Impact of DNA Repair Gene Polymorphisms (XPD and XRCC1) on the Risk of Breast Cancer in Egyptian Female Patients

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ABSTRACT

The genes involved in DNA repair system play a crucial role in the protection against mutations .It has been hypothesized that functional deficiencies in highly conserved DNA repair processes resulting from polymorphic variation may increase genetic susceptibility to breast cancer. There are multiple pathways to repair the different types of DNA damage and maintain genomic integrity among them is the nucleotide excision repair (NER) and the base excision repair (BER) pathways. ***Aim & methods:*** *The aim of the present study was to examine the relation between the DNA repair gene polymorphisms and breast cancer (BC) risk in Egyptian females and to analyze their relation to clinico-pathological parameters of BC and also to investigate the synergistic effect of both genes on BC susceptibility . Both XPD and XRCC1 polymorphisms were characterized in 100 BC Egyptian females and 100 healthy women who had no history of any malignancy by amplification refractory mutation system -polymerase chain reaction (PCR) (ARMS) method and PCR with confronting two-pair primers(PCR–CTPP) , using DNA from peripheral blood in a case control study.* ***RESULTS****: our results revealed that the frequencies of AA genotype of XPD codon 312 polymorphism were significantly higher in the breast cancer study patients than in the normal individuals($p \leq 0.003$), and did not observe any association between the XRCC1 Arg399Gln polymorphism and risk of developing breast cancer Also, no association between both XPD Asp312Asn and XRCC1 A399G polymorphisms and the clinical characteristics of disease Finally ,the combination of AA(XPD)+AG(XRCC1) were significantly associated with breast cancer risk.* ***In conclusion****, the present results suggest that, XPD gene is an important candidate gene for susceptibility to breast cancer. Also, gene–gene interaction between XPD(AA)+XRCC1(AG) polymorphism may be associated with increased risk of breast cancer in Egyptian women.*

Keywords: *nucleotide excision repair****,*** *base excision repair, breast cancer, XPD, XRCC1.*

INTRODUCTION

Breast cancer remains the most frequent cancer and the second leading cause of cancer death among women in the world [1]. In Egypt, It accounts for nearly 35.1% of cancers among the Egypt National Cancer

Institute patients [2] with an age-adjusted incidence rate of 49.6/100 000 population [3] and an increase of about 11 folds in breast cancer incidence rate between 1972 and 2001[4].

Cells of the body constantly exposed to mutagenic assault from radiation, chemical carcinogens, alcohol, estrogen, and diet, which produce reactive oxygen species, oxidized bases, bulky DNA adducts, and DNA strand breaks. Unrepaired or misrepaired DNA may lead to deletions, amplifications, and/or mutations of critical genes that contribute to breast carcinogenesis [5].

There are multiple pathways to repair the different types of DNA damage and maintain genomic integrity. Among these pathways is nucleotide excision repair (NER) pathway that repairs a wide variety of DNA damage, including cross-links, oxidative damage and bulky adducts and the base excision repair (BER) pathway that repair small lesions such as oxidized or reduced bases, fragmented or non bulky adducts, and lesions caused by methylating agents[6].

There are over 100 identified DNA repair genes (protecting genes) and most of them are known to have genetic variation in humans[7]. DNA repair gene polymorphisms may alter the protein function and cause reduction in DNA repair capacity that may lead to genetic instability and carcinogenesis [8,9].

The XPD (xeroderma pigmentosium group D) protein participates in NER as it represent an integral member of the basal transcription factor BTF2/TFIIH complex, which is a multi-protein with functions including transcription, NER, transcription-coupled repair, apoptosis, and cell cycle regulation. Also, the XPD gene product has an ATP-dependent DNA helicase activity [10]. Single nucleotide polymorphisms (SNPs) have been identified in several exons of the XPD gene (NER), among which one in codon 312 of exon 10 and the other in codon 751 of exon 23 are commonly studied and result in amino acid changes (Asp312Asn and Lys751Gln, respectively)[11]. These polymorphisms are associated with lower DNA repair capacity and a higher level of DNA adducts [11, 12].

XRCC1 (X-ray repair cross-complementing group 1) is known to participate in base excision repair (BER). The XRCC1 is a multidomain protein that has no known catalytic activity itself but links with a scaffolding protein that associated with other proteins such as DNA polymerase β, DNA ligase III and poly(ADP-ribose) polymerase (PARP) that are needed at the site of DNA damage [13].

