms within these genes may affect cancer susceptibility (Barili et al., 2024). Vitamin D 1,25(OH)2D, the active form of vitamin D, exerts its effects mainly through binding to the nuclear vitamin D receptor (VDR) and then binding to specific DNA sequences and vitamin D response elements. Through this genomic pathway, 1,25(OH)2D regulates the expression of specific genes in a tissue-specific manner. Many studies have shown that 1,25(OH) 2D can inhibit cellular proliferation and angiogenesis, while inducing differentiation and apoptosis in both normal and malignant breast cells (Dubey & Sharma, 2025). The aim of this study was to investigate the associations between breast cancer risk and the biochemical parameter OPG as a diagnostic marker in Iraqi females with breast cancer and other parameters. Patients were classified into subgroups based on prediagnosis and chemotherapy status, and the results were compared with those of the control group. Also, we tested whether breast cancer risk is associated with four amino acid substitution variants in DNA repair genes, including *XRCC1* Arg194Trp and *OGG1* Ser326Cys, which are involved in nucleotide excision repair.

## Materials and Methods

### *Study design*

The current case-control study includes a total of 132 females with an age range of 25-45 years. The samples were classified into three groups: G1 as the control group, G2 as newly diagnosed with breast cancer, and G3 as women who were given the first and/or second doses of chemotherapy, as shown in **Fig. 1.** flow diagram. All females received medical care at Medical City, Oncology Teaching Hospital, Department of Early Diagnosis of Breast Cancer, Baghdad, Iraq. The sample collection period was from January to April 2024.

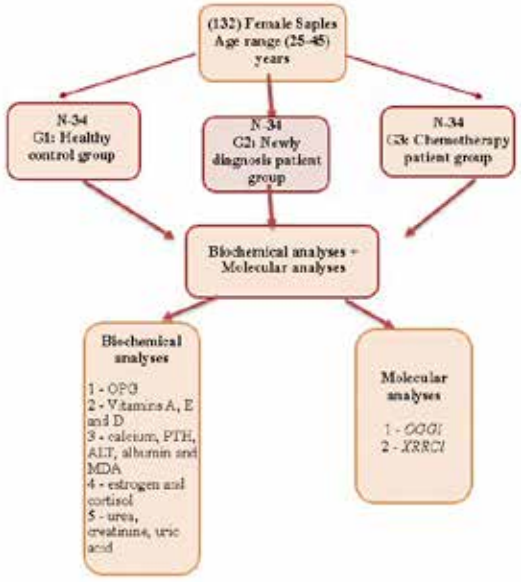


**Fig. 1.** Flow diagram of the study

**Inclusion criteria:** The inclusion criteria for patients were newly diagnosed females with breast cancer, and females who received the first or second dose of chemotherapy, aged between 25 and 45 years old, and before menopause.

**Exclusion criteria:** The exclusion criteria for patients were females at menopause and any cases with chronic inflammatory diseases.

**Sample collection:** After collecting 10 mL of whole blood, 5 mL of the blood were allowed to clot by leaving the sample at room temperature. This procedure usually takes 10-20 minutes. After removing the clot by centrifuging at 2,000-3,000 rpm for 20 minutes, the biochemical assay was performed. The second part (5 mL) was placed into an EDTA tube for molecular testing; the resulting sera and blood were stored at -20 °C until the assay.

### *Methods*

**Biochemical analyses:** Osteoprotegerin was measured using the OPG kit (Catalogue Number: SL1314Hu). The ELIZA method involves pre-coating the microplate with an OPG-specific antibody, adding serum samples to the appropriate microplate wells, and combining them with the specific antibody. This is followed by the addition of horseradish peroxidase-conjugated antibody and, finally, the addition of TMB substrate to stop the reaction.

**Vitamins:** The kits used to measure vitamins A, E, and D were from Human Sunlong/China Cat. No.: SL1833Hu, Sunlong/China Cat. No.: SL2328Hu, and Sunlong/China Cat. No.: SL1834Hu, respectively. The enzymatic method was used to evaluate calcium, PTH, ALT, albumin, urea, creatinine, and MDA. These methods for analyzing these parameters offer improved specificity and accuracy compared to traditional chemical methods.

**Molecular analyses:**

***PCR-RFLP Genotyping Assays*:** Genomic DNA was isolated from 200 µL of whole blood, using the WizPrep™ gDNA Mini Kit (Blood) (Republic of Korea). A qualitative PCR protocol was used to detect the presence of *OGG1* and *XRCC1* genes.

PCR-RFLP involves digesting PCR products with a restriction enzyme, with the conditions depending on the specific enzyme used and requiring a specific number of enzyme units per amount of DNA. A typical protocol includes incubating the PCR product with the enzyme and its corresponding buffer at the enzyme's optimal temperature for a specified time, often between 30 minutes and 3 hours. The amount of enzyme used is critical and is expressed in units, where one unit is the amount of enzyme required to digest a specific amount of DNA.

**Preparation of Primers:** The primers used to identify and amplify the *OGG1* (Ser326Cys) and *XRCC1* (Arg399Gln) polymorphisms by the PCR-RFLP method, as described by Berg et al. (2012), were prepared by Integrated DNA Technologies (IDT) in Canada. Using primers from Alpha DNA USA, a 200 bp fragment of the *hOGG1* gene and a 248 bp fragment of the *XRCC1* gene were amplified using a specific primer. The PCR materials for the *hOGG1* and *XRCC1* genes are shown in **Tab.1**.

