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EXPERIMENTAL WORKS

Role of XPG Gene Polymorphism towards Colorectal Cancer Susceptibility: A Case Control Study

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Abstract—XPG protein is a crucial component of the nucleotide excision repair pathway. Various single nucleotide polymorphisms of XPG gene have been reported to modulate colorectal cancer susceptibility. Therefore, this case control study evaluated the association of XPG (rs1047768 T>C) polymorphism with risk of colorectal cancer. In this study total 175 individuals comprising of age matched one hundred pathologically confirmed colorectal cancer cases and seventy-five controls were genotyped for XPG (rs1047768 T>C) polymorphism. Genotyping was accomplished with "Tetra amplification-refractory mutation system (ARMS) PCR" and PCR products were electrophoretically resolved on agarose gel. To validate the results 10% samples were re-analysed for XPG genotyping. Demographic factors were represented by mean ± SD. Multivariate logistic regression analysis was applied to compute odds ratio and confidence interval considering association between genotypes, demographic factors and colorectal cancer risk. Results were computed by MedCalc and SPSS version 24. Results showed significant association of XPG rs1047768—T>C genotype (OR: 7.51, CI: 1.63–35.02) and increased risk of colorectal cancer. Additionally, smoking and family history were significant contributors in colorectal cancer development. In summary, XPG (rs1047768 T>C) polymorphism is a low susceptibility penetrance gene for colorectal cancer and has never been screened before in Pakistani population.

Keywords: XPG, colorectal cancer, nucleotide excision repair, ARMS-PCR

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INTRODUCTION

Colorectal cancer is the third most pften diagnosed malignancy in all age groups and fourth leading cause of cancer related mortality throughout the world [1]. Underlying mechanism of colorectal cancer development is still not completely understood. Low penetrance susceptibility genes along with environmental factors plays a complex interaction in its 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 associated with increased cancer risk. Genes linked with DNA repair mechanisms have been considered as candidate genes for cancer susceptibility because decreased DNA repair efficiency may initiate genetic instability and carcinogenesis [3, 4].

Nucleotide excision repair (NER) pathway 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. NER pathway comprises of more than thirty proteins and among them seven are xeroderma pigmentosum (XP) complementation groups (ranging from XPA to XPG) representing malfunctioning proteins [5, 6].

XPG gene is an indispensable component of NER pathway. It encodes a "structure-specific endonuclease which catalyses 3' incision and involves subsequent 5' incision" with the help of ERCC1-XPF heterodimer [7]. Few evidences suggested that polymorphism of XPG variant, rs1047768 (T > C) plays an important role in carcinogenesis and yield varied survival outcomes [8]. It results in a coding synonymous polymorphism His46His. Association of gene polymorphism with cancer may be explained by its linkage with various other non-synonymous polymorphisms or its precise impact on enzyme confirmation leading to altered substrate specificity or activity. Association of nucleic

Table 1. Distribution of selected variables between colorectal cancer cases and controls

Variables	Cases (n = 100)		Control (n = 75)		P-value
	n, %		n, %		
Age (mean ± SD)	45.99 ± 11.64		42.57 ± 12.23		
Family history					
Yes	42	42	8	10.7	0.000
No	58	58	67	89.3	
Smoking					
Yes	46	46	23	30.7	0.040
No	54	54	52	69.3	
XPG					
TT	78	78	72	96	
TC	18	18	2	2.7	0.003
CC	4	4	1	1.3	

acid repair genes was evaluated by different studies, but the results were inconclusive [8–11].

Therefore, we planned to explore the involvement of XPG (rs1047768 T>C) polymorphism in colorectal cancer development. Furthermore, association of various clinical parameters with the onset and development of colorectal cancer was illustrated.

MATERIALS AND METHODS

Study was approved from ethical committees of "Fatima Jinnah Women University and Rawalpindi Medical University along with its affiliated hospitals including Holy Family Hospital, Benazir Bhuto Shaheed Hospital and District Headquarter Hospital, Rawalpindi." A total of 100 histopathologically diagnosed colorectal cancer cases along with age matched 75 healthy controls 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.

