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ANALYSIS OF DNA REPAIR GENETIC POLYMORPHISM IN BREAST CANCER POPULATION

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ABSTRACT

The aim of the present study is to analyze the variations in DNA repair genetic pathways in Breast Cancer. The study carried out in three groups, 50 Participants in each group. Females newly diagnosed for breast cancer, females at high risk for breast cancer, and females with normal health are included for the study. The polymorphisms namely Arg 194 Trp, Thr 241 Met, Arg 415 Gln, Asp 1104 His are investigated using their peripheral blood. The three polymorphisms Arg 194 Trp, Arg 415 Gln, Asp 1104 His are presented to be significantly associated with breast cancer in their heterozygous mutant type with P- Value - 0.01, 0.009, and 0.0001 respectively. Females with breast cancer and those categorised as high risk females presented similar polymorphism status. DNA repair genes are regions of DNA which repair the emergent damage rapidly and effectively. Variations in these genes increase the risk of occurrence of breast cancer.

KEY WORDS: Breast Cancer, High Risk Females, Polymorphism, and DNA repair genes.

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INTRODUCTION

Breast cancer is the most common cancer in women. According to International Agency For Research on Cancer (IARC) – WHO's specific cancer agency, the world wide incidence is about 11.9% and in India it is about 30 – 33 per 1, 00,000 population. WHO stresses that the priority should be given to cancer prevention and control measures for breast cancer globally [1]. Cancer that seem to develop due to mutation at gene level is found to have greater impact in india due to its increasing incidence and mortality in recent years[2].Another important life time risk factor is hormone based life events, which plays a major role in the development of breast carcinoma, suggests that breast cancer is somehow affected by prolonged exposure to female sex hormone, such as estrogen[3].DNA repair is a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encodes genome. Base excision repair (BER), Nucleotide excision repair (NER), Homologous recombination (HR) and Excision repair cross complementation (ERCC) pathways repair damaged DNA and polymorphisms in these genes might affect breast cancer susceptibility. BER and NER are two mechanisms that repair single stranded DNA by excising damaged sequences and using the complementary DNA strand as a template to fill the resulting gap. DNA damage can cause lesions that may or may not distort the double helix structure, and these are repaired by BER and NER pathways respectively [4]. The DNA repair genes encode proteins with specialized functions for the repair of damaged DNA. The present study investigated the associations between genes in NER, BER, HR pathways and risk of breast cancer. Polymorphisms in these genes may alter the function of proteins encoded by XRCC1, XRCC3, ERCC4 and ERCC5, leading to the possibility that women with variant alleles may have reduced repair capacity and increased susceptibility to breast cancer. The present study aimed to evaluate the association between SNPs in four DNA repair genes namely XRCC1 Arg 194 Trp (rs 1799782), XRCC3 Thr 241Met (rs 861539), ERCC4 Arg 415 Gln (rs 1800067), ERCC5 Asp 1104 His (rs 17655) and the risk of breast cancer.

MATERIALS AND METHODS

The study is being conducted among 150 females in three groups, each comprising of 50 females, in the Department of Anatomy, ACS Medical College and Hospitals, Dr.MGR University, Department of Industrial Biotechnology, Dr. MGR Engineering and Research Institute, Dr.MGR University, Tamilnadu, India, and Saveetha Medical College and Hospital, Tamilnadu, India. The participants are age matched between 35-60 years. The study commenced after getting approval from the Institutional Human Ethical Committee, Saveetha University, Chennai, Tamilnadu. The participants are given detailed explanation about the procedure and their co-operation and willingness is obtained with an informed consent. The participants are grouped based on selection criteria. Group I includes females diagnosed histopathologically for breast cancer as their primary site of carcinoma and not affected by any other major diseases or disorders. Group II includes females who are a categorized as high risk for breast cancer based on their family history for breast cancer (mother, sister or daughter) or any two criteria based on their endogenous exposure to estrogen which includes Menstrual history (early menarche below 12 years , late menopause above 50 years) Parity status (first full term pregnancy(FFTP) above 30 years of age, Nulliparity), Personal history of fibroadenoma, obesity, hormone replacement therapy (HRT). Group III includes normal healthy females free of any diseases and disorders. The data collection procedure includes collection of 3ml of peripheral blood in EDTA (Ethylene diaminetetraaceticacid) coated test tubes through Venipucture. The procedure includes Extraction of DNA, followed by amplification of specific gene segments using Polymerase Chain Reaction (PCR) and identification of polymorphism using Restriction Fragment Length Polymorphism (RFLP).

