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Genetic polymorphism in *ERCC5* and breast cancer risk

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ABSTRACT

ERCC5 plays crucial role in excision repair DNA damage induced by UV in NER pathway. Single neuleotide polymorphism in *ERCC5* were responsible for different cancers. Therefore, current study evaluated the relationship between *ERCC5* (rs1047768 T>C) polymorphism and the risk of breast cancer in Pakistani population. The rs1047768 polymorphism was screened among 175 females including one hundred breast cancer cases and age matched seventy-five healthy controls. Genotyping was performed with Tetra amplification-refractory mutation system (ARMS) PCR and products were observed through electrophoresis. Multivariate logistic regression was used to calculate odds ratio (OR) and 95% confidence interval (95% CI) investigating relationship between genotypes, clinical parameters and risk of breast cancer. Statistical analysis exhibited significant relationship between the TC genotype (OR=7.2, 95% CI=1.5-34.3) and increased breast cancer risk. Moreover, family history (OR=6.25; 95% CI= 2.61-15.00) and late menopause (OR=2.41; 95% CI=1.20-4.83) were found to be breast cancer associated risk factors. In conclusion, *ERCC5* (rs1047768 T>C) polymorphism may contribute towards increased risk of breast cancer in Pakistani population.

Keywords: *ERCC5*; breast cancer; NER; ARMS-PCR

INTRODUCTION

Breast cancer is commonest malignancy among women and foremost cause of cancer related morbidity and mortality throughout the world [1]. Underlying mechanism of breast carcinogenesis is still not completely understood. Low penetrance susceptibility genes along with environmental factors plays a complex interaction in breast cancer development [2]. DNA repair systems play fundamental roles in the maintenance of genome integrity and protecting normal cells against genetic alterations. Various genetic polymorphisms among genes responsible for DNA damage responses contribute towards cancer development and linked with proliferated cancer risk. Genes linked with DNA repair mechanisms have been considered as candidate genes for cancer susceptibility because decreased DNA repair efficiency may initiate

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carcinogenesis [2, 3]. Nucleotide excision repair pathway (NER) is most adaptable and multifaceted DNA repair mechanism and is involved in the removal of helix distorting DNA lesions from the genome. It counteracts the harmful effects caused by mutagenic exposure of cells by recognizing the lesion, protein binding, excision of oligonucleotides and reconstruction of DNA fragment. The NER pathway comprises of more than thirty proteins and among them seven are xeroderma pigmentosum (XP) complementation groups illustrating malfunctioning proteins [4, 5].

ERCC5 gene is an indispensable component of NER pathway and encodes a "structure-specific endonuclease which catalyses 3` incision and involves subsequent 5` incision" with the help of *ERCC1-ERCC4* heterodimer [6]. Few evidences suggested that polymorphism of *ERCC5* (MIM # 133530) variant, rs1047768 (T>C) plays an important role in carcinogenesis and yield varied survival outcomes [7]. It results in a coding synonymous polymorphism His46His. Association of gene polymorphism with carcinogenesis might be described by its linkage with various other non-synonymous polymorphisms or its precise impact on confirmation of enzyme leading to altered substrate specificity or activity. Association of nucleic acid repair genes was evaluated by different studies, but the results were inconclusive [3, 5, 7, 8]. Therefore, we designed a study to explore the relationship of *ERCC5* (rs1047768) polymorphism with breast cancer development. Furthermore, relationship of several clinical factors with the onset and development of breast cancer was illustrated.

MATERIALS AND METHODS

At first study gets approval from "IRB (institutional review board) and ethical committees of Fatima Jinnah Women University and different hospitals including Holy Family Hospital, Benazir Bhuto Shaheed Hospital and District Headquarter Hospital, Rawalpindi". In the current study 100 diagnosed breast cancer patients along with age matched 75 healthy females were recruited over a period of one year (May 2017– April 2018). Blood samples and detailed clinical history was collected by interviewing the patients and controls after taking informed consent.

The rs1047768 was positioned in DNA region with average CG in exon 2 of *ERCC5* placed on chromosome 13 and has T>C polymorphism. Tetra ARMS-PCR primers were made [9] with default primer settings. DNA was extracted manually by phenol chloroform extraction method [10] and the genotypes of rs1047768 was detected by Tetra ARMS-PCR [11]. Amplified PCR product was visualized using gel electrophoresis.

Hardy Weinberg equilibrium was used to test goodness of fit by chi square test for genotype distribution. Quantitative variables were expressed by mean ± standard deviation. Multivariate logistic regression analysis was used to calculate odds ratio (OR) and 95% confidence interval (95% CI) investigating relationship between genotypes, clinical parameters and risk of breast cancer. Correlation of other clinical features like age, age at menarche, family history and menopause with breast cancer was also evaluated. $P<0.05$ was considered as significant and calculated using 2 tailed test. SPSS version 24 and MedCalc were used to perform statistical analysis.