The XRCC1 Arg399Gln polymorphism is located in the area coding for a PARP binding site. PARP is a zinc-finger containing enzyme that detects DNA strand breaks. Arg399Gln is non-conservative amino acid changes and occur in evolutionarily conserved regions. That is, the wild-type and variant residues have dissimilar physical and/or chemical properties, suggesting that these substitutions may affect protein structure and potentially have functional relevance[14].

Because DNA damages are repaired by multiple repair pathways, a genetic variant in multiple repair pathways may have synergistic effect on breast cancer risk. The aim of the present study was to evaluate the association of genetic polymorphisms in 2 DNA repair pathways, XPD (Asp312Asn) as NER and XRCC1(A399G) as BER, with breast cancer susceptibility. We further investigate the potential combined effect of these DNA repair variants on breast cancer risk.

SUBJECTS & METHODS

Subjects:

The current study included one hundred Egyptian women (mean age 50.5±9.8 years), with histologically proven diagnosis of BC between January 2009 and June 2010, they were among the attendants of Surgical Clinics of Zagazig University Hospital at Sharkia, Egypt. The control group were 100 age- matched women (mean age 51.2±11.4) with no signs or symptoms of malignancy, they were randomly selected from various clinics at Sharkia, Egypt. The clinicopathological features of the patients and controls were summarized in Table 1. BC cases were graded according to the modified criteria as described by **Bloom and Richardson**[15], and they were staged according to the American Joint Committee on Cancer (AJCC) staging system [16].

The study protocol was approved by the ethical committee of Faculty of Medicine, Zagazig University, and informed consent for the experimental use of specimens was obtained from all participants.

Table 1: The clinicopathological features of breast cancer patients (n=100).

Patient characteristic	*Number*
Age (years)	50.5±9.8
≤50	67
51-60	33
Tumor size	
T1 (≤2 cm)	54
T2 (>2 cm–5 cm)	27
T3 (>5 cm)	13
T4 (tumor of any size that has broken through (ulcerated) the skin, or is attached to the chest wall)	6
Lymph node status	
N0	52
N1	48
AJCC Pathological stage	
I	40
II	35
III	14
IV	11
Histological grade (modified Bloom-Richardson score)	
I (Well differentiated)	18
II(Moderately differentiated)	52
III(Poorly differentiated)	30

Blood sample collection:

Half- ml of Blood was collected in EDTA-treated tubes for DNA extraction, and one ml in plain tube for serum separation for CA15.3 and CEA assay.

Measurement of CEA and CA15.3:

Serum levels of CEA and CA15.3 were measured by an enzyme-linked immunosorbent assay (ELISA) in duplicate using ELISA commercial kit (DRG Diagnostics, GmbH, Germany) according to the manufacturer's instructions. The assay employs the quantitative sandwich enzyme immunoassay technique.

DNA preparation and genotype analysis:

EDTA-blood samples were obtained from all participants, coded and analyzed in a blind manner for genomic DNA extraction using QIAGEN genomic DNA extraction kit (QIAGEN, Clinilab, Egypt) as described in the user manual. The quality of the genomic DNA was tested using agarose gel electrophoresis.

Samples were genotyped for detecting SNP OF XPD codon 312 and XRCC1 codon 399 in genomic DNA.PCR, for both polymorphisms, was performed in a final volume of 25 µl containing 100 ng of genomic DNA, 1X PCR mix (20mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 0.3mM dNTPs and 2U Taq DNA polymerase) and 0.2 µM of each primer .The amplification was carried out using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA).

A tetra-primer amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR) assay was used for detection of XPD codon 312 polymorphism as described by **Rybicki et al. (2004)** [17], The ARMS assay is an allele-specific PCR method that uses two primers, each with the 3′ terminal base complementary to one of the alleles to be identified. An internal control primer pair was included in each reaction (ARMSA: 5′-CCC ACC TTC CCC TCT CTC CAG GCA AAT GGG-3′; ARMSB: 5′-GGG CCT CAG TCC CAA CAT GGC TAA GAG GTG-3′) at a 1:5 dilution relative to the allele-specific primers. A 150-bp PCR fragment was generated with the following primers: reverse primer 5′-CAG GAT CAA AGA GAC AGA CGA GCA GCG C-3′; G allele forward specific primer 5′-GTC GGG GCT CAC CCT GCA GCA CTT CGG C-3′; A allele forward specific primer 5′-GTC GGG GCT CAC CCT GCA GCA CTT CGA T-3′. Cycling conditions were initial denaturation of 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 61 °C and 50 s at 72 °C and finally 7 min at 72 °C.

