RESEARCH COMMUNICATION

Simplified MSI Marker Panel for Diagnosis of Colorectal Cancer

Atena Irani Shemirani, Mahdi Montazer Haghighi*, Saman Milani Zadeh, Seyed Reza Fatemi, Mohammah Yaghoob Taleghani, Narges Zali, Zahra Akbari, Seyed Mohammad Hossein Kashfi, Mohammad Reza Zali

Abstract

Background: Colorectal cancers (CRCs) tumors are diagnosed by microsatellite instability (MSI) due to accumulation of insertion/deletion mutations in tandem repeats of short DNA motifs (1–6 bp) called microsatellites. Microsatellite instability (MSI) is not only a hallmark marker for screening of hereditary nonpolyposis colorectal cancer (HNPCC), but also a prognostic and predictive marker for sporadic colorectal cancer. Our objective was to determine and study of five mononucleotide microsatellite markers status among Iranian patients with HNPCC and sporadic colorectal cancer. Material and Methods: In the current investigation 80 sporadic CRC and 80 HNPCC patients were evaluated for MSI. The pentaplex panel including 5 quasimonomorphic mononucleotide repeats (NR-21, BAT-26, BAT-25, NR-27 and NR-24) was used. Results: Our findings showed that the NR-21 was the most frequent instable marker among the other markers. 53% and 25.6% specimens had instability in sporadic CRC and HNPCC, respectively. Furthermore, the frequencies of instability BAT-25 was determined in 20% sporadic CRC and 23% HNPCC samples. Interestingly our results demonstrated that the frequency of instability NR-24 was similar 20% sporadic CRC and 20.5% HNPCC. Moreover, percentage of NR-27 in HNPCC was 19.2 and 0% in sporadic CRC. Finally, BAT-26 was instable in 21.8% HNPCC patients while we could find 6.6% instability for BAT-26 in sporadic cases. Conclusion: It seems that among 5 mononucleotides markers NR-21 was the most useful marker for diagnosis HNPCC and sporadic cancer. Following NR-21, BAT-25 and NR-24 are the most reliable markers. Therefore using a triplex panel including 3 aforementioned MSI markers should be more promising markers for identifying MSI status in both patients with HNPCC and/or sporadic colorectal cancer.

Keywords: HNPCC - mononucleotide markers - MSI - sporadic colorectal cancer

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Introduction

Colorectal cancer is the third most common cancer in the world and recently becomes the most common cancer in Asia. It has an incidence approximately one million cases and mortality of more than 500,000 per year .The number of cases will increase over the next two decades as a result of the aging and expansion of populations in developed and developing countries (Lin et al., 2011). Thereby, the importance of CRC as a major worldwide health concern is also increasing in our population (Moghimi-Dehkordi et al., 2008) (Haghighi et al., 2009).

The great majority (80%) of patients with colorectal cancer have sporadic disease with no evidence of having inherited the disorder. The rest of patients (20%), a potentially definable genetic component exists (Al-Sukhni et al., 2008). Microsatellite instability is considered as a defect in mismatch repair (MMR) system. The main function of the MMR gene products is to identify and correct mismatches as well as short insertion or deletion loops, which occur during replication and recombination (Aaltonen et al., 1998). Disabled MMR machinery, cause to errors the contraction/expansion of tandemly repeated sequences. Detection of MSI has been a useful prescreening laboratory tool for the recognition of suspected colorectal cancer cases (Hampel et al., 2008). MSI is approximately detected in about 15% of all colorectal cancers due to a germline mutation in one of the mismatch repair genes (MLH1, MSH2, MSH6 and PMS2) or to epigenetic silencing of MLH1. About 3% are of these are associated with HNPCC or Lynch syndrome and the other 12% are caused by sporadic (Boland et al., 2010). In the other hand, 15% to 20% of sporadic and most (90%) patients with Lynch syndrome, show microsatellite instability. Therefore it considers as a feasible marker for the disease and a hallmark of mismatch repair deficiency (Berginc et al., 2009). MSI is a widespread instability in

*Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran *For correspondence: mah_haghighi@hotmail.com*

coding and noncoding microsatellite sequences, due to mismatch repair (MMR) deficiency (Aaltonen et al., 1998; Hampel et al., 2008; Kristen 2010).

