Original Article

Investigating of microsatellites instability in patients with hereditary non-polyposis colorectal cancer in Isfahan

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Abstract

Background: Microsatellites or simple sequence repeats are repeating sequences of deoxyribonucleic acid (DNA). Mutation in mismatch repair (MMR) genes can cause microsatellites instability (MSI) in some tumors. In familial disorder of hereditary non-polyposis colorectal cancer (HNPCC), there is a defect in the mechanism of MMR and clearly defective MMR cause unstable microsatellites. This study has been conducted for investigating the instability of microsatellites in alleles of BAT-26 of MSH2 gene in patients with HNPCC in Isfahan, which is an important prognostic biomarker for the prediction of the treatment outcome.

Materials and Methods: DNA extraction from forty HNPCC patients peripheral blood samples were performed by using the DNA extraction kit. The polymerase chain reaction (PCR) reaction to amplify BAT-26 was performed. The PCR products were studied by electrophoresis on agarose gel.

Results: The size of specific band was 121 bp out of 40 HNPCC samples and based on the above method, it was shown that 12 cases (30%) demonstrated MSI. Chi-square test showed this difference is statistically significant ($P < 0.05$).

Conclusions: The present study was conducted to evaluate the MSI in HNPCC patients. It was determined that the polymorphisms in BAT-26 of MSH2 gene could detect MSI with high sensitivity. Previous reports as well as our results have shown that the use of BAT-26 alone would be sufficient to identify HNPCC-associated MSH2 gene. Identifying MSI in these genes as a marker for prognosis, according to the present study and other researches is important to predict the treatment outcomes.

Key Words: Hereditary non-polyposis colorectal cancer, microsatellites instability, MSH2, mismatch repair

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INTRODUCTION

Microsatellites or simple sequence repeats are repeating sequences of deoxyribonucleic acid (DNA). The length of microsatellite varies from person-to-person. Everyone has a set of microsatellites with its own special layout, that are normal and common.[1] Microsatellite instability is a change in the DNA of certain cancer cells, in which the number of microsatellites repeats are different from non-cancerous cellular DNA.[2] Naturally, the accuracy of the genome is regulated by multiple mechanisms, which one of them is the correction of DNA replication errors entitled as DNA mismatch repair (MMR) mechanism.[3] Mutation in MMR genes can cause microsatellites instability (MSI) in some tumors.[3] Alterations in

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the size of microsatellites in DNA of tumor cells compared with non-tumor cells can be caused by either additions or deletions.[4] Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant genetic disease that is associated with the risk of developing colon cancer. It has shown that in familial disorder of HNPCC, there is a defect in the mechanism of MMR and clearly defective MMR cause unstable microsatellites.[4] One of the methods of determining the MSI is the investigation of the repeated units of DNA in microsatellites normal and cancerous tissues by polymerase chain reaction (PCR). BAT-26 is a poly-A containing string, which is located in the 3' end of MSH2 gene and is considered as the most sensitive and most specific marker for MSI.[5] BAT-26, in comparison to many other markers has several advantages in the study of MSI. This marker can effectively be used for the detection of tumors. It shows very high instability in tumors, but shows very little changes between alleles in an individual [6-9] Many studies have supported the usefulness of BAT-26 alone in the diagnosis process of tumors.[10] This study has been conducted for investigating the instability of microsatellites in alleles of BAT-26 of MSH2 gene in patients with HNPCC in Isfahan, which is an important prognostic biomarker for the prediction of the treatment outcome.

MATERIALS AND METHODS

Forty HNPCC patients, after genetic counseling and based on their family history were entered to this study. DNA extraction from peripheral blood samples were performed by using the DNA extraction kit and concentration of the extracted DNA was determined using a spectrophotometer at a wavelength of 260 nm as well as electrophoresis of 10 micro-liters of the extracted DNA on 1% agarose gel. The PCR reaction to amplify BAT-26 contained 100-300 ng DNA, 1.5 mM, $MgCl_2$, 0.2 mM of each of the dATPs and 0.2 mM of each primers. The sequences of each of the primers were as follows:
Forward: 5 'TGACTAC T T T T GAC T T CAGCC 3'
Reverse: 5 'AACC ATT CAACA TTTTTAACCC 3'