SNP rs1047768 was situated in DNA region with average CG in exon 2 of XPG gene placed on chromosome 13 and has T>C polymorphism. Tetra ARMS-PCR "tetra-primer amplification refractory mutation system-polymerase chain reaction" primers were designed using a program developed by Ye et al. [12] and all default primer settings were used for genotyping. DNA was extracted by phenol chloroform extraction method [13]. In tetra ARMS-PCR, fragment generated by outer primers was used as a template for inner primers to produce allele specific fragments [14]. Therefore, outer primers were first optimized separately, and then complete reaction was performed with both outer and inner primers. Amplified PCR product was visualized using gel electrophoresis.

Age for both colorectal cancer cases and controls was expressed by mean ± standard deviation. Multivariate logistic regression analysis was applied to compute odds ratio and 95% confidence interval exploring association between genotypes, clinical details and colorectal cancer risk. Correlation of other clinical features like age and family history with colorectal cancer was also evaluated by 2 tailed test considering $P \leq 0.05$ as statistically significant. SPSS version 24 and MedCalc was used to perform statistical analysis.

RESULTS

Clinicopathological features of colorectal cancer cases and controls are briefed in Table 1 including age, family history of cancer and XPG rs1047768 polymorphism. Mean age of colorectal cancer cases and controls were 45.99 ± 11.64 and 42.57 ± 12.23 years respectively. There was non-significant difference between colorectal cancer cases and controls in term of age as we tried to enrol age matched cases and controls. Colorectal cancer cases were more likely to have positive family history of respective cancer as compared to controls. Greater percentage of cases were smokers than controls. Further, genotype distributions of XPG polymorphism were more prevalent for TC (heterozygous) and CC (homozygous mutant) among colorectal cancer cases as compared to controls (Fig. 1).

Multivariate logistic regression analysis was used to find out the associated risk factors of colorectal cancer. Results showed that TC genotype was significantly associated with increased risk of colorectal cancer (OR 7.51; 95% CI 1.63–35.02). Though homozygous mutant i.e., CC genotype was found to be more common among colorectal cancer cases than controls, but it was calculated to be statistically non-significant (OR: 3.7, CI: 0.34–39.26). Moreover, positive significant association was found for family history of col-

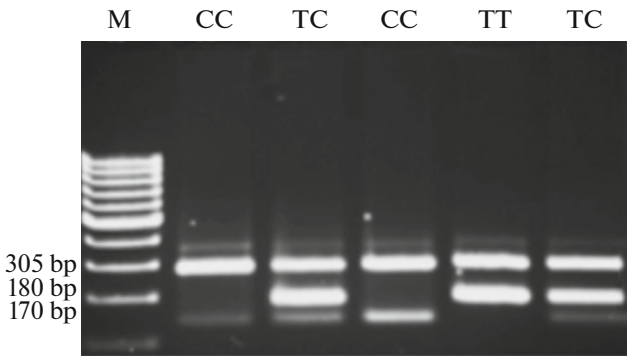

Fig. 1. Electropherogram representing XPG rs1047768 polymorphism in colorectal cancer cases.

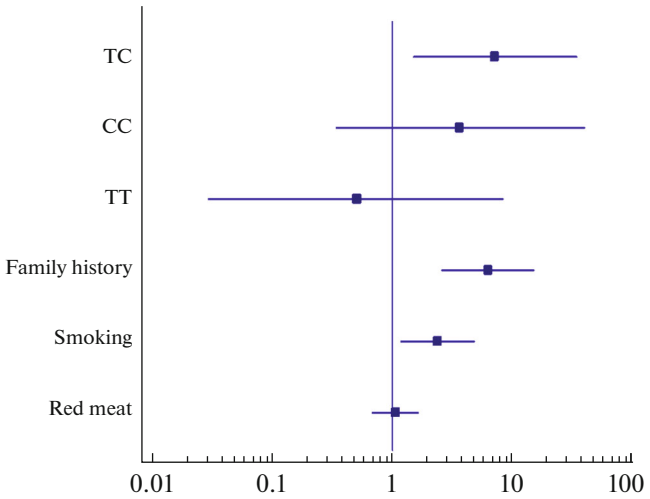

Fig. 2. Forest plot measuring the risk factor of colorectal cancer.

