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Association of Adenometous Polyposis Coli (APC) I1307K VariantPolymorphism with Colorectal Cancer among Selected Pakistani Population

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ABSTRACT

Colorectal cancer is the result of abnormal growth of cells in colon and rectum. It is the major cause of mortality and morbidity globally. Among all cancer occurrence, CRC accounts for over 9%. In women, colorectal cancer is the seventh most common cancer and the ninth most common cancer in men, in Pakistan. Numerous risk factors are responsible for colorectal cancer, mainly age, smoking, alcohol consumption, family history and physical inactivity. Colorectal cancer due to genetic alterations occurs approximately with rate of 10% while sporadic colorectal cancer occurs with the rate of 90%. Adenomatous Polyposis Coli (APC) is a tumor suppressor gene and mutation in this gene downregulates the β-catenin which in turn activates the transcription of oncogenes. The objective of the present study is to analyze the significant association of APC I1307K (T→A) polymorphism with colorectal cancer in selected Pakistani population. This study includes 100 sporadic samples of CRC and 50 healthy controls. DNA was extracted from all CRC samples and controls. ARMS-PCR was performed to genotype APC I1307K polymorphism. Among three genotypes, TT, TA, AA, only two genotypes TT and TA were found in current study. Occurrence of APC I1307K was found to be significantly greater in CRC patients compared to controls (P-value 0.0000). Calculated allelic frequency of A allele was higher in patients (0.65%) than controls (0%). On the basis of significant statistical results, we may say that in our selected population, an association between APC I1307K variant and colorectal cancer was present.

Introduction:

Colorectal cancer is one of the utmost malignancies of humans. Among all cancer occurrences, CRC accounts for over 9% [1]. Several risk factors are responsible for colorectal cancer, mainly age, smoking, alcohol consumption, family history and physical inactivity. It is recognized as a multifactorial cancer because of the involvement of both genetic and environmental factors [2]. Colorectal cancer due to genetic alterations occurs approximately with rate of 10% while sporadic colorectal cancer occurs with the rate of 90% [3].

Colorectal cancer accounts for 6% of heritable germ line mutations [4]. Mutation in Adenomatous Polyposis Coli (APC) gene (tumor suppressor) is inherited in Familial Adenomatous Polyposis patients (FAP). APC is present on chromosome 5q22.2 [5]. This tumor suppressor gene contains 15 exons encoding 2843 amino acids and spans 58kb [6]. 75% of coding sequence of APC lies in the region of exon 15 (7). A large protein encoded by APC gene is involved in suppressing tumor activities and various cellular functions (8). 1A and 1B are the two promoter regions of APC gene (9). The 1A promoter of APC gene has been shown to be heavily methylated in colorectal tumors (10). Tumors with promoter hyper methylation fail to express the transcripts of APC (11). There is no evidence for epigenetic mechanism with the APC 1B promoter. It suggests that APC promoter 1A hyper methylation may provide an alternative mechanism of APC inactivation in the early stages of colorectal tumorigenesis..

Two types of mutations have been found in APC gene including germline mutations and somatic mutations. Mutations between codons 1061 and 1309 are most common germline mutations in APC that account for a third of all the germline mutations (12,13). Loss of the wild type APC allele causes somatic mutations in most of the sporadic cancer (12,14). Colorectal adenomas of <5mm in size have been seen to show somatic mutations (15). There is a region in the coding sequence of APC gene between codons 1286 and 1513, termed as mutation cluster region (MCR), where over 60% of all somatic mutations occur in APC (12). A truncated APC protein is formed when a mutation occur in MCR of APC gene, this truncated protein lacks all the binding sites for axin and one or two of its 20 amino acids of beta-catenin binding sites.

Numerous Single Nucleotide Polymorphisms (SNPs) of APC gene have been identified. In a particular population, biological and epidemiological data showed that several SNPs attributes to the development of increased risk of CRC (2). Variant I1307K of APC causes the formation of cancer and in Ashkenazi Jewish population it is directly involved in 3-4% of all colorectal cancer (16). APC I1307K is a missense mutation and is involved in the substitution of Isoleucine (I) by Lysine (K). Three variants of APC gene, E1317Q (G→C), D1822V (A→T) and I1307K (T→A), have been found to be associated with the occurrence of CRC in previous studies. In studies of association between APC gene and risk of CRC occurrence, E1317Q and I1307K variants of APC gene are most common (17,18). It indicates that this site might be a functional variant. On the basis of previous studies, variant at position I1307K (rs1801155) of APC gene is selected to conduct this population study.