**Table 1.** Sequences of primers used in this study

| Target gene | Sequence of primers | Allele and size of PCR products (bp) | GC (%) | Tm (°C) |
|---|---|---|---|---|
| *OGG1* Ser326 Cys | F1: 5'-GGA AGG TGC TTG GGG AAT-3' | For the C allele (200 bp) | 55 | 62 |
| | R1: 5'-ACT GTC ACT AGT CTC ACC AG-3' | | 55 | 62 |
| *XRCC1* Arg399 Gln | F1: 5'-CAA GTA CAG CCA GGT CCT AG-3' | For the G allele (248bp) | 55 | 56 |
| | R1: 5'-CCT TCC CTC ATC TGG AGT AC-3' | | 50 | 60 |

**PCR-RFLP and genotyping:**

**A) rs1052133/*OGG1* Gene:** Simple PCR-RFLP was used to detect the Ser326Cys variant. The primers used for amplification of the *OGG1* gene exon 7 are A-OGG1 forward: 5'-GGA AGG TGC TTG GGG AAT-3 and B-OGG1 reverse: 5'-ACT GTC ACT AGT CTC ACC AG-3'. Consequently, the ser/ser (homozygote), ser/cys (heterozygote), and cys/cys genotypes result in 200 bp, 200 and 100 bp, and 100 bp digestion fragments, respectively. Restriction fragment length polymorphism (RFLP) is a technique that cuts a DNA sequence using restriction enzymes (such as Fnu4H1) into specific pieces. **Fig. (2-6)** represent the electrophoresis patterns in the studied groups of the *OGG1* and *XRCC1* genes.

**B) *XRCC1* Gene:** The distribution of genotyping of codon 399 of *XRCC1* following primers is designed to encompass the Arg399Gln polymorphism site. A- *XRCC1* forward: 5'-CAA GTA CAG CCA GGT CCTAG-3 B- *XRCC1* reverse: 5'-CCT TCC CTC ATC TCG AGT AC-3: Consequently, the Arg/Arg (homozygote), Arg/Gln (heterozygote), and Gln/Gln genotypes result in 89 and 159 bp; 89, 159, and 248 bp; and 248 bp digestion, respectively. RFLP cuts a DNA sequence into pieces by using restriction enzymes NcilI; the action of this enzyme is specific.

**Statistical analysis:** Version 26 of SPSS was utilized for data analysis. Mean ± standard deviation (SD) was used to illustrate the results. The data were compared using independent-sample t-tests. Correlation was tested using the Spearman rank correlation test. Significance was set at $p<0.05$. The demographic characteristics of the cases and controls were compared using Fisher's exact tests, Student's t-tests, and $X^2$ tests. After adjusting for subject characteristics, ORs and 95% CIs were computed using logistic regression. Each two-way interaction between genotypes was taken into consideration. The model was eliminated of non-significant interactions by applying a backward-stepping algorithm. The

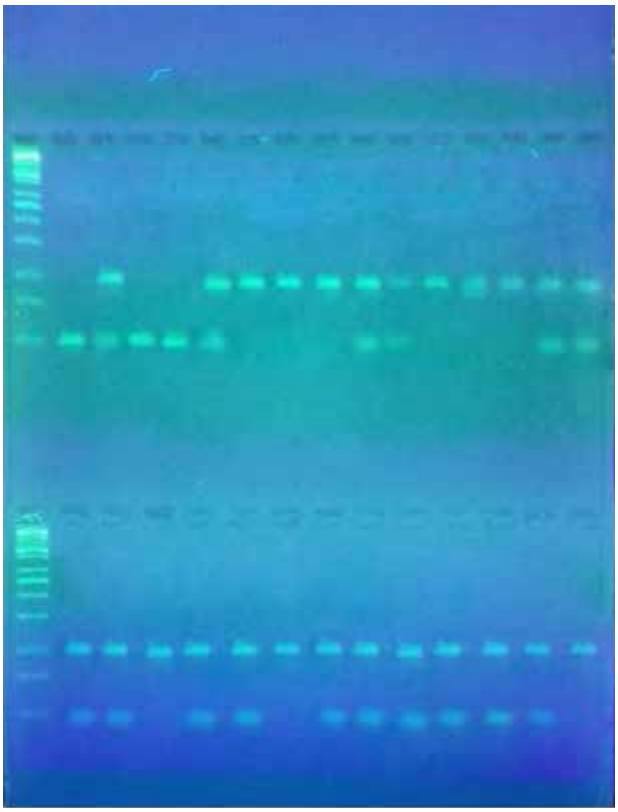

**Fig. 2.** Electrophoresis pattern of 200 bp PCR product of healthy females, digested with Fnu4HI restriction enzyme (2.5% agarose gel). Lanes 1, 3, 4 homozygous: GG genotype (100 bp); Lanes 6, 7, 8, 11, 12, 13, 18, 21, 28 homozygous: CC genotype (200 bp). Lanes 2, 5, 9, 10, 14, 15, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27 heterozygous: GC genotype (100/200 bp). M: DNA molecular marker 100 bp in size. Bands in the gel were stained with red stain.