orectal cancer (OR 6.25; 95% CI 2.61–15.00) and smoking (OR 2.41; 95% CI 1.20–4.83) with increased colorectal cancer risk (Table 2). We are unable to find any association between intake of red meat (1 medium bowl per week) and colorectal cancer in our study population (Fig. 2).

DISCUSSION

In this study we explored the relationship of XPG rs1047768 polymorphism with colorectal cancer and associated risk factors. Current study reported that increased colorectal cancer risk was correlated with family history of colorectal cancer concordant with already reported literature [15]. While evaluating that smoking and red meat intake are associated with increased colorectal cancer risk it was found that smoking was statistically associated with increased risk of the disease [16]. Being an essential part of Western diet, red meat consumption like beef, lamb, pork and mutton is fairly high in most of the developed countries [17]. Therefore, in those countries red meat was reported to be a contributor in increasing incidence of colorectal cancer [18]. But we are unable to find any such association in our population probably because of less intake of red meat due to its high rates. As a developing and resource poor country, it's not possible for everyone to include meat in their weekly diet. Another possible reason might be the small sample size of the study.

Genetic alterations which affect gene expression regulation can contribute 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. XPG is a multi-functional gene in NER pathway encoding for a structure specific endonuclease [19]. Single nucleotide polymorphisms in coding region of XPG results in elusive alteration of XPG activity which may lead to increased cancer susceptibility [20]. Literature have reported association of XPG gene polymorphisms with various cancers [7]. Liang et al. reported that XPG rs7655 may not contribute in the development of lung cancer [21] whereas, it significantly associated with increased risk of laryngeal cancer illustrated by Lu and colleagues [22]. Similarly, another case-control study showed significant association of rs229647 and rs751402 polymorphisms with gastric cancer [23]. A meta-analysis reported significant association of rs1047768 with lung cancer in a stratified analysis [7]. It was reported that XPG rs1047768 polymorphism with C allele promotes sensitivity to platinum-based chemotherapy [24]. Another study reported significant association of rs751402 polymorphism with risk of oral cancer [25]. A meta-analysis showed that XPG gene polymorphism contribute towards the development and severity of colorectal cancer [5]. Literature had shown inconsistent results and unable to generate a conclusion. Discrepancies

Table 2. Multivariate logistic regression analysis of risk factors associated with colorectal cancer

Variables	Colorectal cancer	*P* (Wald's test)
	OR (95% CI)	
TC	7.51 (1.63 to 35.02)	0.0137**
CC	3.68 (0.34 to 39.26)	0.2793
TT	0.515 (0.03 to 8.34)	0.641
Family history	6.25 (2.61 to 15.00)	0.0001***
Smoking	2.41 (1.20 to 4.83)	0.0131**
Red meat#	1.07 (0.69 to 1.65)	0.7490

***$P < 0.001$, **$P < 0.01$. CI: Confidence interval, #one medium bowl per week.
Null model –2 log Likelihood 239.018.
Full model –2 log Likelihood 200.576.
Cox & Snell R2: 0.1972.
Nagelkerke R2: 0.2648.

between results of already reported studies may be due to differences in study populations, design and tumour types.

CONCLUSIONS

Current study reported statistically significant association of XPG heterozygous T>C polymorphism with colorectal cancer. Whereas, no association was found for homozygous mutant CC genotype, although it was more common in cases than controls. Literature is limited in this area therefore, studies with larger sample and more precision are prerequisite to get pronounced results. Briefly, XPG rs1047768 polymorphism may contributes towards increased colorectal cancer risk. Furthermore, family history and smoking are contributing factors in colorectal cancer development.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans. All applicable international, national, and/or institutional guidelines were followed.

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