Methodology:

This is a case control study comprising of 100 CRC patients (59 males and 41 females) along with 50 healthy unrelated individuals. Study was performed on selected Pakistani population. An informed consent was obtained from all the blood donors. The clinical data (age, gender, family history of CRC, smoking history) of CRC patients were obtained through medical records and through patients. For the analysis of association of APC with colorectal cancer, genomic DNA was extracted from all the samples (CRC patients and healthy individuals). For genotyping of APC *I1307K*, PCR with Amplification refractory Mutation System **(**ARMS) assay was used. For the identification of APC *I1307K*, two forward primers were used; one specific for common allele (5'-CTAATACCCTGCAAATAGCAGAAGA-3'), other specific for mutant allele (5'-CTAATACCCTGCAAATAGCAGAAGT-3') and a common reverse primer (5'-TGAGTGGGGTCTCCTGAACATA-3') was used for both forward primers. Reactions for wildtype and mutant allele were prepared in separate tubes. Initial denaturing temperature of ARMS-PCR was 96°C for 1min, annealing temperature was 57°C for 1min followed by 30 cycles and the final extension temperature was 72°C for 5min. Products obtained from ARMS-PCR were analyzed on 3% agarose gel stained with ethidium bromide. Gel was prepared by inserting two combs. Products for wildtype allele were loaded in upper row and products for mutant allele were loaded in lower row to differentiate both alleles easily. To analyze the band size 100bp DNA ladder (O'GeneRuler) was used. After loading the samples, voltage was supplied and then gel was analyzed on Gel Documentation System.

Statistical Analysis:

For the interpretation and analysis of data, IBM SPSS software was used. Chi-square and p-value for age, gender and histopathology were calculated. Genotypic and allelic frequencies were also calculated. A minimum level of statistical significant value was considered at a p level of <0.05.

Results:

This study showed three genotypes, two genotypes, homozygous major TT and heterozygous TA have been identified in patients and only one genotype, homozygous major TT have been found in control cases and not a single homozygous minor genotype AA have been seen in both patients and controls. Genotypic and allelic frequencies of the APC I1307K polymorphism in CRC patients were calculated. Analysis showed that patients had higher frequency of TA genotype (65%) compared to controls (0%). To determine the significant association of APC I1307K with CRC, t-test was applied. The value of t-test was 0.000 ($p<0.005$). The resultant value showed that this is a significant study. Allelic frequencies of CRC patients showed a significantly higher frequency of APC *I1307K* A allele (0.335%) compared to controls (0%). This analysis revealed significant statistical difference.

All the three genotypes were also analyzed in male and female CRC patients and controls. Genotype TT was found to be 21 and 14 in CRC males and females respectively while TA genotype was found to be 38 and 27 in CRC males and females respectively. TA genotype was not found in control group. Genotype AA was not present in any of the member from patient and control groups. The value of odd ratio for gender was, OR 0.938 with 95% CI (0.406-2.167). Chi-square test was applied to gender to find out association between gender and CRC. The value of Chi-square was 0.22 with p-value 0.881. It was found that p-value was greater than the standard significant p-value (<0.05). Among all the 100 CRC individuals, frequency of colon and rectum cancer was also calculated. Number of patients with rectum cancer (54%) was found to be higher than the patients with colon cancer (46%). This result showed that rectum cancer is more prevalent. Analysis of data showed that colorectal cancer is more common between the age group of over 30 and below 65. This age group had both genders. The calculated mean and standard deviation for age was 44.17±12.96. The chi-square value for age was 3.846 and p-value

was 0.697. As p-value was non-significant, it can be concluded that age might not be the most relevant risk factor for developing CRC. In this study, histopathology reports of CRC patients were also collected. According to histopathology reports, 24% patients had well differentiated adenocarcinoma, 27% patients had moderately differentiated adenocarcinoma and 49% patients had poorly differentiated adenocarcinoma. Chi-square value of histopathology was 28.423 with p-value 0.000. This statistical analysis showed that most of the patients found to have poorly differentiated adenocarcinoma.