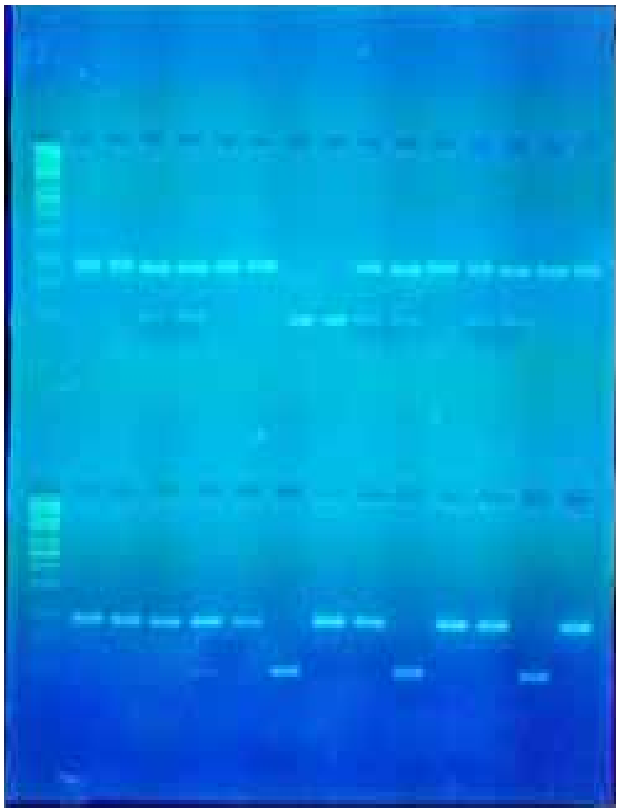

**Fig. 3.** Electrophoresis pattern of 200 bp PCR product of newly diagnosed females, digested with Fnu4HI restriction enzyme (2.5% agarose gel). Lanes 7, 8, 21, 24, 27 homozygous: GG genotype (100 bp); Lanes 1, 2, 5, 6, 11, 14, 16, 17, 18, 20, 22, 23, 25, 26, 28 homozygous: CC genotype (200 bp). Lanes 3, 4, 9, 10, 12, 13, 19 heterozygous: GC genotype (100/200 bp). M: DNA molecular marker 100 bp in size. Bands in the gel were stained with red stain.

online tool https://www.easycalculation.com/health/hardy-weinberg-equilibrium-calculator.Php was used to determine the variance of various genotypes in accordance with the Hardy-Weinberg Equilibrium (HWE) principle.

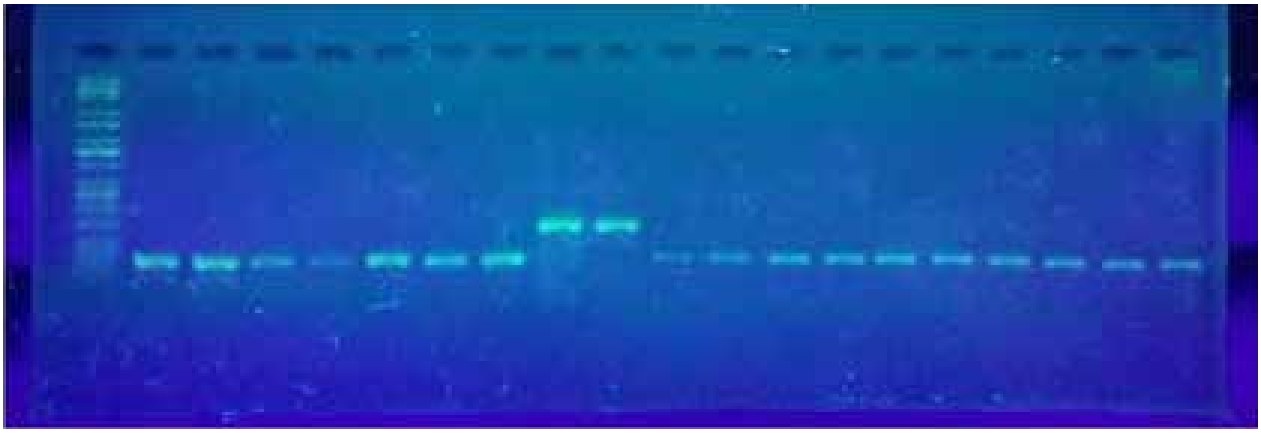

**Fig. 4.** Electrophoresis pattern of 200 bp PCR product of chemotherapy-treated females, digested with Fnu4HI restriction enzyme (2.5% agarose gel): Lanes 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 homozygous: GG genotype (100 bp); Lanes 8 and 9 homozygous: CC genotype (200 bp). M: DNA molecular marker 100 bp in size. Bands in the gel were stained with red stain.

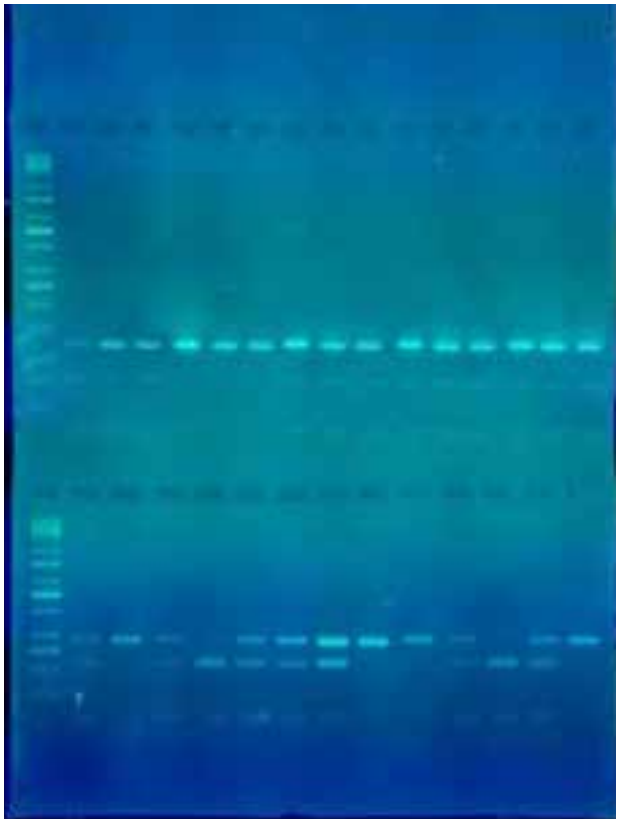

**Fig. 5.** Electrophoresis pattern of 248 bp PCR products of newly diagnosed females digested with NciI restriction enzyme (2.5% agarose gel). Lanes 1-3, 7-16, 18, 20-22, 25, 27 heterozygous: AG genotype (248/159/89 bp); Lanes 19 and 26 homozygous: GG genotype (159/89 bp). Lanes 4, 5, 6, 17, 23, 24, 28 homozygous: AA genotype (248 bp). M: DNA molecular marker 100 bp in size. Bands in the gel were stained with red stain.