DNA EXTRACTION

The samples are assigned unique identifier code. From each study participant 3ml Blood sample is drawn in to EDTA coated test tubes. The strategy followed for isolation of DNA from blood cells includes lysis of cells, separation phases (carrying DNA, RNA and proteins) and precipitation of DNA. The blood cells are lysed using RBC lysis buffer and proteinase K. Separation phases done using Phenol Chloroform Treatment. Followed by precipitation of DNA strands is performed using 95% ethanol in the presence of sodium salt. The DNA pellet is washed with 70% ethanol to dissolve salts. The pellet is air dried and finally reconstituted In TE(Tris EDTA) buffer and stored at -20°C until used for genotyping.

GENOTYPING ANALYSIS

Determination of polymorphisms XRCC1 Arg194 Trp, XRCC3 Thr 241 Met, ERCC4 Arg 415 Gln, ERCC5 Asp 1104 His is performed by PCR thermal cycler. The PCR conditions for the Total reaction volume of 25 µl the working concentration is PCR buffer - 1X, dNTPs -(250 µm) - 0.25 µl, Forward primer (10pm/µl) - 1 µl, Reverse Primer (10 pm/µl) - 1 µl.Taq DNA polymerase (5u/µl) (GeNet Bio) - 0.75 µl, genomic DNA - 2µl, transferred to capillary tubes. The polymerase chain reaction thermal cycling condition is 5min of initial denaturation at 95°C Followed by amplification at 95°C for 1 min 30 sec, annealing temperature at 68°C, 58°C, 56°C for XRCC1, ERCC4, ERCC 5, respectively for 1min 30 sec and 72°C for 30 sec, 35 cycles and final extension at 72°C for 7 min. The PCR reaction for XRCC3 is performed in a total reaction volume of 20 µl containing 10 ng genomic DNA, 0.4 u Taq polymerase (GeNetBio) in 1X PCR buffer, 1.5mM MgCl 2, 50 mM dNTPs in 250 nM each primer. The thermal cycling conditions are initial denaturation step at 95°C for 3 min, 35 cycles of PCR, 72 degree Celsius for 20s, 60°C annealing temperature and 72°C for 20 s, followed by final extension step at 72°C for five min.[5]. The DNA repair gene XRCC1, chromosome position 19q.13.2, Arg 194 Trp (rs- 1799782) polymorphism, C→T transition in exon 6, is determined using following primers, Forward Primer- GCCCCGTCCCAGGTAAGC, Reverse Primer- AGCCCCAAGACCCTTTCACT [6]. Arginine allele at codon 194 creates MspI(Thermo scientific) sites, 491 bp PCR products were digested at 37 ºc for 2 hours and resolved on 3% agarose gel. Arg/Arg, Arg/Trp, Trp/Trp genotypes for codon 194 resulted in 292bp and 313 bp, 491bp, 292 bp and 313bp and491bp and 313 bp respectively. The gene XRCC3, chromosome position 14q.32.3, Thr 241 Met (rs 861539) polymorphism, C→T transition, exon 7, is determined using the following primers, Forward Primer–GCCTGGTGGTCATCGACTC, Revere Primer- ACAGGGCTCTGGAAGGACTGCTCACGCAC C[5]. The Thr allele at codon 241 creates NcoI sites. The 136 bp PCR product is digested at 37°C and using NcoI (Thermo Scientific) for 2 hours and resolved on 3% agarose gel. Thr/Thr, Thr/Met, Met/Met genotypes for codon 241resulted in 136bp and 108bp,136bp, 38bp and 108bp,108bp and 38bp respectively. The ERCC4 gene, chromosome position 16p.13.12, Arg 415 Gln (rs- 1800067) polymorphism, G→A transition, in exon 8 is determined using the following primers, Forward primer- CTTCGGGTGAAGGAATAAG, Reverse Primer- TTCTCAAAGGTTTTCCTGTAG[7] , Arg allele at codon 415 creates PdmI(Thermo scientific) sites, 194 bp PCR products are digested at 37 ºc for 2 hours and resolved on 3% agarose gel. Arg/Arg, Arg/Gln, Gln/Gln genotypes for codon 415 resulted in 77bp, 77bp and 196bp, 196bp respectively. The ERCC5 gene, chromosome position 13q22-q33, G→C transition in exon 15, is determined using following primers, Forward Primer- TTTCAGATTCTAAACGAAAGAATA, Reverse Primer- GAGTTCTGCGAATCTGAAGCAC[7], His allele at codon 1104 creates Hin1II(Thermo scientific) sites, 172 bp PCR products were digested at 37 ºc for 2 hours and resolved on 3% agarose gel. Asp/Asp, Asp/His, His/His genotypes for codon 1104 resulted in 172bp, 172bp and 84 bp, 84bp respectively. All PCR reactions are accompanied by a negative control without genomic DNA to rule out possibility of genomic DNA contamination of reagents. RFLP analysis is accompanied by

