FAMILIAL ADENOMATOUS POLYPOSIS: ANALYSIS OF GENETIC INSTABILITY OF MICROSATELLITES LOCI AND GENETIC ALTERNATIONS OF TUMOR SUPPRESSOR GENES

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ABSTRACT

Familial adenomatous polyposis (FAP) is an autosomal dominant illness with the highest risk for appearance of colorectal cancer’s disease. In our study, we have used Bethesda criteria that define colorectal cancers which can be tested on microsatellite instability. The aim of our study is make an analysis of microsatellite instability (MSI), appearance of RER+ phenotype, genetic alteration of tumor suppressor genes as like as one of responsible factor for genesis of adenomatous polyposis. The base for this study were shown families with clinical diagnosed FAP. In this study two families with clinical diagnosed adenomatous polyposis were involved. Our study of both families showed that three tumor tissues belonged to RER negative phenotype, but only one belonged to RER positive phenotype. Microsatellite analysis showed instability of mononucleotide marker Bat 7 at 7 samples and Bat 5 at 5 samples, but Bat 8 in 1 sample. Dinucleotide marker TP 53 did no show any microsatellite alterations. Genetic alteration of tumor suppressor gene APC appeared at 4 samples, p53 at 3 samples, RB1 at 2 samples and NM23 only at 1 sample, but tumor suppressor genes DCC1 and DCC2 were homozygote. Our results are agree with results of earlier studies and also the got results confirm the fact that loss of heterozygosity of tumor suppressor gene APC and p53 are responsible for genesis of adenomatous polypose and it also represents the characteristic of genetic changes FAP’s patients in our region.

KEY WORDS: Familial adenomatous polyposis, microsatellite instability, loss of heterozygosity
INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant illness with the highest risk for appearance of colorectal cancer’s disease (1); which is appeared in over 100% of unoperated cases. This illness is characteristic of forming with over 100, usually about 1000 polyps of colorectal cancer and often with stomach’s adenoma as like as small intestine’s stomach. The biggest number of persons with FAP illness get the colorectal cancer before their 40 year old. The illness frequency is 1:3:100 000. The illness is hereditary on descendants in 50% of its cases, but it can get sick and more generations in a family. Oftentimes FAP is caused by mutation of APC gene. Persons with hereditary mutated APC gene have a high risk for developing adenoma in their childhood, so that estimated risk is over 90%. Some cancers show p53 mutation before APC mutation. Usually, loss of heterozygosity which is appeared at colorectal cancers through the FAP’s frame is appeared on hromosomes 5, 14, 17, 18 and 22. This point has showed localization of tumor suppressor genes. It seems that the illness of males is some often that the illness of females (54 : 46). There is no positive history of the illness in 45% of diagnosed cases. Defect of MMR gene leads to high level of microsatellites instability (MSI-H) in tumor tissue. So that the microsatellites instability can be found in early stage of the adenoma. It is known that a full develop of a microsatellites mutator phenotype can depend and on accumulation of secondary mutations (2). The microsatellite instability is established in 80% adenoma of hereditary non-polyposis colorectal cancer, and even 66.7% belongs to MSI-H (3). That investigation in this group of MSI-L levels show that there is a relation commonly with loss of hMLH1 or hMSH2 expression, and what is different to situation with MSI-L of sporadic colorectal cancers. According to authors, this phenomenon that MSI-L status is in rank of 57-93% (4-6). Usage of these 26 dinucleotide markers gave results in appearance of low percent detected mutation in microsatellite loci at benign adenoma than at adenomas which have had alteration on malignancy (5). There is relation among MSI-H adenomas and loss of MMR expression at colorectal cancers. It seems that MSI-H adenomas have more tendency to developing of cancers (7). In our study, we have used Bethesda criteria that define colorectal cancers which can be tested on microsatellite instability (8). According to these criterias, there is a cancers classification on microsatellite instability as like as following:

- MSI-L - microsatellite instability-low (MSI in locus less than 30-40 %);
- MSI-H - microsatellite instability-high (MSI in locus more than 40% and replication positive error (RER+)
- MSS cancers- microsatellite stable

The aim of our study is make an analysis of microsatellite instability (MSI), appearance of RER+ phenotype, genetic alteration of tumor suppressor genes as like as one of responsible factor for genesis of adenomatous polyposis. The base for this study were shown families with clinical diagnosed FAP.

