

# Correlation of Homologous Recombination Repair System by Studying a Single-nucleotide Polymorphism in XRCC3 Gene with Initiation and Progression of Colorectal Cancer

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## ABSTRACT

**Background:** Colorectal cancer is the third cause of cancer death in western countries. Age, inadequate diet, obesity, inactivity and genetic changes are some of the risk factors of colorectal cancer. Correlation of genetic diversity in homologous recombination repair system with cancer was evaluated in many recent studies. This study was done to investigate the correlation of T241M polymorphism in XRCC3 gene and colorectal cancers.

**Methods:** In this case-control study, after collecting blood samples and extracting genomic DNA, genotype distribution of the polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

**Results:** A significant correlation was observed between T241M polymorphism and colorectal cancer. Age and family history were also found to be correlated with this cancer. Although no statistically significant relationship has been found between smoking and colon cancer, our findings showed a correlation between smoking and rectum cancer. Furthermore, we observed that the most occurrence of metastatic activity was in the rectum.

**Conclusion:** According to our study, T241M polymorphism in XRCC3 gene from homologous recombination (repair system??) could be a suitable factor for early diagnosis of colorectal cancer, while its co-operation with smoking is also regarded as a risk factor for rectum cancer.

**Keywords:** *Colorectal Cancer, Homologous Recombination Repair System, XRCC3 Gene, PCR-RFLP.*

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## Introduction

Any cancer occurred in the colon, rectum, and appendix is referred to as colorectal cancer. This kind of cancer, with mortality rate over 65,500 people per year, is the fourth most common form of cancer in the United States and the third common cancer resulting in death in Western countries.<sup>1</sup> In Iran, the colorectal cancer is the third and the fourth common cancer diagnosed in men and women, respectively. Based on the previous studies conducted in Iran, the approximate number of individuals with colorectal cancer is 3641 people each year of whom 2,262 people died from this cancer, which comprises 6.3% of the total cancer mortality in Iran.<sup>2</sup> The risk factors of the colorectal cancer are older age, high-fat and low-fiber diets, heavy consumption of alcohol, obesity, inactivity, exposure to radiation, and smoking,<sup>3</sup> whereas it has been shown that a high intake of fiber diet, including fruits and vegetables, along with physical activity and exercise reduces risk of cancer.<sup>4-6</sup> (The highest prevalence of this type of cancer belongs to countries eating foods containing saturated fat and red meat, yet low in fruits and vegetables along with low levels of physical activity, which is similar to diets of the Western countries.<sup>7</sup> High-fat and low-fiber diets along with low levels of physical activity prolong the movement of food through the intestine, and consequently, the epithelial cells of colon and rectum are exposed to mutagens for longer period of time.<sup>8</sup>

The changes in gastrointestinal behaviors and habits, the gastrointestinal dysfunction, and presence of blood in stool due to the presence of a tumor in the rectum are considered to be the major symptoms of the colorectal cancer. The changes in the number of bowel movement and the prolonged time of defecation are also among the symptoms of this type of cancer. Other symptoms of the disease include diarrhea, constipation, and increased flatulence. Furthermore, abdominal pain, anemia, and weight loss may be symptoms of the disease progression and advanced stage of the tumor.<sup>9</sup> The cancer begins with benign adenomatous polyps that can be developed into an advanced adenoma with high-grade dysplasia and then into an invasive form of cancer.<sup>10</sup> Colorectal cancer may become metastatic and spread to the lymph nodes, liver, lungs, peritoneum, ovaries, and brain.<sup>9</sup> Invasive cancers

limited to the colonic wall (the tumor metastatic nodes, stages I and II) are treatable. However, if they are not treated, they spread to the lymph nodes (stage III), then, metastasize to farther parts.<sup>11,12</sup> The most effective treatment for stages I and II is known to be an excision surgical method, whereas the effective treatment for over 73% of the stage III cases involves surgical operations along with adjuvant chemotherapy.<sup>11, 13, 14</sup> Although recent developments of chemotherapy have increased the chance of patient survival, treatment of the colorectal cancer in patients mainly depends on the stage of the disease at diagnosis, while the stage IV of the disease is normally incurable.<sup>11,14</sup> The depth of the invasion of cancer cells into the gastrointestinal tract wall, the presence of these cells in lymph nodes, and involvement of other parts of the body are important factors in diagnosing the disease. Diagnosis of the disease prior to its progression and metastasis of malignant cells outside the gastrointestinal tract and in the lymph nodes is likely to prevent unnecessary surgeries.<sup>15, 16</sup> Almost 50% of the patients with colorectal cancer have been diagnosed at stage I or II, while the cancer cells have been treated only through the surgical operation and excision of the tumor.<sup>17</sup>