The diagnosis of hereditary nonpolyposis colorectal cancer at the molecular level relies on the presence of an alteration germ line mutation in one of the mismatch repair genes. Because cancer morbidity and mortality can be dramatically reduced by colonoscopic screening of individuals with the HNPCC syndrome and by prophylactic surgeries, molecular screening of colorectal cancer patients for HNPCC is now feasible (Domanska et al., 2009; Ionov et al., 1993). High-frequency microsatellite instability (MSI-H) is a genetic instability observed in virtually all tumors from patients with hereditary nonpolyposis colorectal cancer and in a subset of sporadic colorectal cancers (Søreide 2007).

The objective of this study was to detect the most frequent MSI mononucleotide markers among five markers (NR-21, BAT-26, BA T -25, NR-27 and NR-24) in order to devise the simplest diagnostic assay to diagnosis of patients and their kindred with hereditary nonpolyposis colorectal cancer and sporadic CRC at molecular level.

Materials and Methods

Between June 2007 and December 2009, 80 sporadic CRC and 80 HNPCC individuals were selected clinically and genetically. Two biopsy samples from each individual, normal and tumor, were obtained. Pathology unit reconfirmed the colonoscopy results. Both patients group were recruited by the Research Institute for Gastroenterology and Liver Diseases (RIGLD), Taleghani Hospital, Shahid Beheshti University of Medical Sciences. All subjects provide informed consent to the study and the Institutional Review Boards of the RIGLD. The Ethical Committee of the Institute have reviewed and approved this study. Submitted fresh tissue samples (500 µg) from HNPCC and sporadic colorectal cancer patients were immediately frozen at –70 oC after extracted DNA from them, they were examined for MSI status. Genomic DNA was obtained from all colorectal fresh tissue using QIAamp Tissue kit (QIAGEN GmbH, Germany). Concentration and purification of extracted DNA were determined by electrophoresis on 1% agarose gel and spectrophotometric analysis. Determination of MSI status in the total 160 patients was examined using the pentaplex panel consisting of BA T -26, NR-21, BAT -25, NR-27 and NR-24 quasimonomorphic mononucleotide repeat. This panel was first suggested by (Buhard et al., 2004) PCR primers is labeled with either 6-FAM (blue), HEX (green) or NED (black) fluorescent; the 5 markers are distinguished by peak size related to their sequence size and fluorescent labels. Primer sequences and details based on our previous work (Buhard et al., 2004; Haghighi et al., 2010).

PCR was carried out in a total volume of 25 µl using a final concentration of 200 µmol/L deoxyribonucleotide triphosphates (MBI Fermentas, St. Leon-Rot, Germany), 500 nmol/L each sense and antisense primer (Eurogentec, Seraing, Belgium), 1X PCR buffer (60 mmol/L Tris-SO_4, pH 8.9; 18 mmol/L NH_4 SO_4; 2 mmol/L $MgSO_4$). The five markers were synchronously amplified in a multiplex PCR.PCR program was include denaturation at 95 ˚C for 5 minutes, 35 cycles of denaturation at 95 oC for 30 seconds, annealing at 55 ˚C for 30 seconds and extension at 72 ˚C for 1 minute (Buhard et al., 2004). Separation of amplification products were carried out by capillary electrophoresis using an ABI 3130xl Genetic Analyzer (Applied Biosystems) and were analyzed by Genemapper software version 3.7.