MATERIALS AND METHODS

Through the study we involved two families with clinical diagnosed adenomatous polyposis and samples were collected from Gastroenterological of University Clinical Center in Tuzla (Bosnia and Herzegovina). Tumor’s and healthy surroundings tissue were fixed in formalin, and after that tissue was shaped in paraffin blocks. Methods of isolation of genomic DNA is made on deparaffinization of tissue sections as like as on cell proteolises of tissue by proteinase K.(9) Fluoroscent chain synthesis of DNA is a method which has very broad application in tumor detection, and it is specially important in determination of microsatellite instability (MSI) and loss of heterozygosity (LOH) of tumor suppressor gene. We used mononucleotide and dinucleotide microsatellite markers in detection of microsatellite instability. We used in the group of mononucleotide markers following: BAT25, BAT26 and BAT40, but in the group of dinucleotide markers we used following: DS123 and TP 53. for detection of LOH, we used intragene markers for following tumor suppressor genes: NM23, p53, APC, RB1, DCC1 and DCC2.(10) PCR was performed using a PCR Thermocycler 9600 (Perkin-Elmer). The PCR conditions were as follows: after an initial 2 min denaturation step at 94°C, 30
amplifications cycles were performed, each consisting of a 10 s step at 94°C, a 30 s step at 55°C, and a 30 s elongation step at 72°C. Amplification was completed with a final incubation step at 72°C for 7 min. For separating of amplified PCR products, we used automatical sequencer 310 ABI PRISM, Genetic Analyser 310 (Perkin Elmer), which enable separating and quantification of DNA fragments according to principle of capillary electroforesis. Microsatellite analysis presents comparation of healthy and tumor tissue at the same patient by means of Genescan program package for analysis. Software program detects fluorescent peaks and shows them on electropherogramme. Each fluorescent peak is automatic quantified in base size, height and peak field. All samples were tested twice because result confirmation. Loss of heterozygosity was calculated by mathematics (10) for heterozygosity cases, allele ratio was calculated for each pair of normal and tumor tissue according to formula $T_1:T_2/N_1:N_2$, where $T_1$ and $N_1$ are valuable fields of shorter allele length; $T_2$ and $N_2$ are fields of value of longer allele for tumor and healthy samples. The result had rank from 0.00-1.00. If the result was lower or equal to 0.50. Then it is showed a significant loss of heterozygosity and on longer allele. Homozygosity cases cannot be used in calculation.

RESULTS

Our study involved two families with clinical diagnosed adenomatous polyposis. We have made analysis of four members in Family 'A' (two brothers and two sisters). One of brothers (Patient No.1) was operated of FAP with adenocarcinomas of rectosigmoid region in his 50 age. Adenomas were tubular and tubulovillous type. Other three members were phenotypely healthy. According to the history of illness of this family, we have concluded that mother of these four children had colorectal cancer. Microsatellite analysis at three members of the Family 'A' did not find any change, but at their sick member that had developed cancer are found changes of mononucleotide markers both Bat 25 and Bat 40. Loss of heterozygosity analysis has shown a genetic alteration of tumor suppressor genes both APC and RB1, as like as homozygosity of DCC1 and DCC2 locus at sick patient (Table 1). Family 'B' has six members (five brothers and one sister). One of brothers (Patient No.1) was operated FAP adenocarcinomas flexurae hepatalis in his 34 years old. Adenomas of this patient were pathohistological Adenoma tubulare and Adenoma tubulovilious. Sister (Patient No.5) was operated FAP adenocarcinomas colonis rectosgymoidei in her 32 years old. Polypes were also Adenoma tubulare and adenoma tubulovilious. One of brothers (Patient No.3) had diagnosed polype Adenoma tubulare. Other three brothers are phenotypely healthy. It has analyzed the familial history of their mother who died in her 52 years old from colorectal carcinoma. Mothers sister also died from cancer and one mothers brother was operated colorectal cancer and he is still alive. Microsatellite analysis of the Family 'B' has showed that it three members have no alteration, but that alteration exists at other it three members. The microsatellite instability analysis of adena (Patient No.3) has shown alteration of markers both Bat 26 and Bat 40.