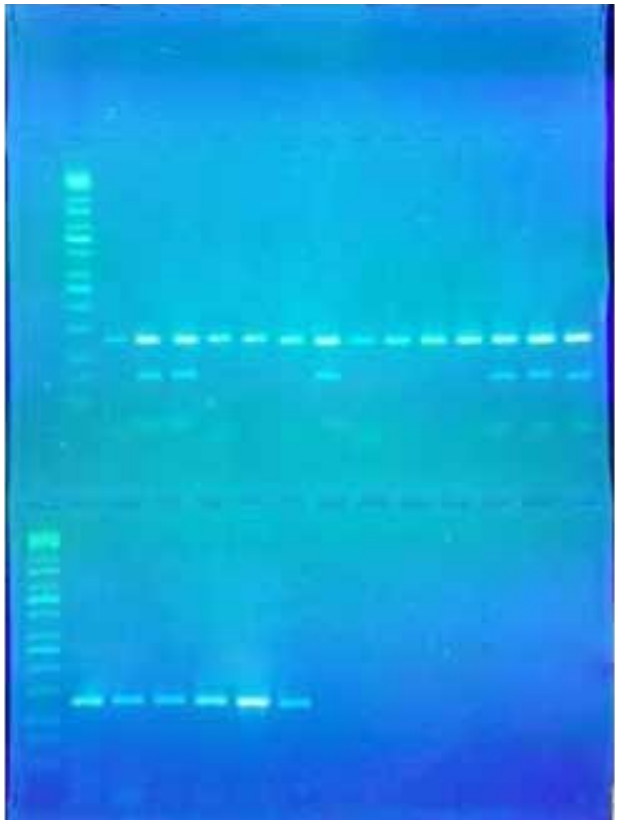

**Fig. 6.** Electrophoresis pattern of 248 bp PCR product (chemotherapy) digested with NciI restriction enzyme (2.5% agarose gel). Lanes 2, 3, 7, 12, 13, 14 heterozygous: AG genotype (248/159/89 bp); Lane zero homozygous: GG genotype (159/89 bp). Lanes 1, 4, 5, 6, 8, 9, 10, 11, 15, 16, 17, 18, 19, 20 homozygous: AA genotype (248 bp). M: DNA molecular marker 100 bp in size. Bands in the gel were stained with red stain.

## Results

The Body Mass Index and age in **Tab. 2** show that there was no significant difference between the patient groups and the control group ($p>0.05$). **Tab. 3** shows that all parameters except calcium, OPG, ALP, estrogen and albumin had significant differences between the groups, especially for vitamin E and D.

***OGG1* polymorphisms in newly diagnosed patients: Tab. 4** presents the frequency distribution of *OGG1* gene polymorphisms in the newly diagnosed group compared to the healthy control. The allele C/C (homozygous) genotype was more likely to have a risk of breast cancer ($p<0.05$), showing an odds ratio (OR) of 3.5-fold higher risk (95% CI = 1.22–10.01) when compared with the control group. The allele C/G was associated with a low level of breast cancer risk, with a $p<0.05$, an odds ratio (OR) of 0.2, indicating a 0.2-fold higher risk compared to the control group. In contrast, the allele G/G had an OR of 1.8 with a non-significant $p$-value ($p>0.05$). The genotype distribution in the *OGG1* gene in the healthy group (A) and the newly diagnosed group (B) is shown in **Fig. 7**.

***OGG1* polymorphisms in the chemotherapy group:** The frequency distribution of *OGG1* gene polymorphism of the chemotherapy group compared with the healthy control is represented in **Tab. 5**. The allele C/C was more common among chemotherapy patients, with a significantly higher risk (OR=3.5, $p<0.05$) compared with the control

**Table 2.** Age and BMI in the studied groups

| Parameters | Group Mean ± SD | | | *p*-value | | |
|---|---|---|---|---|---|---|
| | (G1) N=34 | (G2) N=34 | (G3) N=34 | G1 & G2 | G1 & G3 | G2 & G3 |
| **Age, years** | 38.0 ± 4.38 | 44.04 ± 3.18 | 37.04 ± 3.38 | 0.20 | 0.36 | 0.12 |
| **BMI, kg/m²** | 26.04 ± 3.38 | 26.80 ± 7.68 | 28.14 ± 6.43 | 0.86 | 0.33 | 0.63 |

*p* - significant at $p\leq0.05$, highly significant at $p<0.001$, Non-significant at $p>0.05$
G1: Control group, G2: Newly diagnosed, G3: Chemotherapy group

