MONO NUCLEOTIDES DETERMINES THE FREQUENCY OF UNSTABLE MICROSATELLITES BIOMARKERS IN PATIENTS WITH NON-HEREDITARY COLORECTAL CANCER POPULATION OF THE CITY OF TABRIZ IN IRAN

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ABSTRACT
Short Tandem Repeats motifs DNA microsatellites instability in the accumulation of mutations in genes that occurs due to repair-deficient nucleotide pairing is done. So can be used as an alternative biomarker for assessing gene function in repairing the defect base pair in the colon is bad. Also, instability microsatellites not only a biomarker for colorectal cancer screening is hereditary, but in 15% of cases of non-hereditary colon cancers can also be evaluated. The aim of this study was to determine the frequency and evaluation 5 biomarker microsatellites monomorphic in patients with non-hereditary colon cancer in the city of Tabriz. Under the project 110 patients with non-hereditary colon cancer were evaluated in terms of instability microsatellites. In this study, a panel of five biomarkers multiplex Penta microsatellites monomorphic (BAT-25, NR-24, NR-27, NR-21, BAT-26) were used. The results showed that the most abundant biomarker biomarker NR-21 was unstable compared with other biomarkers. % 74/2 patients in the study were divided panel of five biomarkers were unstable Mono-nucleotide. The frequency instability of two biomarkers BAT-25 and NR-24% 17/8 was determined and the frequency of instability biomarkers BAT-26% 8/3 and NR-27% 1/7 respectively. According to the results, we can conclude that biomarkers NR-21, BAT-25, NR-24 as a biomarker in the panel are unstable.

KEYWORDS: colorectal cancer, instability of microsatellites, biomarkers mono-nucleotide.
INTRODUCTION

Colorectal cancer is the third most common cancer in the world and most recently in Asia is the most common cancer.[1] Every year 1 million patients are diagnosed with this cancer and more than 50,000 deaths due to this cancer occurs in the world.[2] The number of cancer patients due to aging and population growth in developing countries will increase in the next twenty years.[3] Thus the importance of colon cancer as a global public health crisis is important.[4] Clinical diagnosis of patients with hereditary colorectal cancer with colonoscopy is not you and tumor tissue samples using standard pathology is confirmed. Using the results, it was found that 515 of the patients with repetitive nucleotide sequences are unstable. In other words, people who have two or more biomarkers demonstrate instability (MSI-H) and if only one of the biomarkers are unstable (MSI-I) as patients with biomarker known to be unstable. The aim of this study was to determine and compare the frequency of Biomarkers MSI mono nucleotide (NR-21, NR-24, NR-27, BAT-25, BAT-26) is the result of a biomarker unstable microsatellites can be determined.

Figure 1: Schematic view of the colorectal cancer biomarkers in human MSI-frequency generator.

MATERIALS AND METHODS

The selection of patients for pilot projects and 110 patients with non-hereditary colon cancer who were randomly selected genetic and clinicopathological tumor. Per person with two biopsy (a sample of normal and tumor samples as negative control) were prepared. Colonoscopy Colonoscopy results were confirmed by standard unit. After obtaining informed
consent from patients to participate in this project, newly obtained tissue samples from patients and controls from non-hereditary colorectal cancer patients after extracting DNA from patients were frozen at -70 and then were tested to determine the MSI status. Genomic DNA of all new colorectal tissue using the kit Diagene product Germany was obtained. Concentration and purity of DNA extracted by agarose gel electrophoresis technique and spectrophotometric analysis were determined.

Table 1: sequence gene loci primers and markers.

<table>
<thead>
<tr>
<th>Size</th>
<th>Gene</th>
<th>Genbank Number</th>
<th>Repeat</th>
<th>Primer Sequences</th>
<th>Amplicon Size ( bp )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-27</td>
<td>Inhibitor of apoptosis protein-1</td>
<td>AF070674</td>
<td>A5’UTR27</td>
<td>F: AACCATGCTTGCAAAACCACTR: CGATAATACTAGCAATGACC</td>
<td>94</td>
</tr>
<tr>
<td>NR-21</td>
<td>SLC7A8</td>
<td>XM033393</td>
<td>T5’UTR21</td>
<td>F: GAGTCGCTGGGACAGTCTA R: CTCGACTCGCTGTATTACAA</td>
<td>114</td>
</tr>
<tr>
<td>NR-24</td>
<td>Zinc finger2</td>
<td>X60152</td>
<td>T3’UTR24</td>
<td>F: GCTGAATTTACCTCTGAC R: ATTGTGCCCATTGCATTCCAA</td>
<td>128</td>
</tr>
<tr>
<td>BAT-25</td>
<td>C-KIT</td>
<td>X06182</td>
<td>T intron1625</td>
<td>F: TACCAGGTGGCAAAGGGCA R: TCTGCAATTTAACTATGGCTC</td>
<td>161</td>
</tr>
<tr>
<td>BAT-26</td>
<td>Hmsh2</td>
<td>U04045</td>
<td>A intron526</td>
<td>F: CTCGGGTAATCAAGTTTTTAA R: AACCATTCAACATTTTAAAACC</td>
<td>195</td>
</tr>
</tbody>
</table>

PCR primers by FAM-6 blue, HEX green and black NED were labeled with fluorescent properties. 5 biomarkers through peak size to the size of the sequence and fluorescent labels are detectable. PCR process in a final volume of 25 micromoles using a total concentration of 200nm / L of each primer DTP500μm for password encryption and anti-sense and antisense 1X buffer SO42mmol / L, NH418mmol / L of Tris-SO460mmol / L was prepared by Ph8.9 + MgSO4.

Figure 2: Nucleotide sequence of five biomarkers in Multiplex PCR.
Unstable tumors in two or more of the biomarkers (MSI-H) were found, and if a positive locus MSI-L or will be weak and if they do not show any instability as stable microsatellites or MSS tumors are determined.

![Figure 3: The stable and unstable five biomarkers in tumor cells.](image)

**RESULTS**

The data showed that the most abundant biomarker biomarker NRR-21 Mono-nucleotide instability CTNI non-hereditary CRC was reported in 27%. BAT-25 was the second highest this biomarker in non-hereditary CRC instability was 24%.

![Figure 4: Schematic view of the pattern of bond formation in c-kit protein.](image)
Figure 5: Schematic view of how biomarkers target nucleotide sequence in the present study.
Figure 6: Schematic diagram of the statistical analysis of biomarkers. The aim of this study was modified alleles.

Figure 4: Frequency instability of microsatellites in the world from 1865-Dec-01 to 2009-Dec-20.
DISCUSSION
Although it seems that colorectal and gastric cancer clinicopathological characteristics are different, but compared to the situation microsatellites, MSI-H tumors are similar. Based on the results it can be suggested that non-hereditary colon cancer patients to determine the status of MSI, MSI measured using a fluorescent triplex which contains NR-21, NR-24, BAT-25 is, can decrease MSI testing time and costs as well as increase the accuracy and reliability necessary. Use of this panel can be widespread screening for colorectal cancer in patients with unstable microsatellites ease.

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REFERENCES