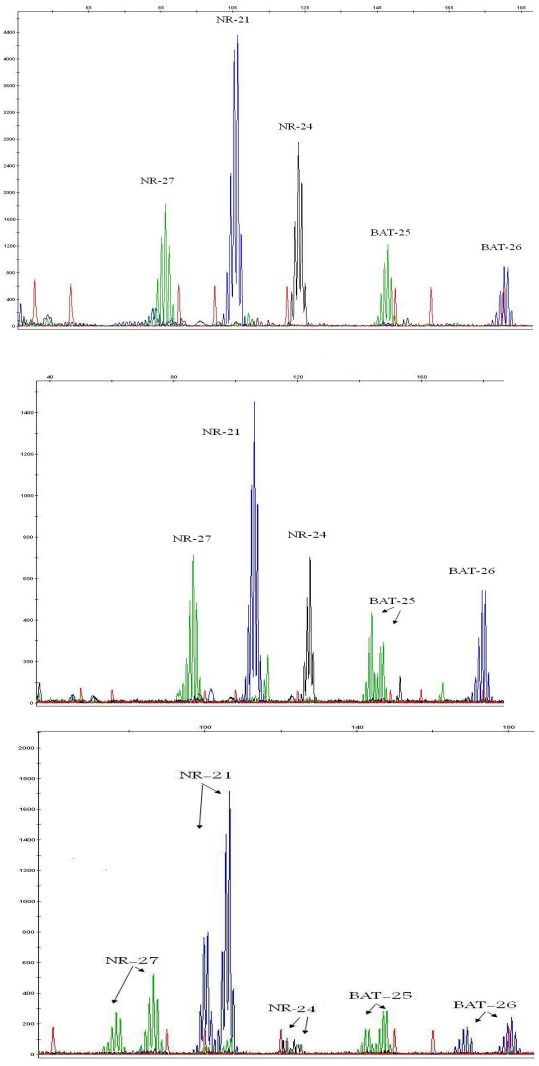

Figure 1. Normal MSI Status: Top, MSS; Middle, MSI-L Bottom, MS-H

Tumors with instability at two or more of these markers were defined as being MSI-High (MSI-H), MSI- low (MSI-L) if one locus is positive; while those with showing no instability were determined as Microsatellite Stable (MSS) tumors (Brennetot et al., 2005; Haghighi et al., 2010) (Figure 1).

Results

Our finding revealed that the most frequent mononucleotide marker was detected in NR-21. Instability of the marker was 53% in sporadic CRC and 25.6% in HNPCC. BAT-25 was in the second position. Instability of the marker was 20% in sporadic CRC and

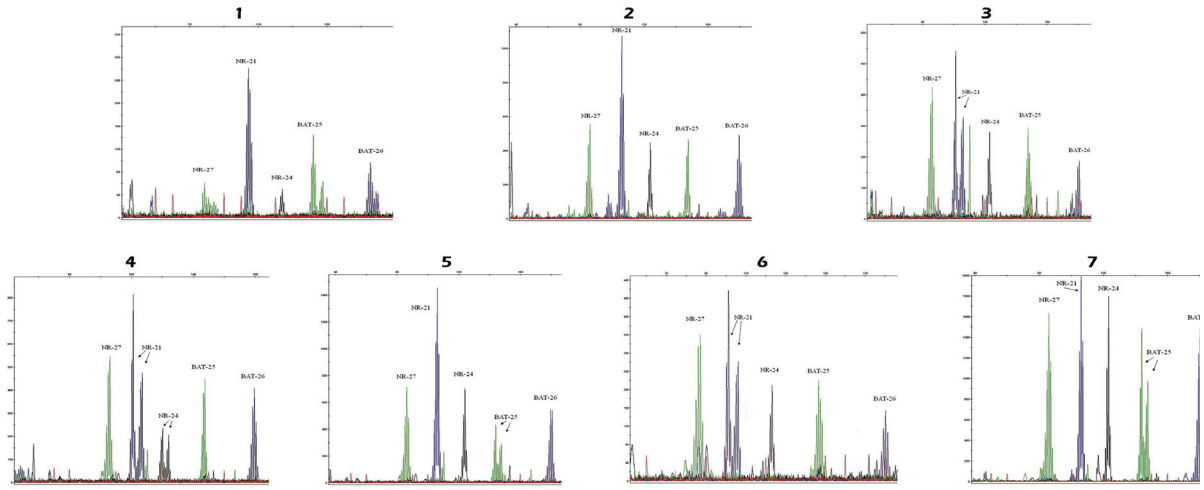

Figure 2. Some MSI Results in Sporadic Colorectal Cancer 1. MSI-H, 2.MSI-L, 3.MSI-H, 4.MSI-H, 5.MSI-L, 6.MSI-L, 7.MSI-L