Discussion:

Third most frequently occurring cancer is colorectal cancer and it is considered as fourth foremost reason of mortality rate globally (19,20). APC gene is a tumor suppressor gene and when altered causes cancer. It is evident from a study that APC gene contains germ-line variants that can give rise to development of colorectal cancer (21).

The aim of this study was to determine the role of APC I1307K with Colorectal Cancer in selected Pakistani population. Total 100 samples were collected to conduct the study along with the 50 controls. Current study showed association of APC I1307K with CRC by giving P-value 0.00 that was less than the standard P-value 0.05. A study on population of Egypt also showed significant association of APC I1307K with CRC (22). Previously, 8.7% I1307K mutation was found in patients of CRC, this study also reported that mutation was found to be greater (11.2%) in Ashkenazi Jews (23). Another study also reported that Ashkenazi Jews had 6.1% of APC I1307K mutation (24,25). Previously, Ashkenazi Jews (10.4% patients of CRC) have been identified to carry this mutation (26). Some studies are contradicted to the above mentioned studies reporting no association of APC I1307K with CRC. No association was found between APC I1307K and CRC in Tunisian population (27) and a study conducted also showed null association between APC I1307K and CRC (28).

In present study, allelic frequencies were calculated for patient and controls. Allele T showed frequency of 0.675% in CRC patients and 2% in controls while A allele showed 0.65% frequency in CRC patients and 0% frequency in controls. According to a study, A allele of APC I1307K was found higher in Patients (10.4%) than controls (4.5%) (22). Two genotypes, TT and TA were identified in present study. These genotypes were analyzed in patients and controls

along with the comparison in gender. In males, TT genotype was higher (0.21%) than females (0.14%) in CRC patients. TA genotype was also found higher in males (0.38%) than females (0.27%) in CRC patients and this genotype was absent in control cases (male and female). Risk of occurrence of colorectal cancer is higher in men (4.7%) than women (4.4%). In men, CRC occurrence found to be more common (30-40%) than women. In current study, rectum cancer (54%) was found to be more frequent than colon cancer (46%). It has been found that colorectal cancer occurs in different ratios with the advancing age. Previously it has been reported in a study of 100 samples that 52% patients had colon cancer and rest of 48% had rectum cancer (29).

In present study, age was one of the parameter. This study showed that most of the patients with the age group above 30 and below 65 were found to have more mutations than the age group below 30. The calculated mean and standard deviation for age was 44.17±12.96. It is reported that with age, risk of CRC and mortality rate gets higher. In colon cancer, median age was 69 in males and 73 in females, in case of rectal cancer; average age was 63 in males and 65 in females (Howlader *et al.*, 2013 (30). According to previous study, APC I1307K mutation was found with mean age at 65.0 +/– 9.7 years (31). Previously a study showed that I1307K mutation was more frequent at the mean age 70.2 ± 1.2 years (25). This study showed that most of the patients carrying I1307K allele were poorly differentiated (49%) while 24% and 27% patients were found to have well differentiated and moderately differentiated adenocarcinoma, respectively. In a study, most of the patients of CRC were moderately differentiated (74.2%), 4.2% and 8.3% were found to have well differentiated and poorly differentiated adenocarcinoma (22).

The APC I1307K polymorphism may confer increased risk of colorectal cancer in different ethnic populations. It is evident from studies that loss in functionality of APC leads to the development of colorectal cancer. Current study was designed to determine the association of APC gene with colorectal cancer in selected Pakistani population that has not been performed earlier. By viewing at statistical results and discussion mentioned above, it can be concluded that APC I1307K variant is involved in developing risk of colorectal cancer. Results of the study provide more systematic picture of the role of APC polymorphisms in the risk of developing colorectal cancer and may provide genetic insight into possible strategies for the prevention of colorectal cancer. In conclusion, taken together, this study supports the association of APC I1307K variant with an increased risk of CRC among selected Pakistani population.

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Table 1: Clinical and demographic characteristics of APC I1307K variant among the patients of colorectal cancer

Characteristics	CRC Patients
Age, years, M ± SD	44.17±12.96.
Gender • Male • Female	 59 41
Tumor Location • Colon • Rectum	 46 54
Histological Grade • Well Differentiated • Moderately Differentiated • Poorly Differentiated	 24 27 49

Table 2: APC I1307K genetic polymorphism among CRC patients

Genotypes	CRC Patients
TT	35
TA	65
AA	0
Allelic Frequency	**CRC Patients**
T	0.675%
A	0.335%