**Table 3.** Biochemical parameter levels in the studied groups

| Parameters | Group Mean ± SD | | | *p*-value | | |
|---|---|---|---|---|---|---|
| | (G1) N=34 | (G2) N=34 | (G3) N=34 | G1 & G2 | G1 & G3 | G2 & G3 |
| **Vitamin A (µg\dL)** | 57.9±16.5 | 62.6 ±15.8 | 58.2 ±18.1 | **0.044** | 0.33 | **0.045** |
| **Vitamin E (µg\dL)** | 484.1 ±190.2 | 385.9 ±195.6 | 587.3 ±256.6 | **0.016** | **0.019** | **0.01** |
| **Vitamin D (ng\dL)** | 20.04±2.80 | 4.98 ± 1.67 | 9.38 ± 1.43 | **0.001** | **0.001** | **0.001** |
| **Calcium (mg\dL)** | 9.28 ± 0.43 | 9.23 ± 0.38 | 9.21 ± 0.45 | 0.866 | 0.780 | 0.986 |
| **OPG (pg\mL)** | 324.11 ± 104.73 | 315.0 ± 123.98 | 313.38 ± 109.02 | 0.941 | 0.919 | 0.998 |
| **Urea (mg\dL)** | 27.5 ±8.4 | 38.3 ±9.6 | 27.7 ±6.6 | **0.011** | 0.32 | **0.02** |
| **Creatinine (mg\dL)** | 0.7 ±0.2 | 1.0 ±0.2 | 0.7 ±0.2 | **0.04** | **0.04** | **0.04** |
| **ALP (U\L)** | 100.39 ± 20.76 | 107.70 ± 31.09 | 105.09 ± 22.97 | 0.462 | 0.725 | 0.906 |
| **Cortisol (nmol\L)** | 265.94 ± 81.82 | 294.89 ± 99.06 | 345.85 ± 97.66 | 0.409 | **0.002** | 0.067 |
| **Estrogen (pg\mL)** | 87.93 ± 37.01 | 75.60 ± 24.99 | 76.74 ± 21.87 | 0.185 | 0.247 | 0.985 |
| **PTH (pg\mL)** | 68.52 ± 20.44 | 167.79 ± 35.21 | 136.52 ± 58.56 | **0.001** | **0.001** | 0.779 |
| **Uric acid (mg\dL)** | 3.9± 0.8 | 4.8 ±1.3 | 5.0 ±1.1 | 0.19 | **0.013** | 0.18 |
| **Albumin (g\dL)** | 4.8±0.25 | 4.7 ±1.2 | 4.5 ±0.95 | 0.13 | 0.09 | 0.17 |
| **MDA (mg\dL)** | 6.4 ±4.3 | 4.5 ±3.2 | 3.0 ±2.3 | 0.1 | **0.01** | 0.15 |

Bolded: significant at $p\leq0.05$, highly significant at $p<0.001$, Non-significant at $p>0.05$
G1: Control group, G2: Newly diagnosed, G3: Chemotherapy group

**Table 4.** Genotype distribution of the *OGG1* gene C/C, C/G, and G/G polymorphism in newly diagnosed and healthy control groups

| Polymorphism in *OGG1* | Groups | | | | *p*-value | % | $X^2$ | OR | CI 95% |
|---|---|---|---|---|---|---|---|---|---|
| | G1 N=34 | | G2 N=34 | | | | | | |
| | Observed | Expected | Observed | Expected | | | | | |
| **CC** | 13 | 10.8 | 18 | 15.4 | $p<0.05$ | 51.3 | 5.5 | 3.5 | 1.22-10.01 |
| **CG** | 18 | 14.4 | 11 | 12.2 | $p<0.001$ | 40.7 | 8.3 | 0.2 | 0.07-0.61 |
| **GG** | 3 | 4.8 | 5 | 2.4 | $p>0.05$ | 8 | 0.5 | 1.8 | 0.4-8.11 |

G1: Control group, G2: Newly diagnosed patients group, OR: Odds Ratio, *p*-value: Probability considered significant at level $p<0.05$, CI: Confidence Interval

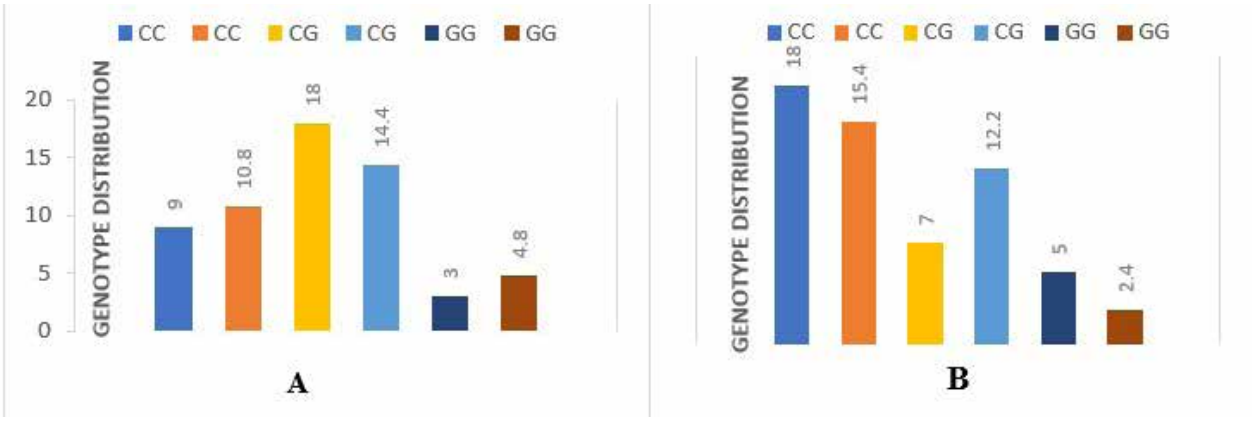


**Fig. 7.** Genotype distribution in the *OGG1* gene in the healthy group (**A**) and the newly diagnosed group (**B**)

group. On the other hand, a significant difference was also observed in allele C/G ($p<0.05$), but with a low OR of 0.2. A significant association was also observed for the C/G genotype ($p<0.05$), but with a low odds ratio (OR=0.2), indicating a reduced risk of breast cancer. The G/G genotype showed a higher risk (OR=1.8); however, this association was not statistically significant ($p>0.05$). The distribution of *OGG1* gene genotypes in the control group (A) and the chemotherapy-treated group (B) is presented in **Fig. 8**.