PCR cycling conditions were as follows: One cycles of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 1 min, extension at 72°C for 1 min and a final cycle of 72°C for 5 min. The PCR products were studied by electrophoresis on agarose gel. About 8-10 μl of the PCR product along with 1 μl of loading dye on 1.5% agarose gel and electrophoresed in 0.5 × tris-borate-Ethylendiamintetraacetic acid (EDTA) buffer and observed on an ultraviolet-transiluminator. Preparation of acrylamide electrophoresis gel and silver staining was performed based on the following protocol: At first, the glass frame was prepared and then 30 g acrylamide and 0.8 g bis-acrylamide were added into 90 ml distilled water and placed at 72°C for 10 min in order to prepare the stock solution of 30.8% acrylamide – bis-acrylamide. Then, 1.5 ml of 10% glycerol and 175 μl of 1% ammonium persulfate and 80 μl of tetramethylethylenediamine were added to the solution of 10% acrylamide – bis-acrylicamide. The prepared solution were then was poured between the glass frame. After electrophoresis, the gel was stained with silver nitrate. Polyacrylamide gel was separated from the glass frame and was placed for 15 min in a solution containing 10% of acetic acid. Then, it was placed for 30 min in silver nitrate. Before rinsing eventually, NaOH along with 37% dialdehydes were added. After the color appeared, gel was washed with distilled water and the results were evaluated. In the study of MSI, if the genome is not stable, different cells have different microsatellite length. Therefore, it provides a variety of bands on the gel ladder shape bands. In situations where there is no MSI, the genome is stable and microsatellite's lengths are the same in different cells without being observed for many times (one or a few bands).

RESULTS

Specific and non-specific bands were observed in any of the samples on electrophoresis gel. The size of specific band was 121 bp [Figure 1]. Non-specific bands were seen above or below the specific band. In some of the cases, ectopic bands were observed close to the main band and in other cases; it was seen in more distant from the main band. Out of 40 HNPCC samples and based on the above method, it was shown that

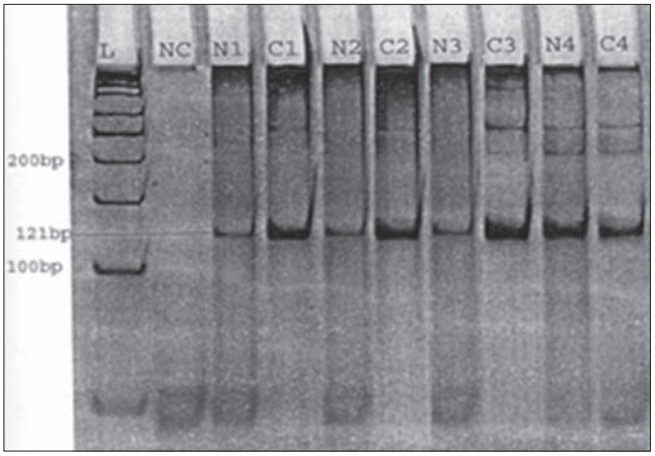

Figure 1: Detection of microsatellites instability by polymerase chain reaction (PCR) amplification in MSH2 gene BAT-26 allel. PCR product electrophoresis on 15% acryl amid gel and stained with silver nitrate. L: Deoxyribonucleic acid ladder, NC: Negative control, N: Normal, C: Cancer

12 cases (30%) demonstrated MSI and this was two cases (5%) for 40 control subjects [Figures 2 and 3]. Chi-square test showed this difference is statistically significant ($P < 0.05$). In other words, there is a significant relationship between HNPCC and MSI.