The colorectal cancer actually results from progressive accumulation of genetic and epigenetic abnormalities, leading to transformation of the normal colonic epithelium into adenoma, and consequently, into carcinoma<sup>18,19</sup> At least two major pathways are known to cause colorectal cancer. About 85% of colorectal cancer cases are caused by the events that lead to chromosomal instability, aneuploidy and early inactivity of adenomatous polyposis coli (APC), which is similar to the incidence of the familial adenomatous polyposis (FAP). The remaining 15% of colorectal cancer cases are caused by the events resulting in microsatellite instability and dysfunction of mismatch repair genes. This case is similar to hereditary non-polyposis colorectal cancer (HNPCC).<sup>19-21</sup> Based on some studies, certain low-penetrance genes are influential in incidence of colorectal cancer when combined with some dietary or lifestyle factors.<sup>22, 23</sup> Studies have been performed on the correlation of some polymorphisms in low-penetrance genes with cancers, including colorectal cancer. One of those genes is X-ray repair complementing defective repair in Chinese hamster cells 3 (XRCC3) participating in the homologous recombination (HR) in-

involved in DNA repair pathway.<sup>24</sup> XRCC3 is a protein essential for chromosomal stability and producing cellular resistance against ionizing radiation and some chemical agents. Besides the important role of XRCC3 in repairing the DNA double-strand breaks (DSBs) through the homologous recombination (HR), little is known about its biochemical properties and its specific function.<sup>25, 26</sup> The principle polymorphism in that gene is the replacement of the organic base thymine (T) for cytosine (C), and this includes the transformation of the amino acid threonine into the amino acid methionine at codon 241 in exon 7.<sup>27, 28</sup> In recent years, some studies have been conducted on the correlation of that polymorphism with different cancers, including the colorectal cancer. In this study, the correlation of T241M polymorphism with metastatic form of colorectal cancer and its interaction with some factors, such as age, sex, and smoking, were investigated in patients with colorectal cancer in the Seyed-al-Shohada Hospital in the city of Isfahan, Iran.

## Method and Methods

In this case-control study, blood samples of 90 male and female patients (case group) with colorectal cancer were collected from the Seyed-al-Shohada Hospital, Isfahan, Iran, when???. Also, 83 healthy people (control group) were selected as their age distribution matched to that of those patients to some extent. In order to extract the DNA from whole blood [with ethylenediaminetetraacetic acid (EDTA, modified Miller's salting out method was used.<sup>29</sup>

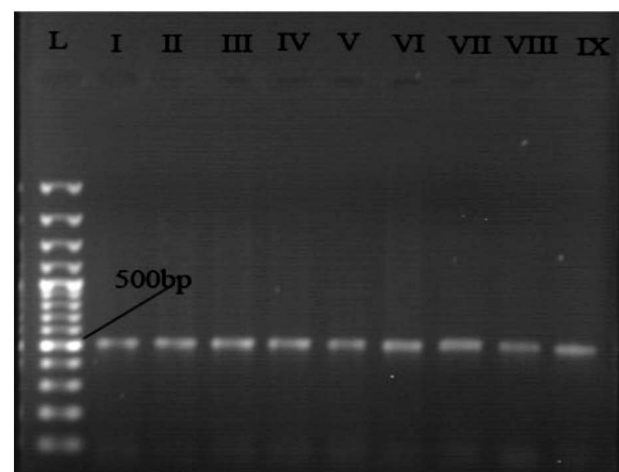
To replicate and to study the fragment containing T241M polymorphism in XRCC3 gene, a pair of primers was designed using the National Center for Biotechnology Information (NCBI) and search engines. In this regard, a 503-bp fragment of DNA was replicated around the polymorphic site using a polymerase chain reaction (PCR). **Figure 1** shows the shape of the replicated band on the agarose gel electrophoresis.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to determine the genotype of patient and control groups, and the PCR products were then digested by restriction enzyme N1aIII (Amount?; Company?, Country?). The recognition and cutting site of the enzyme was a 4-nuclotid sequence (5'-CATG-3'). In this study, recognition and

cutting were done at the polymorphic site for the individuals with T allele, whereas the cases with C allele had no recognition site for. N1aIII. Therefore, the genotype of the studied people was simply determined through the enzymatic cutting site, only in one polymorphic allele. Moreover, other factors and their interaction with the polymorphism were investigated using both the information collected through a questionnaire given to the participants and through their medical records. In statistical analysis of the results, the difference between genotypic distribution of the patients with colorectal cancer and that of the healthy people was examined using  $\chi^2$  test. In all analyses, the significance level was  $p < 0.05$ .