RESULTS

Clinicopathological characteristics of breast cancer cases and controls are explained in Table 1. Mean age of breast cancer cases and controls were 45.9 ±11.6 and 42.5±12.2 years respectively. There was non-significant difference among cases and controls in term of age and age at menarche. Breast cancer cases were more likely to have positive family history of breast cancer, and late menopause as compared to controls. Further, genotype distributions of *ERCC5* was found more prevalent for TC and CC among breast cancer cases as compared to controls.

Table 1: Distribution of selected variables between breast cancer cases and controls

Variables	Cases (n=100)		Control (n=75)		P- Value
	n	%	n	%	
Age (mean ± SD)	45.9±11.6		42.5±12.2		
Age at menarche (mean ± SD)	12.0±0.8		12.1±0.7		
Family history					
Yes	42	42	8	10.7	0.000
No	58	58	67	89.3	
Menopause					
Yes	46	46	23	30.7	0.040
No	54	54	52	69.3	
ERCC5					
TT	78	78	72	96	0.003
TC	18	18	2	2.7	
CC	4	4	1	1.3	

Multivariate logistic regression analysis was used to find out the linked risk factor of breast cancer. Results showed that TC genotype was significantly associated with elevated risk of breast cancer (OR=7.16; 95% CI=1.49-34.25). Moreover, positive significant association was found for family history of breast cancer (OR=6.25; 95% CI+2.61-15.00) and late menopause (OR 2.41; 95% CI 1.20 to 4.83) with breast cancer risk. Whereas, age at menarche was not significantly linked with breast cancer risk (Table 2). Forest plot analysis was used to depict associated risk factors for breast cancer.

Table 2: Multivariate logistic regression analysis of risk factors associated with breast Cancer

Variables	OR	95% CI	P (Wald's test)
TC	7.16	1.49-34.25	0.013
CC	3.68	0.34-39.26	0.279
TT	0.515	0.03-8.34	0.641
Family history	6.25	2.61-15.00	<0.001
Menopause	2.41	1.20-4.83	0.013
Age at menarche	1.07	0.69-1.65	0.749

DISCUSSION

Current study explored the relationship of *ERCC5* rs1047768 polymorphism with breast cancer and associated risk factors. Current study reported that increased breast cancer risk was linked with positive family history of breast cancer concordant with already reported literature [12]. While evaluating that late menopause and early menarche were linked with increased breast cancer risk it was found that late menopause was statistically related with increased risk of breast cancer [13].

Genetic alterations which affect gene expression regulation can impart to the differences among individuals in susceptibility to risk of disease and its severity. Regulation of nucleotide excision repair pathway is crucial to maintain genome integrity. *ERCC5* is a multi-functional gene in NER pathway encoding for a structure specific endonuclease [14]. Single nucleotide polymorphisms in coding region of *ERCC5* results in elusive alteration of *ERCC5* activity which may lead to increased cancer susceptibility [15]. Studies have described association of *ERCC5* gene polymorphisms with various cancers [6]. Liang et al. reported that *ERCC5* rs7655 may not contribute in the development of lung cancer [16] whereas, it was significantly linked with increased laryngeal cancer risk [17]. Similarly, another case-control study showed

significant association of rs229647 and rs751402 polymorphisms with gastric cancer [18]. A meta-analysis reported significant association of rs1047768 with lung cancer in a stratified analysis [6]. It was reported that *ERCC5* rs1047768 polymorphism with C allele promotes sensitivity to platinum-based chemotherapy [19]. Another study reported significant association of rs751402 polymorphism with risk of oral cancer [20]. A meta-analysis showed that *ERCC5* gene polymorphism contribute towards the development and severity of colorectal cancer [4]. Literature had shown inconsistent results and unable to generate a conclusion. Discrepancies between results of already reported studies may be due to differences in study populations, design and tumour types.

Current study reported statistically significant association of *ERCC5* rs1047768 polymorphism with breast cancer. Whereas, no association was found for CC genotype, although it was more common in breast cancer patients in comparison with controls concurrent with already reported literature [21]. Na et al., had reported no association of *ERCC5* rs1047768 polymorphism with breast cancer among Chinese population [8]. Literature is limited in this area therefore, studies with larger sample and more precision are prerequisite to get pronounced results.

In conclusion, *ERCC5* (T>C) polymorphism may contributes towards amplified breast cancer risk. Furthermore, family history and late menopause are contributing factors in breast cancer development.

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Conflict of Interest: None

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