The amplified products were analyzed on 2% agarose gels stained with ethidium bromide. Genotypes were typed as GG and AA depending on the development of bands when primers specific for allele G (as GG) or allele A (as AA) were used. Samples were typed as heterozygotes (GA) when bands were seen with both the primers (Fig. 1).

PCR–CTPP (PCR with confronting two-pair primers) method was performed for detecting the XRCC1 codon 399 variantas

descriped by **Ito et al. (2004)**[18]. The extracted DNA was amplified with the four primers by 'F1, 5′-TCC CTG CGC CGC TGC AGT TTC T-3′; R1, 5′-TGG CGT GTG AGG CCT TAC CTC C-3′; F2, 5′-TCG GCG GCT GCC CTC CCA-3′; and R2, 5′-AGC CCT CTG TGA CCT CCC AGG C-3′. PCR conditions were 1-min denaturation at 94°C followed by 30 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, with a 10-min extension at 72°C. Primer pairs F1 and R1 for the G allele (399Arg) and F2 and R2 for the A allele (399Gln) produced allele-specific bands of 447- and 222-bp, respectively, as well as a 630-bp common band (Fig. 2).

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences version 11 (SPSS Inc., Chicago, USA**)**. The significance of association between the observed and expected number of the genotypes for a population in the Hardy-Weinberg equilibrium was analyzed using the Pearson's two-sided chi-square test. The differences of genotype and allele frequencies between the cases and controls were determined using Chi-square(X^2) test. The associations between genotypes and BC risk were analyzed by calculating the crude Odds Ratios (OR) and 95% Confidence Intervals (CI) by logistic regression method. The adjusted odds ratio was calculated using the multivariate logistic regression method with an adjustment for age. P value of <0.05 was considered statistically significant.

RESULTS

CEA and CA15.3 assay and BC risks

In the breast cancer patients the CEA ranged from 4.5 to 8.5 ng/ml, with a mean value ± SD (6.7±0.13 ng/ml), while in the control group CEA ranged from 2.5-3.1 ng/ml with a mean value of 2.8±0.01 ng/ml, there was significant difference as regard CEA levels (P<0.001).

As regard CA15.3 there was significant difference between breast cancer cases and the control group (P<0.001), as the mean level of CA15.3 ± SD in the cases was 66.24±1.27 IU/l with a range of 42-86 IU/l, while in the control group CA15.3 ranged from 2.5 to 3.1 IU/l, with a mean value ± SD of 2.8±0.02 IU/l. Table 2

Table 2: The serum levels of CEA and CA15.3 in breast cancer patients (n=100) and in the control subjects (n=100).

Parameters (Mean ± SD)	***Breast cancer patients (n=100)***	***Control subjects (n=100)***	***p-value****
CEA(ng/ml)	6.7±0.13	2.8±0.01	P<0.001
CA15.3(IU/l)	66.24±1.27	2.8±0.02	P<0.001

* calculated by unpaired t test; CEA= carcino-embryonic antigen; CA15.3= Cancer antigen

XPD Asp312Asn polymorphism and BC risk

The genotype frequencies of homozygous (GG), heterozygous (AG), and homozygous mutated (AA) were 12%, 45%, and 43% in patients with BC respectively; and 25%, 50%, and 25% in controls respectively.

The frequency of A allele was 65.5% in BC patients and 50% in controls. These data suggest that the A allele was significantly associated with an increased risk of BC (ORadj: 1.8, 95% CI, 1.2-2.8) (P=0.002). The homozygous mutant genotype (AA) significantly increased the risk of BC (ORadj: 3.5, 95% CI, 1.5-8.3) (P=0.003) in comparison with those with (GG) genotype. However, the (AG) genotype showed no significant difference between patients and control groups (p=0.08) (ORadj: 1.8, 95% CI, 0.8-4.1).