## Results

In this study, the age ranges of the patients with colorectal cancer and the healthy people were near to each other. Mean age values of the control and the case groups were 53.32 years and 53.16 years, respectively. Once the genotypes of the case and the control groups were determined, the genotypic distributions of the three genotypes were compared between two groups. In this regard, the results showed a significant difference between genotypic dis-



**Figure 1:** Agarose gel electrophoresis of PCR products. The figure shows nine bands of 503-bp replicated by reciprocal primers from nine genomic DNA samples selected randomly from the case and control groups. The samples I to V belonged to the case groups and samples VI to IX belonged to the control group. L: A 100 bp long DNA marker. The concentration of the agarose gel was 1%, and the electrophoresis procedure happened under constant voltage of 65 V within 1 hour and 30 minutes.

tributions of the two groups. Among the 90 patients, the genotypes T, CT, and CC involved 10, 45, and 35 people, respectively, while among the 83 healthy people, the three genotypes comprised 21, 37, and 25 people, respectively.

The number of individuals with one or two C alleles in the case group was higher than that in the control group. A more accurate analysis was performed to compare genotypic distributions of the genotypes CC and TT between the case and control groups. In this analysis, the odds ratio (OR) and confidence interval values were obtained as 2.940 and 95% (1.194-7.22), respectively, indicating the frequency of the genotype CC in the patients was three times more as compared to the related value in the healthy people ( $p=0.018$ ). In this respect, our findings revealed that the genotype CC increased the risk of the colorectal cancer three times as did the genotype TT. Furthermore, in comparison of the genotype CT with the genotype TT between the two groups, the OR and confidence interval values were obtained as 2.554 and 95% (1.083-6.009;  $p=0.032$ ), respectively, indicating that the presence of only one C allele increased the risk of the disease over 2.5 times.

Our findings also showed that only 6 individuals of control group and 38 individuals of case group had a family history of cancer (OR=9.378). The above result showed that having a family history of cancer increased the risk of the colorectal cancer over 9 times. In addition, 11 individuals of control group and 21 individuals of case group were regular smokers. Despite the higher frequency of smokers in the case group and the increased risk of the cancer in smokers by 2 times (OR=1.992), the difference between the two groups in this regard was not significant ( $p=0.088$ ). However, the significant point in examining the correlation of smoking with the colorectal cancer was detected when the patients with cancer in colon and those with cancer in rectum were studied separately. Only 3 patients out of the 21 smokers had colonic cancer and the remaining 18 people had rectal cancer. This result showed that although the colonic cancer did not correlated with smoking, the risk of rectal cancer in smokers was three times higher as compare to the related value in other patients (OR=2.945;  $p=0.010$ ).

Of the patients, 50 people were male, and 40 people were females, whilst males and females of the control group comprised 54 and 29 individuals, respectively

( $p=0.202$ ). In case group, 9 (10%) individuals showed that the tumor established metastases in other tissues. Of these 9 patients, 7 had rectal cancer, and 2 had primary colonic cancer. This showed that the incidence rate of metastases in the rectal cancer was 2 times more as compared to the related value in the colonic cancer. Moreover, of the 9 patients, 6 had genotype CC, and 3 had genotype TT. Considering the genotypic distribution of the patients, and the lower number of genotype TT than genotype CC, there was no significant difference between the two genotypes in terms of increasing the risk of the disease ( $p=0.835$ ).

## Discussions

Results of this study revealed the correlation of threonine allele (C allele) from T241 M polymorphism with incidence rate of the colorectal cancer, leading to increase in risk of the disease by approximately 3 times in people with genotype CC and over 2.5 times in people with genotype CT, whilst there was no significant relationship between the metastatic tumor and the polymorphism. Although there was no significant correlation between smoking and colonic cancer, smoking increased the risk of rectal cancer by 3 times. Furthermore, results of this study showed no significant correlation between sex of patients and metastases of the colorectal cancer.

In addition, the family history of cancer was revealed to be a major risk factor for the colorectal cancer as the existence of a cancer patient in close relatives increased the risk of the disease over 9 times. Final conclusions of this study require the examination of much larger populations. Further studies should deal with the correlation of XRCC3 gene with other important factors, including the diet, being accustomed to specific drugs and chemical materials, environmental factors, and the interaction among various genes leading to cancer.

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