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<thead>
<tr>
<th>No. of patients</th>
<th>Microsatellite alterations</th>
<th>Genetic alterations</th>
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<tr>
<td></td>
<td>Bat25 Bat26 Bat40 TP53 DS123 NM23 P53 APC RB1 DCC1 DCC2</td>
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aMSI - microsatellite instability  **N – without microsatellite instability  AI – allele imbalance or loss of heterozygosity (LOH)
NAI – without loss of heterozygosity  H – homozygous

TABLE 1. Microsatellite and genetic alteration of the Family 'A'

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<td>3.</td>
<td>N N MSI MSI N N N N N N</td>
<td>AI AI AI AI</td>
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<td>4.</td>
<td>N N N N N N N N N N</td>
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<td>5.</td>
<td>MSI N MSI N N N</td>
<td>NAI AI AI AI NAI NAI</td>
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<td>6.</td>
<td>N N N N N N N N</td>
<td>NAI NAI NAI NAI NA NAI</td>
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aMSI - microsatellite instability  **N – without microsatellite instability  AI – allele imbalance or loss of heterozygosity (LOH)
NAI – without loss of heterozygosity  H – homozygous

TABLE 2. Microsatellite and genetic alteration of the Family 'B'
as like as LOH at NM23, P53 and APC. One of broth- 
ers (Patient No.1) getting sick FAP with developed cancer has found alteration both Bat 26 and Bat 40 as like as loss of heterozygosity P53, APC and RB1. The sister (Patient No.5) getting sick FAP with developed cancer had alteration Bat 25, Bat 40 and DS 123, as like as LOH P53 and APC tumor suppressor gene. All members of family has showed homozygous locus' tumor suppressor gene DCC1 and DCC2 (Table 2).

Discussion

Accepted fact is that familial adenomatous polyposis is a form of colon tumor, which is caused by mutation of tumor suppressor APC gene. The risk for cancer developing is 100% if detected polypes are not removed in time (11). Authors concluded that mutations of APC gene have main role in early development of colorectal neoplasms. It is also concluded that 60% of colorectal tumors as like as 60% of adenomas have mutation in APC gene (12). APC mutation exists in earlier stages of tumor development. Sequence analysis showed that the highest number of adenomas as like as cancers have APC mutation (13). 20% APC mutation exists in earlier stages tumor development. Loss of APC gene is found at 20% adenoma of FAP patients (14). RER tumor phenotype involves 3% adenomas and 24% cancers. From RER positive tumors only 6% showed mutation of APC locus, p53 as like as loss of heterozygosity which had significantly less frequency. Authors conclude that RER positive phenotype comes in late phases and that tumors pass different changes in adenoma to carcinoma sequence what make a high percent of development of APC mutation (4). Researching of germline mutation show that 80% of patients have exist mutation of APC gene (15). Different germline mutations in APC locus exist in 72% samples of Finnish families (16). Families without detected APC mutation differentiate on the families having mutation. The difference involves following: age of diagnosis making was high (about 38 ages) and expression of extra colonal illness’ was also high. Authors cite a significant of predicative tests on mutation at positive families and they stimulate further analysis of mutation at negative families. Appearances of low frequently colon cancers can be described as an unusual occurrence of “second hit” which is necessary for polyp developing or it is low occurrence of APC locus mutation (17).

Mutation in the region of APC tumor suppressor was researched in Czechs populations (18). APC mutations researched at 46 FAP families and 9 ‘possible’ FAP families. Authors have found 25 germline mutations of APC locus. Many colon cancer syndromes have been characterized based upon their phenotype genetic changes. Among them, the most common and highly studied colon can familial adenomatous polyposis and hereditary nonpolyposis colorectal which are caused by mutations in APC and MMR genes, respectively (19). Mutation in APC locus are considered as one the initiation and progression of colorectal cancer. Our study of both families showed that three tumor tissues belonged to RER negative phenotype, but only one belonged to RER positive phenotype. Microsatellite analysis showed instability of mononucleotide marker Bat 73 at 7 samples and Bat 53 at 5 samples, but Bat 25 and in 1 sample. Dinucleotide marker TP 86 did no show any microsatellite alterations. Genetic alteration of tumor suppressor gene APC appeared at 4 samples, p53 at 3 samples, RB1 at 2 samples and NM23 only at 1 sample, but tumor suppressor genes DCC1 and DCC2 were homozygote. Microsatellite analysis and genetic alteration all members of FAP’s Families “A” and “B” with developed colorectal cancer have shown the identical changes as like as at the cancer.

Conclusion

Our results are agree with results of earlier studies and also the got results confirm the fact that loss of heterozygosity of tumor suppressor gene APC and p53 are responsible for genesis of adenomatous polyposis and it also represents the characteristic of genetic changes FAP’s patients in our region.

List of Abbreviations

FAP - familial adenomatous polyposis
MSI - microsatellite instability
REFERENCES