**Amplification of the *XRCC1* gene and polymorphisms:** A specific region of the genome was amplified by using PCR. The gel electrophoresis technique is used to visualize the amplified fragment, which is 248 bp in size. A 100 bp DNA ladder was used as a molecular weight marker, and *XRCC1* polymorphisms were genotyped using a PCR method and primers. The forward primer 5'-CAA GTA CAG CCA GGT CCT AG-3', and the reverse primer 5'-CCT TCC CTC ATC TGG AGT AC-3', were used to amplify the genomic DNA, and this process was followed by restriction enzyme digestion. A single band of 248 bp indicated the AA (homozygote), two bands of 89/159 indicated the GG (homozygote), and three bands of 248/159/89

**Table 5.** Genotype distribution of the *OGG1* gene C/C, C/G, and G/G polymorphism in healthy controls and chemotherapy group

| Polymorphism in *OGG1* | Groups | | | | *p*-value | % | $X^2$ | OR | CI 95% |
|---|---|---|---|---|---|---|---|---|---|
| | G2 N=34 | | G1 N=34 | | | | | | |
| | Observed | Expected | Observed | Expected | | | | | |
| **CC** | 9 | 10.8 | 11 | 0.2 | $p<0.05$ | 1 | 2.8 | 3.5 | 1.22-10.01 |
| **CG** | 18 | 14.4 | 5 | 3.6 | $p<0.001$ | 18 | 18.8 | 0.2 | 0.07-0.61 |
| **GG** | 7 | 4.8 | 18 | 16.2 | $p>0.05$ | 81 | 31.5 | 1.8 | 0.4-8.11 |

G1: Control group, G2: Chemotherapy patient group, OR: Odds Ratio, *p*-value: Probability considered significant at level $p<0.05$, CI: Confidence Interval

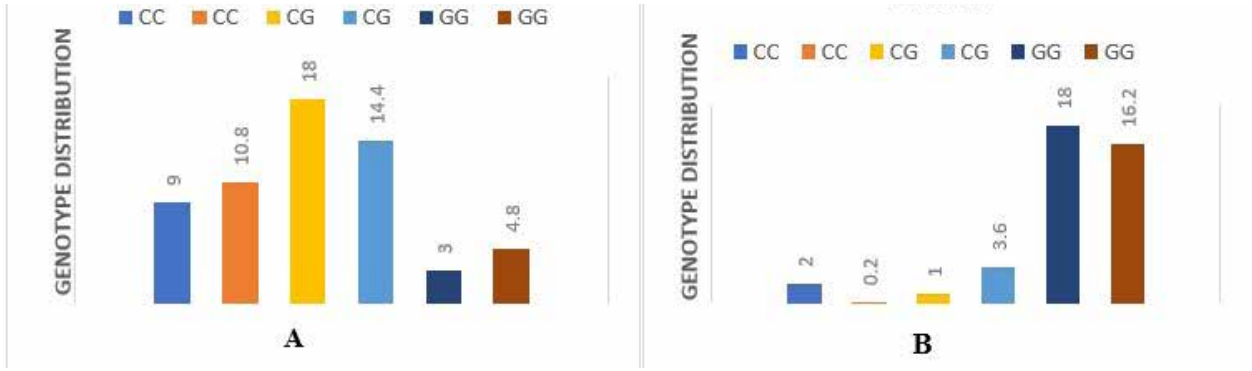


**Fig. 8.** Genotype distribution in the *OGG1* gene in the control group (**A**) and in the chemotherapy patient group (**B**)

bp indicated the AG genotype (heterozygote).

The frequency distribution of *XRCC1* polymorphisms in newly diagnosed female patients compared to a healthy control is represented in **Tab. 6**. A higher risk for breast cancer was observed in the A/G allele, with a highly significant $p \leq 0.01$ when compared with the control group. The A/A genotype showed a significant difference ($p \leq 0.01$) but was linked to a lower risk (OR = 0.11), while the G/G genotype showed no significant association ($p \geq 0.05$, OR = 0.5, 95% CI). The genotype distribution in the *XRCC1* gene for the control (A) and newly diagnosed patient groups (B) is shown in **Fig. 9**.

***XRCC1* polymorphisms in the chemotherapy group: Tab. 7** presents the frequency distribution of *XRCC1* gene polymorphisms in the chemotherapy group compared to the healthy control group. The heterozygous genotype A/G showed a higher breast cancer risk with an OR of 2.79, while for A/A and G/G, they were non-significant with 0.85 and 0.14, respectively. When compared with the control group, the $p \geq 0.05$ in all three genotypes, indicating no statistically significant difference. The genotype distribution in the *XRCC1* gene in the control group (A) and chemotherapy group (B) is shown in **Fig. 10**.

## Discussion

**Biochemical parameters:** The potential role of vitamins A, E, and D in preventing and treating tumors is a subject of growing interest, supported by a combination of preclinical and clinical studies, as well as safety considerations. Vitamins play complex roles in cancer prevention, antioxidant resistance, inflammatory modulation, and epigenetic regulation, which can enhance outcomes by affecting cell differentiation, apoptosis, and proliferation, as well as combating oxidative stress and DNA damage (Talib et al., 2024). Patients who had breast cancer are more likely to have uremia than the control group. Increased migration and invasion of breast carcinoma cells are key events in the development of metastasis to lymph nodes and distant organs, which can lead to an elevated level of urea in the blood (Yang et al., 2025). Increased MDA concentration has been reported in breast cancer, gastric and lung cancer, and several studies present evidence that ROS are involved in the progression and etiology of this type of cancer (Gubaljevic et al., 2018).