AA genotype increased the risk of BC by (2.26 CI = 1.2-4.1) between pts and control group. The distribution of XPD Asp312Asn allele and genotype frequencies in breast cancer patients and the control subjects are represented in Table 3.

Table 3: Distribution of XPD Asp312Asn allele and genotype frequencies in breast cancer patients (n=100) and the control subjects (n=100)

GENOTYPE	*Breast cancer patients n (%)*	*Control subjects n (%)*	*Adjusted OR (95% CI)*	*P**
GG(ref)	12 (12%)	25 (25%)		
AG	45 (45%)	50 (50%)	1.8 (0.8 – 4.1)	0.08
AA	43 (43%)	25 (25%)	3.5 (1.5 – 8.3)	0.003**
G Allele	69(34.5%)	100(50%)	1.8 (1.2 – 2.8)	0.002**
A allele	131(65.5%)	100(50%)		

* calculated by Chi Square test; Adjusted OR=Odds Ratio was adjusted to the age, 95% CI= 95% confidence interval.

XRCC1 A399G polymorphism and BC risk

The genotype frequencies of homozygous (AA), heterozygous (AG), and homozygous mutated (GG) were 37%, 51%, and 12% in patients with BC respectively; and 50%, 40%, and 10% in controls respectively.

The frequency of G (mutant) allele was 37.5% in BC patients and 30% in controls. However, that difference didn't show statistical significant difference between patients and control groups (ORadj: 1.4, 95% CI, 0.9-2.1) (P=0.06). The homozygous mutant genotype (GG) and the heterozygous (AG) genotype showed no significant difference in patients and control groups (p=0.3) (ORadj: 1.6 95% CI, 0.6-4.1).and (p=0.07) (ORadj: 1.7 95% CI, 0.9-3.1) in relation to (AA) genotype. The distribution of XRCC1 A399G allele and genotype frequencies in breast cancer patients and the control subjects are represented in Table 4.

Table 4: Distribution of XRCC1 A399G allele and genotype frequencies in breast cancer patients (n=100) and the control subjects (n=100)

GENOTYPE	*Breast cancer patients n (%)*	*Control subjects n (%)*	*Adjusted OR (95% CI)*	*P[x]*
AA(ref)	37 (37%)	50 (50%)		
AG	51 (51%)	40 (40%)	1.7 (0.9 – 3.1)	0.07
GG	12 (12%)	10 (10%)	1.6 (0.6 – 4.1)	0.3
GG+AG	63(63)	50(50%)	1.7 (0.9 – 2.9)	0.06
A allele	125(62.5%)	140(70%)	1.4 (0.9- 2.1)	0.06
G Allele	75(37.5%)	60(30%)		

[x] calculated by Chi Square test; Adjusted OR=Odds Ratio was adjusted to the age, 95% CI= 95% confidence interval.

Polymorphism in XPD Asp312Asn and XRCC1 A399G and BC risk

Regarding the association of combined polymorphism in XPD Asp312Asn and XRCC1 A399G and the risk of development of BC, the GG(XPD)+AA(XRCC1) wild type genotype were taken as references. The analysis showed that the AA(XPD)+AG(XRCC1) combination were significantly associated with breast cancer risk (ORadj: 3.36 95% CI, 0.9-11.3).and (p=0.04). However no association were found between other compound polymorphisms and breast cancer risk (no significant difference between patients and control group. the distribution of combined polymorphism among patients and control groups are represented in table 5.

Table 5: Distribution of combined polymorphism among patients ad control groups

GENOTYPE XPD	*XRCC1*	*PATIENTS n (%)*	*CONTROL n (%)*	*Adjusted OR (95% CI)*	*P[x]*
GG	AA	6 (6%)	11 (11%)	Reference 1	-----
GG	AG	5 (5 %)	4 (4%)	2.3(0.5-4.9)	0.3
GG	GG	1 (1%)	10 (10%)	0.18(0.01-1.8)	0.1
GA	AA	16 (16%)	26 (26%)	1.1(0.3-3.6)	0.8
GA	AG	24 (24%)	24(24%)	1.8(0.85-5.7)	0.2
GA	GG	5(5%)	0(0%)		
AA	AA	15(15%)	13(13%)	2.1(0.6-7.3)	0.2
AA	AG	22(22%)	12(12%)	3.36(0.9-11.3)	0.04
AA	GG	6(6%)	0(0.0%)		

[x] calculated by Chi Square test; Adjusted OR=Odds Ratio was adjusted to the age, 95% CI= 95% confidence interval.