DISCUSSION

During the replication of DNA, short segments of the repeated bases of DNA, which are found throughout the human genome (as microsatellites) are the subject of insertion or deletion types mutations that can change the length of these microsatellites. Most of these errors are corrected by polymerase enzymes, but a small number of these errors cannot be corrected by MMR system.[11] Almost all the MSI negative tumors are with known features including being less invasive, having better prognosis and showing little changes compared with MSI positive tumors. About 160,000 patients with colorectal cancer (CRC) are added each year to the population, 7% of them are diagnosed with HNPCC and about 70-90% of HNPCC cases demonstrate MSI.[12] Recently, the instability of highly polymorphic microsatellites has been widely investigated in several studies and the results showed that the presence of MSI could be useful for early detection of cancer.[1,2,5,10] MSI is used to study different types of cancers such as stomach, lung, ovarian, breast, prostate, bladder, thyroid, head and neck, melanoma and uterus.[2,5] Thibodeau *et al.* found that MSI-is clearly important in relation with tumor location in the primary area of the colon ($P = 0.003$), increasing the patient survival ($P = 2\%$). Thibodeau *et al*. reported that MSI had a role in the pathogenesis of bladder cancer.[13] In another study on sporadic basal cell cancers, Thibodeau *et al*. found the length changes of mono-nucleotide A8 in HMSH3 gene in 5% of the cases.[13,14] They reported that despite the low frequency of MSI in their samples, it appeared that probably microsatellite instability would be important in the development of basal cell carcinoma. Interestingly similar results were obtained in the present study. In the case of hereditary CRC samples,[14] Siah *et al.* in a study that was performed on patients with CRC, indicated that investigation of BAT-26 allele alone was sufficient to interpret microsatellite instability. Many researchers have reported better prognosis for MSI negative patients. The advantage of better prognosis in CRC with MSI negative phenotype has been reported in the second and third stages of the disease.[2,13,15] The present study was conducted to evaluate the MSI in HNPCC patients and similar results were obtained by study of sBAT-26 allele of the MSH2 gene. It was determined that the polymorphisms in BAT-26 of MSH2 gene could detect MSI with high sensitivity.[2] It is important to note that using more markers may contribute to better diagnosis; although, it is not a cost-effective.[15] Thus, previous reports as well as our results have shown that the use of BAT-26 alone would be sufficient to identify HNPCC-associated MSH2 gene.[16] One goal of these studies in cancer is to evaluate different molecular markers, which could help to predict which could help to predict the outcome of treatment and disease progression. Finding such markers is important in order to identify patients who benefit from the chemotherapy. Determining the polymorphism and mutations of MSH2 gene have already been shown that it is associated with prognosis.

Thus, identifying MSI in these genes as a marker for prognosis, according to the present study and other researches is important to predict the treatment outcomes.

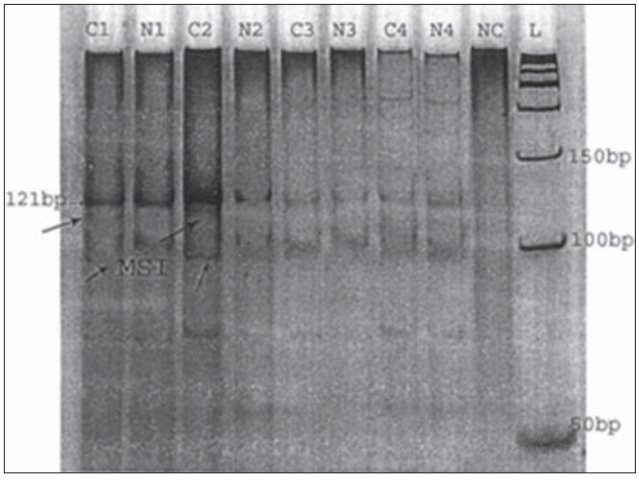

Figure 2: Detection of microsatellites instability (MSI) by polymerase chain reaction (PCR) amplification in MSH2 gene BAT-26 allel. PCR product electrophoresis on 15% acryl amid gel and stained with silver nitrate. In C1 and C2 there is MSI in variable bands

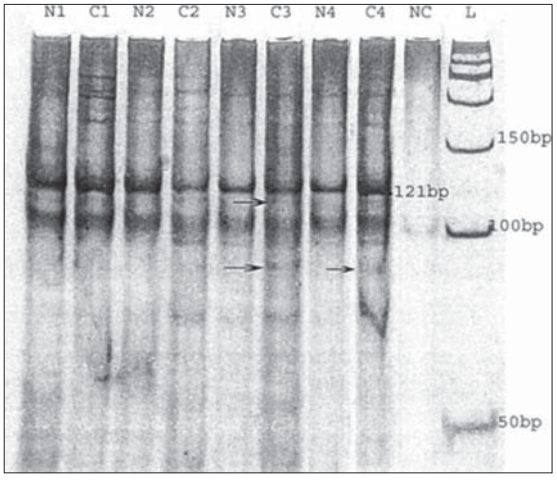

Figure 3: Detection of microsatellites instability (MSI) by polymerase chain reaction (PCR) amplification in MSH2 gene BAT-26 allel. PCR product electrophores on 15% acryl amid gel and stained with silver nitrate. In C3 and C4 there is MSI

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