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Microsatellite instability detection in hereditary colorectal cancer: is it possible in a public hospital?

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Abstract. Microsatellite analysis is emerging as an important tool in the study of cancer. The addition of novel microsatellite alleles is referred as microsatellite instability (MSI) indicating possible mutations in cellular DNA repair mechanism. The detection of these genetic changes demonstrates the presence of a clonal population of cells that share altered genetic information, which is characteristic of cancer cells. In this study, we analyzed the five MSI markers: D2S123, D5S346, D17S250, BAT 25, BAT 26 recommended at the 1997 National Cancer Institute in a patient with suspicious familiar history of Non-Polyposic Hereditary Colorectal Cancer (NHPCC), Lynch syndrome, and high instability MSI-H was confirmed.

Keywords: Colorectal cancer; DNA; PCR; Microsatellite instability; NHPCC

1. Introduction

Colorectal cancer is one of the principal causes of cancer-related death in Caucasian society. Non-Polyposic Hereditary Colorectal Cancer (NPHCC) [1–3], one of the hereditary syndromes related with this disease, is an autosomal dominant disorder accounting for about 3–5% of all colorectal cancer cases caused by mutation of one of the DNA mismatch repair genes. Clinical features include familiar history of colorectal, endometrial, ovarian and transitional tumors. The genetic hallmark is microsatellite instability (MSI), a replication error in genomic repeated sequences of one or two nucleotides present all along chromosomes and DNA sequences enlarges or gets shorter, but cells have mechanisms to repair this damage. HNPCC is cause by germiline mutation of the DNA mismatch repair genes (hMLH1, hMLH2, hPMS1, hPMS2, hMSH6).

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Microsatellite instability (MSI) is found in the colorectal cancer DNA of most individuals with germiline mismatch repair genes mutations [4,5].

The aim of our study was to detect MSI in a patient with history that suggests Lynch syndrome (NPHCC) [1–3].

2. Materials and methods

A patient with suspicious familiar history of Lynch syndrome signed an informed consent form. DNA sample was extracted by salting out method [7,8], tumoral DNA from paraffin-embedded tissue with QIAmp DNA kit (QIAGEN). DNA samples were amplified in a Perkin Elmer Gene Amp PCR System 9700® (PE Applied Biosystems) for the following loci D2S123, D5S346. D17S250, BAT-25, BAT-26, BAT-40, TP53, D18S58, D18S69, D13S175, and D10S197 markers recommended at the 1997 National Cancer Institute (NCI)-sponsored conference on MSI [4]. The PCR products were detected and

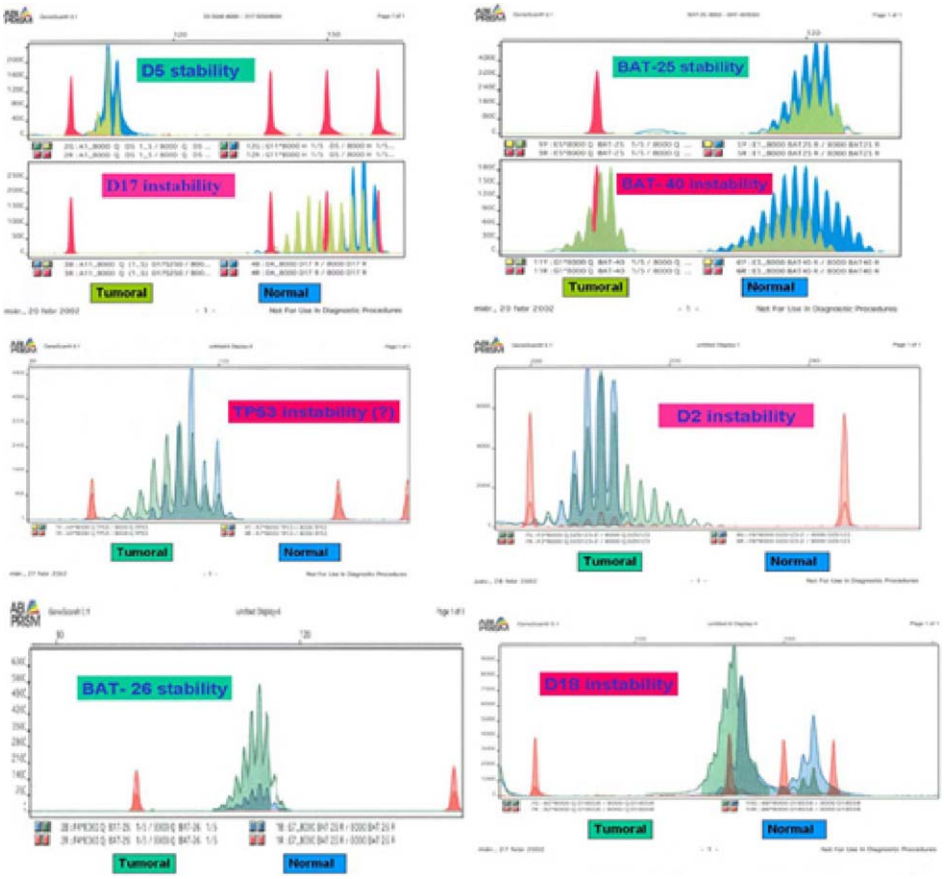

Fig. 1. Microsatellite markers studied.

analyzed using Capillary electrophoresis in an ABI PRISM™ 310 Genetic Analyzer and GeneScan® Analysis software 3.1 (PE Applied Biosystems) [7,8].

3. Results

In 1997, the National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome proposed specific markers for MSI testing [4,6]. The microsatellite markers used for the diagnosis of MSI recommended by NCI were: D2S123, D5S346, D17S250, BAT-25, BAT-26 and defined MSI-high (MSI-H) when the tumor DNA sample present shifting of at least two of the five loci.

MSI-low (MSI-L) is identified by only one marker showing instability and more loci have to be analyzed. MSI testing using these markers should be performed on the tumor tissue of individuals who meet the Bethesda criteria [4], modified putatively affected with HNPCC. A result of MSI-high in tumor DNA usually leads to consideration testing by sequencing for mutation in DNA repair genes. In our case, the tumor sample of the patient showed MSI at two loci: D2S123, and D17S050, which means high instability (MSI-H), but also other microsatellite markers were studied and loci BAT 40 and TP 53, were also instable (Fig. 1).

4. Conclusions

The detection of MSI was successful and possible in our hospital; we could detect MSI-H in the patient studied. The method based on capillary electrophoresis and fluorescent markers detect 10% of tumoral cells in the sample, which means that the high sensitivity of this technology compares to the standard polyacrylamide gel electrophoresis and radioactive detection. Consensus panel and expert opinion suggests evaluation for MSI as the first step in the genetic work-up of pedigrees suspected to be affected by HNPCC.

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