Relation of XPD Asp312Asn and XRCC1 A399G polymorphisms and clinicopathological parameters of the BC patients

The association between genotypes and clinical characteristics of breast cancer patients were analyzed using six clinicopathological parameters; age, primary tumor size, presence of lymph node metastasis, AJCC stage tumor grade, and histological type

(modified Bloom-Richardson score) Both XPD Asp312Asn and XRCC1 A399G polymorphisms didn't show any association with the clinical characteristics of disease among breast cancer patients (Table 6,7)

Table 6: Association of XPD Asp312Asn polymorphism with clinical characteristics.

Parameters	*n*	*XPD Asp312Asn polymorphism genotypes*			
		GG n (%)	GA/AA n (%)	p-value	Adjusted OR (95% CI)
Patients	100	12	88	------	
Age (years)					0.6(0.17—1.17)
≤50	67	7(58.3%)	60 (68.2%)		
51-60	33	5 (41.7%)	28 (31.8%)	0.49	
Lymph node status					2(0.5—7.1)
N0	52	8 (66.7%)	44(50%)	0.2	
N1	48	4 (33.3%)	44 (50%)		
Tumor size					1.2(0.2—5.9)
T1 &T2	81	10(83.3%)	71 (80.7%)		
T3 &T4	19	2 (16.7%)	17 (19.3%)	0.8	
AJCC pathological stage					1.7(0.3-8.6)
I&II	75	10 (83.3%)	65 (73.9%)		
III&IV	25	2 (16.7%)	23 (26.1%)	0.4	
Histological Grade					1.1(0.2—4.9)
I&II	72	9 (75%)	63 (71.6%)		
III	28	3 (25%)	25 (28.4%)	0.8	

[x] calculated by Chi Square test; Adjusted OR=Odds Ratio was adjusted to the age, 95% CI= 95% confidence interval.

Table 7: Association of XRCC1 A399G polymorphism with clinical characteristics

Parameters	*n*	*XRCC1 A399G polymorphism genotypes*			
		AA n (%)	AG/GG n (%)	p-value	Adjusted OR (95% CI)
Patients	100	37	63	------	
Age (years)					0.49(0.19—1.24)
≤50	67	21 (56.7%)	46 (73%)	0.09	
51-60	33	16 (43.3%)	17(26.9%)		
Lymph node status					0.57(0.23—1.4)
N0	52	16 (43.3%)	36(57.1%)	0.1	
N1	48	21 (56.7%)	27 (42.9%)		
Tumor size					
T1 &T2	81	29(78.3%)	52 (82.5%)	0.6	0.77(0.25—2.3)
T3 &T4	19	8 (21.7%)	11 (17.5%)		
AJCC pathological stage				0.7	0.4(0.2-1.8)
I&II	75	26 (70.3%)	49 (77.8%)		
III&IV	25	11 (29.7%)	14 (22.2%)		
Histological Grade					1.1(0.4—2.9)
I&II	72	27(72.9%)	45(71.4%)		
III	28	10 (27.1%)	18 (28.6%)	0.8	

[x] calculated by Chi Square test; Adjusted OR=Odds Ratio was adjusted to the age, 95% CI= 95% confidence interval.

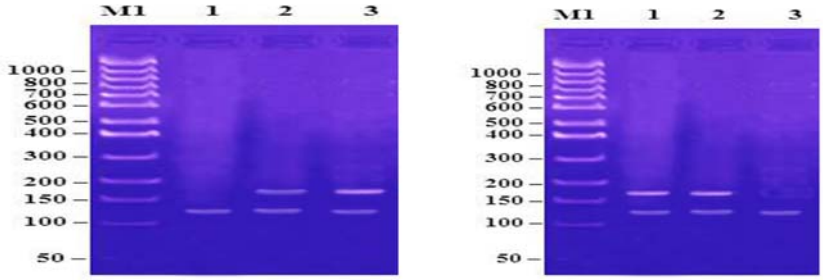

Fig. (1): Representative results for XPD Asp312Asn polymorphism by the ARMS method. Left gel for A genotype, right for G genotype Lane M, marker, lane 1, GG, lane 2, AG, lane 3, AA.