negative and positive controls that lack or have restriction site of target restriction enzyme. Approximately 10% of samples are reanalyzed to rule out genotyping errors and found 100% concordance.

STATISTICAL ANALYSIS

Genotyping consistency for Hardy Weinberg Equilibrium is analyed using Chisquare test. The associations of DNA repair gene polymorphism and the Breast Cancer Risk is analysed using Odds Ratio and P value for level of significance.

RESULTS

Table 1
Association between polymorphism status of DNA repair gene and Breast cancer risk

SI	GENE	SNP	GENOTYPE	CONTROL(%)	BREAST CANCER (%)	OR (95% CI)	P-VALUE
1	XRCC1 R194W	rs1799782	R/R	47(94)	36(72)	6.0926 (1.626 -22.815)	0.007*
			R/W	3(6)	13(26)	0.1817 (0.0482-0.6850)	0.01*
			W/W	0	1(2)	0.3267 (0.0130-8.2151)	0.49
2	XRCC3 T241W	rs861539	T/T	40(80)	42(84)	0.7619 (0.2732-2.12480)	0.6033
			T/M	8(16)	7(14)	1.170 (0.3895-3.5148)	0.77
			M/M	2(4)	1(2)	2.0147 (0.1792 – 23.267)	0.5653
3	ERCC4 R415Q	rs1800067	R/R	48(96)	34(6)	11.29 (2.435-52.38)	0.002*
			R/Q	2(4)	12(24)	0.1250 (0.0263-0.5937)	0.009*
			Q/Q	0	4(8)	0.1023 (0.0054-1.9524)	0.1297
4	ERCC5 D1104H	rs17655	D/D	47(54)	20(40)	23.50 (6.4233-85.9766)	0.0001*
			D/H	3(6)	26(52)	0.0589 (0.0162-0.2145)	0.0001*
			H/H	0	4(8)	0.1023 (0.0054-1.9524)	0.1297

All genotypes are tested for Hardy Weinberg Equilibrium and they showed no significant difference between the referent and expected genotype. * Statistically Significant.