23% in HNPCC samples. Furthermore, interestingly our results demonstrated that the frequency of instability of NR-24 was very similar 20% in sporadic CRC and 20.5% in HNPCC. In the case of, BAT-26our finding revealed 21.8% instability in HNPCC patients and just 6.6% instability for BAT-26 in sporadic cases. Interestingly, frequency ofinstability NR-27 showed a huge difference between two groups 19.2% and 0% in HNPCC and sporadic CRC (see Figure 2 for the latter).

Discussion

Since more than 90% of hereditary non-polyposis colorectal cancers and 20% of sporadic cases show MSI, the microsatellite status has become a common diagnostic marker for colorectal cancer. Several studies have been suggested the role of MSI in colorectal cancer prognosis (Schmeler et al., 2006; Vasen et al., 2007).

Many investigations have been conducted on MSI markers to find best marker as a biomarker tool for diagnosis. In 2002, the NCI proposed the revised Bethesda guideline criteria for CRC screening (Laghi et al., 2004; Umar et al., 2004; Pinol et al., 200) .It was suggested that mononucleotide repeats improving the sensitivity of MSI detection in CRC. The use of mononucleotide repeat microsatellites for MSI characterization has been shown to be advantageous over many dinucleotide microsatellites due to the quasimonomorphic nature of both loci and their high sensitivity to MSI (Buhard et al., 2004). However, the current standard method of MSI analysis using five quasi mononucleotide markers is still time-consuming and expensive. Furthermore, microsatellite markers are examined in DNA amplified from tumor and normal tissues, and amplification might be difficult due to the limited amount of available tissues and DNA.

The aim of the current investigation was to find the most promising mononucleotide MSI marker to detect HNPCC and sporadic colorectal cancer in order to present a shirked panel for diagnosis. Several investigations like our previous study have been investigated frequency markers just in one group HNPCC or sporadic. Our results demonstrated that NR-21 and BAT-25 markers have the most instability in HNPCC and sporadic colorectal cancer. While two group reported that BAT-26 and BAT-25 have the highest sensitivity and specificity in the similar panel in HNPCC patients.(Brennetot et al., 2005; Pastrello et al., 2005).Furthermore, another study support the previous results was performed on the pentaplex panel demonstrated that the fidelity each marker as follow 100% for BAT-26, 96.9% for BAT-25, 87.5% for NR-24, 97.0% for NR-21, and 97% for NR-27 (Søreide, 2011) nonetheless, the results were in contrast of our finding. In a new approach 5 quasimonomorphic markers including BAT-25, BAT-26, NR-21, NR-22, and NR-27 in Slovenian population tested. The frequencies of polymorphisms 0.07%, 1.4%, 2.1%, 1.4%, and 1.4% for BAT-25, BAT-26, NR-21, NR-22, and NR-27 were identified, respectively. Their finding revealed that NR-21 was the most polymorphic marker and their finding was compatible with our results (Berginc et al., 2009).

We believe that to determine the MSI status of HNPCC and sporadic patients, the use of a single triplex fluorescent MSI assay including NR-21, BAT-25 and NR-24 reduces the time and costs involved in MSI testing with increased reliability and accuracy .Using the panel should facilitate widespread screening for microsatellite instability in patients with HNPCC and sporadic colorectal cancers. It is suggested that these tree markers are the most reliable and useful markers for diagnosis of colorectal cancer in our population rather than the Bethesda panel. Nonetheless, more accurate MSI testing ought to perform to improve prognostic and predictive panel for colorectal cancer in other populations.

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