**OPG in breast cancer:** According to the study in the European Prospective Investigation for Cancer and Nutrition (EPIC)(Sarink et al., 2017), elevated serum OPG is linked to higher overall and specific mortality rates for breast cancer. Its action promoted cell

**Table 6.** *XRCC1* gene A/A, A/G, and G/G polymorhism genotype distribution in the newly diagnosed brest cancer patients and healthy controls

| Polymorphism in *XRCC1* | Groups | | | | *p*-value | % | $X^2$ | OR | CI 95% |
|---|---|---|---|---|---|---|---|---|---|
| | G1 N=34 | | G2 N=34 | | | | | | |
| | Observed | Expected | Observed | Expected | | | | | |
| **AA** | 22 | 20.8 | 7 | 10.2 | $p<0.001$ | 34 | 15.0 | 0.11 | 0.04-0.35 |
| **AG** | 4 | 8.3 | 21 | 14.6 | $p<0.001$ | 48.7 | 19.8 | 15.2 | 4.18-55.02 |
| **GG** | 8 | 0.9 | 6 | 5.2 | $p>0.05$ | 17.3 | 0.7 | 0.5 | 0.08-2.67 |

G1: Control group, G2: Newly diagnosed patients group, OR: Odds Ratio, *p*-value: Probability considered significant at level $p<0.05$, CI: Confidence Interval

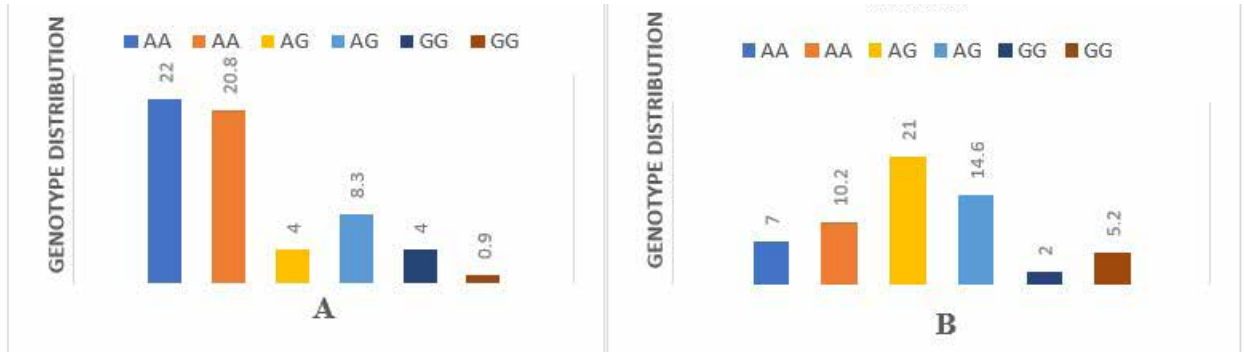


**Fig. 9.** Genotype distribution in the *XRCC1* gene in control group (**A**) and newly diagnosed patient group (**B**)

apoptosis and extended the survival of inflammatory cells by interacting with the endoplasmic reticulum (ER) chaperone and master regulator of the unfolded protein response, glucose-regulated protein/binding immunoglobulin protein (GRP78/BiP). In orthotopic breast cancer xenografts, recombinant OPG (rOPG) inhibited tumor development and metastasis by reducing the activity of the β-catenin pathway, thereby limiting breast cancer stemness (Liu et al., 2015). Additionally, studies have shown that OPG may have indirect tumor-promoting effects on the development of breast cancer in addition to its direct proliferative effects on breast cancer cells, and in breast cancer tumorigenesis, one hypothesis implies that OPG's pro-angiogenic activities encourage the growth of tumors. Another hypothesis suggests that OPG causes proliferation and aneuploidy in normal mammary epithelial cells, thereby reprogramming them into a tumorigenic state and accelerating cancer development (Infante et al., 2019). The mechanism behind the association between BMI and breast cancer risk is controversial; the positive association between BMI and breast cancer risk was speculated to result from the higher level of estrogen derived from the aromatization of androstenedione within the larger fat reserves of women of higher BMI. The negative relationship between higher BMI and breast cancer risk may come from a protective effect of increased weight in the early premenopausal years, which is a predictor of longer anovulatory cycles and a lower level of progesterone and estrogen (Radhi et al., 2025) .

***OGG1* polymorphisms in the newly diagnosed patients group:** According to studies done by

**Table 7.** Genotype distribution of the *XRCC1* gene A/A, A/G, and G/G polymorphism in healthy controls and female breast cancer chemotherapy groups

| Polymorphism in *XRCC1* | Groups | | | | *p*-value | % | $X^2$ | OR | CI 95% |
|---|---|---|---|---|---|---|---|---|---|
| | G1 N=34 | | G2 N=34 | | | | | | |
| | Observed | Expected | Observed | Expected | | | | | |
| **AA** | 22 | 20.8 | 14 | 14.5 | *p*>0.05 | 72.5 | 0.07 | 0.85 | 0.25-2.89 |
| **AG** | 8 | 8.3 | 12 | 5 | *p*>0.05 | 25 | 2.1 | 2.79 | 0.69-11.22 |
| **GG** | 4 | 0.9 | 8 | 0.5 | *p*>0.05 | 2.5 | 2.9 | 0.14 | 0.01-2.63 |

G1: Control group, G2: Chemotherapy patients group, OR: Odds Ratio, *p*-value: Probability considered significant at level *p*<0.05, CI: Confidence Interval