Recessive inheritance model where two variant allele were required to confer risk showed no significant difference for any genotype between groups . For Co-Dominent and Dominent model (one variant allele required to confer risk), three SNPs XRCC1(rs 1799782), ERCC4 (rs 1800067), and ERCC5 (rs 17655) had significant association with Breast cancer, where as the result of XRCC3 (rs 861539) was inconsistent. The frequency of association of variant alleles XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) in heterozygous mutant of healthy females when asocciated with breast cancer are for Dominant Inheritance OR (95% CI)- 0.16 (0.43- 0.61), 0.08 (0.01-0.41), 0.04 (0.01-0.15) respectively. XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) for Co-dominent model OR (95% CI) - 0.17 (0.04-0.66), 0.11 (0.02-0.56), 0.04 (0.01-0.18) respectively. One vareint ERCC5 rs 17655 showed significant difference in homozygous mutant type for Co-Dominant model. For Overdominent and Additive model the SNPs XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) showed significant association for breast cancer with OR 95% CI for healthy population - 0.18, 0.13, 0.05 for Overdominent model and 0.15, 0.07, 0.03 for Additive model respectively.

Table II
Association between variant (minor) allele of DNA repair gene and Females at High Risk for Breast cancer

SI	GENE	SNP	GENOTYPE	CONTROL(%)	HIGH RISK (%)	OR (95% CI)	P-VALUE
1	XRCC1 R194W	rs1799782	R/R	47(94)	40(80)	3.9167 (1.0079-5.2201)	0.05*
			R/W	3(6)	10(20)	0.255 (0.0657-0.9922)	0.01*
			W/W	0	0	-	-
2	XRCC3 T241W	rs861539	T/T	40(80)	40(80)	1 (0.3753-2.6645)	1
			T/M	8(16)	9(18)	0.867 (0.3051-2.4675)	0.79
			M/M	2(4)	1(2)	2.04 (0.1792-23.267)	0.565
3	ERCC4 R415Q	rs1800067	R/R	48(96)	34(68)	11.29 (2.4352-52.3804)	0.002*
			R/Q	2(4)	16(32)	0.0885 (0.0191-0.4106)	0.002*
			Q/Q	0	0	-	-
4	ERCC5 D1104H	Rs17655	D/D	47(94)	31(62)	9.6022 (2.6185-35.27)	0.0006*
			D/H	3(6)	18(36)	0.1135 (0.0309-0.4173)	0.0018*
			H/H	0	1(2)	0.3267 (0.0130-8.2151)	0.49

*** statitically significant.***

Under Reccessive model of inheritance where two variant allele required to confer risk none of the variant allele showed significant elevated risk for breast cancer. For Codominant and Dominant model where one variant allele required to confer risk, the three SNPs XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) showed significantly elevated risk for breast cancer, even in high risk females population, with OR (95% CI) for Co-Dominant model in the healthy population were 0.25 (0.06-0.99), 0.08 (0.01-0.41), 0.10 (0.02-0.42) respectively for the heterozygote mutant type. None of the variant allele showed significant difference for homozygous mutant type in Codominant model. For Dominant model of inheritance the odds ratio for healthy population was 0.16, 0.08,0.0001 for XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) respectively. For Overdominent and Additive model the SNPs XRCC1(rs 1799782), ERCC4 (rs 1800067), andERCC5 (rs 17655) showed significant association for females high risk for breast cancer with OR 95% CI for healthy population - 0.05, 0.002, 0.001 for Overdominent model and 0.05, 0.002, 0.0005 for Additive model respectively.

Table III
Association of variant (minor) allele of DNA repair gene between Females High Risk for Breast cancer and breast cancer population