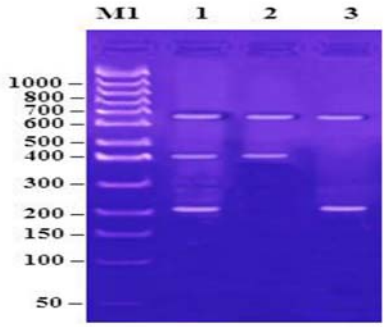

Fig. (2): Representative results for XRCC1 Arg399Gln polymorphism by the PCR-CTPP method. Lane M, marker, lane 1, A/G, lane 2, GG, lane 3, AA.

DISCUSSION

One of the most commonly diagnosed cancers over the world is the Breast cancer, but its etiology is still largely unknown. Several environmental factors, as radiation, diet, smoking, and endogenous or exogenous estrogens, are associated with DNA damage. Unrepaired or misrepaired DNA results in gene mutations, chromosomal alterations, genomic instability and carcinogenesis[19]. Earlier studies have demonstrated a strong association of higher levels of DNA damage and deficient DNA repair capacity in breast cancer patients[20,21]. Genetic polymorphisms on DNA repair genes are very common events, and there are conflicting reports on the influence of these polymorphisms on DNA repair capacity and cancer susceptibility[22-24].

The cause of breast cancer is complicated and can not be explained by means of only a single selected marker. Therefore, the goal of the

present study was to examine the relation of two DNA repair genes XPD and XRCC1 polymorphisms with the risk of BC in Eastern Egyptian females .Also to determine the gene-gene interaction aiming to increase our understanding of the interaction between potential carcinogenic environmental exposure and genetic factors in the pathogenesis and predisposition to BC disease risk.

As regard to XPD codon 312 polymorphism, our results revealed that the frequencies of AA genotypes and A allele were significantly higher in the breast cancer patients than in the normal individuals ($p \leq 0.003, p \leq 0.002$ respectively). It was also found that participants homozygous Asn at XPD Asp312Asn had 3.5-fold higher risk of breast cancer.

Our findings are consistent with the results of **Zhang et al. (2005)**[25] who reported that XPD AA genotypes were significantly different between BC patients and control group in Chinese population. Another study in Taiwanese patients by **Wang et al. (2010)**[26] stated that the heterozygotes and homozygotes of the A allele of XPD asp312asn associated with BC development. Also, in France [27] a study demonstrated an increase in the risk of breast cancer in individual heterozygous for ERCC2 (XPD) Asp312Asn in women receiving menopause substitution treatment.

However, a study by **Kuschel et al. (2005)**[28] reported no evidence of association of ERCC2(XPD) polymorphism and breast cancer risk. Our results supported an African – American study who found that subjects with an Asp-Asn or Asn-Asn polymorphic type in codon 312 of XPD had elevated levels of PAH-DNA adducts compared to subjects with Asp-Asp genotype [29].

As regards XRCC1 polymorphism in the present study, the frequency of G(mutant) allele was 37.5% in BC patients and 30% in controls. However, this difference did not show statistical significant difference between patients and control groups (P=0.06). The homozygous mutant genotype (GG) and the heterozygous (AG) genotype showed no significant difference between patients and control groups (p=0.3) and (p=0.07).

Our results are consistent with the results of previous studies who found no significant role for XRCC1 Arg399Gln polymorphic variants in breast carcinogenesis[30- 34]

Furthermore, contrary to our results, **Chacko et al. (2005)**[35],and **Syamala et al**. (2009)[36], found an increased risk for sporadic breast cancer in Indian individuals with 399Gln allele of the XRCC1 .However , **Dull et al.(2001)** [37]found a correlation between XRCC1(A allele) codon 399 polymorphism and breast cancer susceptibility in African-American women, but not in Caucasian women. Also, studies on Asian populations [38-40] provided an evidence of significant association between XRCC1 399 Arg homozygosity and BC susceptibility. A study by **Zipprich et al. (2010)** [41] reported that although the main effects of the genotype XRCC1 were not significantly associated with familial cancer risk, the XRCC1 399A may alter mRNA expression and DNA repair phenotype.

Despite these inconsistent findings, which could be explained by ethnic factors or interaction between genetic and environmental factors, functional significance of polymorphism in XRCC1 gene is evident[42-44].