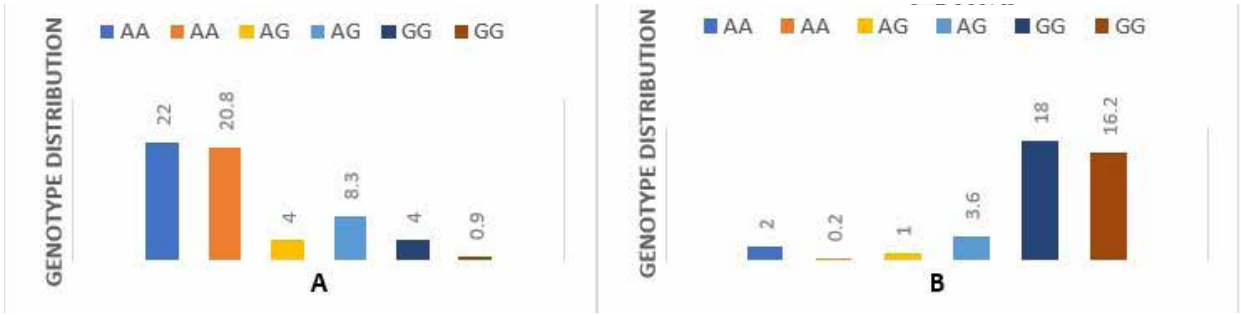


**Fig. 10.** Genotype distribution in the *XRCC1* gene in the control group (**A**) and chemotherapy group (**B**)

Sharma et al. (2020) and Huang & Zhou (2021), many DNA repair genes have different alleles, some of which may alter DNA repairing capacity; clarifying the etiology of cancer may be facilitated by the characterization of these genotypic differences in DNA repair functions and their association with cancer. Our results imply that variations in amino acid substitution of DNA repair genes in two repair pathways, especially when combined, may increase the risk of breast cancer and also revealed that female patients with an allele G/G (homozygous) genotype with a risk factor of 4% were more likely to have a disease compared to patients who carry allele A/A (homozygous) genotype and allele A/G (heterozygous) genotype. The Arg399Gln mutation is located in a hydrophobic region of the *XRCC1* gene, which resides between the domains that interact with ADP-ribosyltransferase and DNA polymerase. The functional significance of the Arg194Trp region remains unclear, and further research is needed to elucidate its role in DNA strand break repair and common oxidative damage. Current data indicate that genetic variants in *XRCC1* Arg399Gln correlate with breast cancer risk, aligning with the findings of Ruan et al. (2025). Nonetheless, the *XRCC1* Arg194Trp variant was not associated with breast cancer risk in an alternative investigation by Zoubi et al. (2015). In this study, the OR of 81.0 could be explained by genetic instability and impaired DNA repair, which significantly increase an individual's susceptibility to cancer due to the accumulation of mutations in critical genes.

**Association between the risk of breast cancer and rs1052133 single-nucleotide polymorphisms:** Three genetic variants in *OGG1* (Ser326Cys, 7143A/G, and 11657A/G) were linked to the incidence of breast cancer, as shown by Rossner and colleagues (Rossner et al., 2006). It is yet unknown how oxidative stress affects the risk of breast cancer. *OGG1*, which catalyzes the removal of DNA's 8-oxodeoxyguanosine, the most prevalent lesion resulting from oxidative stress, 8-oxodeoxyguanosine, is highly mutagenic. Our aim was to study the association between the polymorphism in the *OGG1* gene (SNP: rs1052133 (Ser326Cys)) on exon 7 and genetic variability in *OGG1*. A variety of human malignancies commonly lose the *OGG1* genetic locus (3p26.2); *OGG1* mutations, on the other hand, indicate that the observed disease development may be due to factors other than the gene's role in carcinogenesis in the 3p26 chromosome region (Ali et al., 2015). Different gene sequence mutations can have either no effect or limit the activity of the gene, and this disorder may arise from the majority of these changes that impact gene activity. The mechanism of protecting the genome's integrity, resulting in minimizing the quantity of mutations caused by carcinogens, involves the cellular DNA repair process, which stabilizes the genome (Clarke & Mostoslavsky, 2022). Our findings concurred with the results of Yuan et al. (2010), who found that the *hOGG1* Ser326Cys polymorphism is associated with a high risk of breast cancer in a meta-analysis study. According to a study of Wang et al. (2012), women who carry a variant allele of *hOGG1* Ser326Cys are likely to be at a higher risk of developing breast cancer. Other research has shown that the genotype distribution of the *hOGG1* Ser326Cys polymorphism may not have a significant impact on breast cancer incidence (Cai et al., 2006).

**Association between the risk of breast cancer and *XRCC1* single nucleotide polymorphisms:** *XRCC1* is believed to be involved in the multistep base excision repair pathway, which eliminates "non-bulky" base adducts created by methylation, oxidation, reduction, or fragmentation of bases by ionizing radiation or oxidative damage, according to several studies on the gene's association with breast cancer risk (Hindi et al., 2021; Datkhile et al., 2023).

The distributions of *XRCC1* genotypes may affect an individual's vulnerability to breast cancer, as demonstrated by the study of Filip et al. (2025), who investigated an association between DNA repair and breast cancer histopathological subtypes. Other research has demonstrated that the *XRCC1* gene polymorphism's allele frequency distribution may not significantly affect American white women's chance of developing breast cancer (Falagan-Lotsch et al., 2009).

## Conclusion

Osteoprotegerin has important roles in breast cancer by promoting tumor growth and spread, but also in the prevention of bone breakdown from metastases. Low concentrations of vitamin D are associated with an enhanced risk of breast cancer by affecting cell growth and estrogen release. The *OGG1* gene plays a crucial role in repairing DNA damage, and its polymorphism, Ser326Cys (rs1052133), is associated with an increased risk of breast cancer due to the decreased efficiency of the *OGG1* protein and impaired DNA repair activity. The *XRCC1* Arg399Gln polymorphism has been related in a significant way with increased breast cancer risk, particularly in newly diagnosed and chemotherapy-treated females, as it affects DNA repair function, leading to genomic instability and cancer development. In our study, BMI did not show a significant difference between the patient and control groups, although it affected insulin levels and estrogen production.

**Ethical approval:** The Applied Chemistry Department, College of Applied Sciences, University of Technology, Iraq, approved this study.

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