SI	GENE	SNP	GENOTYPE	HIGH RISK (%)	BREAST CANCER (%)	OR (95% CI)	P-VALUE
1	XRCC1 R194W	rs1799782	R/R	40(80)	36(72)	1.5556 (0.6149-3.9350)	0.350
			R/W	10(20)	13(26)	0.71 (0.2785-1.8176)	0.47
			W/W	0	1(2)	0.3267 (0,0130-8.2151)	0.49
2	XRCC3 T241W	rs861539	T/T	40(20)	42(84)	0.7619 (0.2732-2.1248)	0.6033
			T/M	9(18)	7(14)	1.3484 (0.4596-3.9565)	0.5862
			M/M	1(2)	1(2)	1 (0.0608-16.4446)	1
3	ERCC4 R415Q	rs1800067	R/R	34(68)	34(68)	1 (0.4316-2.3272)	1
			R/Q	16(32)	12(24)	1.4902 (0.6181-3.5925)	0.37
			Q/Q	0	4(8)	0.1023 (0.0054-1.9524)	0.1297
4	ERCC5 D1104H	Rs17655	D/D	31(62)	20(40)	2.4474 (1.0954-5.4680)	0.02*
			D/H	18(36)	26(52)	0.5192 (0.2331-1.1565)	0.1087
			H/H	1(2)	4(8)	0.2347 (0.0253-2.1782)	0.2023

**** Statistically significant.***

The genotype frequency showed a similar presentation in population with breast cancer and the population who are at risk for breast cancer. Under Codominant, Recessive and Over- Dominant model none of the variant allele showed significant difference. Under Dominant model and Additive model the ERCC5 rs 17655 showed significant difference for variant allele in homozygous wild type with frequency in the high risk population for Dominant model OR (95% CI)- 0.40 (0.18-0.91) and for the Additive model 0.37(0.17-0.83).

DISCUSSSION

The present study suggested that the three DNA repair genes XRCC1, ERCC4, and ERCC5 in their variant alleles Arg 194 Trp, Arg 415 Gln, and Asp 1104 His respectively, showed significant association for breast cancer. SNPs Arg 194 Trp, Arg 415 Gln presented to be associated significantly with females at high risk for breast cancer. XRCC3 Thr 241 Met does not show significant association for breast cancer risk. According to Jorgensen TJ et al [8] two SNPs XRCC1 and ERCC4 genotypes showed statistically significant association for high risk females for breast cancer. The results of the present study support the contention that the variant allele in ERCC4 and XRCC1 DNA repair genes may be linked to Benign Breast Disease which was an intermediate physical representation within breast carcinogenesis pathway. Several studies have investigated the association between the XPG Asp 1104 His Polymorphism and breast cancer however the result have been inconsistent. Kumar et al [9] found that genotype with C allele (His) was associated with a 1.5 fold increased risk for breast cancer in Europeon subjects (OR-1.5 95% CI- 1.04-2.16) in 2003. By contrast Ming Shiean et al [10] G allele variant(Asp) to be significantly associated with breast cancer in Asian population (OR-1.42, 95% CI-1.08-1.97), which was in agreement with present study. XIAO-Ming XO

et al 2014 concluded no association between the polymorphism and breast cancer. But large scale epidemiological studies were required to validate these conclusions. Sangrajray, 2007 suggested that XRCC3 Thr 241 Met polymorphism was likely to play a modifying role in individual suceptibility to breast cancer among thai women [11]. Smith TR et.al 2003 suggested that aminoacid substitution variant of XRCC1 and XRCC 3 genes may contribute to susceptibily breast cancer [12]. Jacobsen et.al, 2003 [13] concluded that there was no association between XRCC3 241 genotypes and risk of breast cancer which was in agreement with the present study, and the study suggested that the result may reflect that gene–environment interactions are required for that a putative linkage to the effective mutation differ between ethnic groups or that polymorphism was not important for development of basal cell carcinoma. The finding of the present showed no significant association of XRCC3 polymorphism with neither the breast cancer nor the population at high risk for breast cancer, which was not in agreement with some studies[11,12] .

CONCLUSION

DNA repair genes are regions of DNA which repair the damage rapidly and effectively. Polymorphism occurring in only specific regions of these genes is effective not only in gynecological cancers, also in the development of many organ tumors. Herein, in our study this gene polymorphism is found to be statistically significant at higher rate in patients with breast cancer.

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