Regarding the association of clinical characteristics and different genotypes in breast cancer patients, our results showed no association between both XPD Asp312Asn and XRCC1 A399G polymorphisms and the clinical characteristics of disease. This data is consistent with results of **Syamala et al.(2009)**[36] in their study on XRCC1 codon 399 polymorphism in sporadic and familial breast cancer patients.

Although the effect of an individual SNP is generally small, it is believed that the genetic effect of combinations of functionally relevant SNPs may additively or synergistically contribute to increased breast cancer risk. So, we studied the combined polymorphism of XPD and XRCC1 genes. To the best of our knowledge, there are no previous reports available regarding the association between combined polymorphism of XPD and XRCC1 genes and breast cancer from Egypt. Our results revealed that only combination of AA(XPD)+ AG (XRCC1) might increase the risk of breast cancer (OR 3.36 CI 0.9 ---11.3) ($p \leq 0.04$). However, these results need more investigation to study gene - gene interactions and its relation to breast cancer susceptibility aiming to identify individuals at increased risk of breast cancer and develop preventive strategies .

In conclusion, our results suggest that AA genotype of XPD Asp 312 Asn polymorphism increased the risk of breast cancer.XPD gene is an important candidate gene for susceptibility to breast cancer. Also, gene–gene interaction between XPD(AA)+XRCC1(AG) polymorphism may be associated with increased risk of breast cancer in Egyptian women.

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تأثير أصلاح ال DNA للتحور الجينى XPD و XRCC1 على خطورة سرطان الثدى فى المريضات المصريات

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ان الجينات التى تدخل فى نظام أصلاح ال دى.ان.ايه تلعب دورا حرجا فى الحمايه ضد الطفرات. لقد وضع افتراض ان الأختلالات الوظيفيه فى العمليات العالية المحافظه والألتزام لأصلاح الدى ان ايه قد نتجت من التحورات الجينيه التى قد تزيد من القابليه الجينيه لحدوث سرطان الثدى. هناك طرق متعددة لأصلاح الأنواع المتعددة من تلف الدى.ان.ايه والمحافظة على السلامه الجينيه من بينهم الأصلاح بأزالة النيكلوتيد والأصلاح بأزالة القاعده.

تهدف هذه الدراسه لفحص العلاقه بين اصلاح التحورات الجينيه للدى ان ايه وأحتمال حدوث سرطان الثدى فى السيدات المصريات وكذالك تحليل العلاقه الباثولوجية والسريريه لسرطان الثدى بالأضافه لفحص التأثير المدعم لهذين الجينين على قابلية حدوث سرطان الثدى.

تم فحص التحورات الجينيه لXPD و XRCC1 فى مائة مريضة مصرية مصابة بسرطان الثدى ومائة سيدة سليمة غير مصابه بالمرض وليس عندهن اى تاريخ مرضى لأى اصابه بالسرطان قبل ذالك عن طريق تكبير جهاز الطفرات المقاوم بطريقة االتفاعل البوليميرى والتفاعل البوليميرى المتسلسل بمواجهة اثنان من أوليات الأصلاح (PCR-CTPP) وبواسطة ال دى ان ايه من الدم فى السيدات السليمات.

أظهرت نتائج الدراسة ان حدوث النوع الجينى AA لشفرة دى. ان. ايه للتحور الجينى ٣١٢ لجين XPD كان عاليا وبدلالة أحصائيه فى مرضى سرطان الثدى عنه فى السيدات السليمات ولم نلاحظ اى علاقه بين التحور الجينى XRCC1 Arg339Gln وأحتمال حدوث سرطان الثدى ايضا.

كذلك لم نجد اى علاقه بين التحورات الجينيه XPD Asp312An و XRCC1 A399G والخصائص السريريه للمرض. وأخيرا وجدنا ان ارتباط AA(XPD)+AG(XRCC1) مصاحب لخطر حدوث سرطان الثدى وبدلاله احصائيه.

من نتائج هذه الدراسه يمكن أقتراح ان جين XPD مصاحب مهم لقابلية الأصابة بسرطان الثدى وكذلك التفاعلات الجينيه-الجينيه بين التحورات الجينيه XPD(AA) +XRCC1(AG) قد تكون مصاحبة لزيادة أحتمال حدوث سرطان الثدى فى السيدات المصريات.