MOLECULAR ANALYSIS: MICROSATELLITY INSTABILITY AND LOSS OF HETEROZYGOSITY OF TUMOR SUPPRESSOR GENE IN HEREDITARY NON-POLYPOSIS COLORECTAL CANCERS (HNPCC)

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Abstract

HNPCC (Hereditary non-polyposis colorectal cancers) development is caused by mutation of genes included in system of mismatch repair genes. The mutation exists at 60% of patients in hMSH2 gene, 30% in hMLH1 and 10% both in hPMS1and hPMS2 genes. RER+ exists in about 90% in hereditary non-polyposis colorectal cancer and about 15-28% in sporadic cancers.

The purpose of the study was to determine highly sensitive microsatellite markers which can be fast and efficient way of microsatellite screening for detection of HNPCC patients. Moreover, we have analysed the loss of heterozygosity of tumour suppressor genes which could have the diagnostic value in detection of HPNCC patients.

KEY WORDS: HNPCC, microsatellite instability, loss of heterozygosity, RER phenotype

Introduction

HNPCC is one of the most frequent forms of familial colorectal cancer with about 5-13% of all colorectal cancers (1,2). Its heredity is autosomal dominant, and possibility of its transmission on children is 50:50 regardless of sex (3). If the members of a family are bearers of genetic mutation there is a possibility that cancer will develop during their lives. The risk of development of colorectal cancers is about 80%, endometrium cancers 40% at females, gastric cancers 15% and ovary cancers 10% at females (4). HNPCC develops before the age of 40-45 in regard to sporadic cancers having their own developing periods average about 55-60 ages. There is an opinion (5) that HNPCC and sporadic RER+ colorectal tumours having different paths for cancer development. These tumours have increased mutation level, so called mutator phenotype, which is a result of MMR gene inactivating. There are two paths for genesis of colorectal tumours:

- tumour suppressors path: mutational inactivation both alleles;
- mutator phenotype path: microsatellite instability in HNPCC and sporadic RER + colorectal cancers.

RER+ exists in about 90% in hereditary non-polyposis colorectal cancer and about 15-28% in sporadic cancers. Germline mutation in hMLH1 and hMSH2 genes commonly have for consequence a loss of heterozygosity which is inactivated by MMR gene(6-8). Germline mutation in HNPCC exists in each cell and only one following event, commonly loss of heterozygosity, can inactivate MMR gene. This happens in earlier stage, before inactivation of APC tumour suppressor gene, and results in a fast progression from adenoma to cancer, so called »the adenoma-carcinoma sequence». Loss of functions of APC gene leads towards the other mutations, usually to mutation of „ras" oncogene. Next is the third stage of mutation in tumour suppressor gene, which leads to late adenoma development. Only when the both copies p53 of tumour suppressor gene become defective, will the cells become malignant. At least seven „hits"are necessary for further cancer: two per each tumour suppressor gene (APC, DCC and p53) and one on "ras" oncogene. In 1990 in Amsterdam, an international collaboration group established the Amsterdam Criteria (10) used in diagnostics of HNPCC families, as follows:

- at least three relatives with CRC;
- one should be first degree relative of other two;
- at least two successive generations affected;
- at least one CRC diagnosed before the age of 50;
- FAP excluded;
- diagnosis confirmed by histology.

The Bethesda criteria were used in the study to assist the decision of which colorectal cancers should be tested for microsatellite instability (11). A microsatellite panel must have mononucleotide markers: Bat 25 and Bat 26, and obligatory DS 123 from dinucleotide markers. According to these criteria, there is a cancers classification on microsatellite instability 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+) and MSS cancers- microsatellite stable

Materials and Methods

Through the study we involved 54 patients with clinical diagnosed sporadic colorectal cancer. The Amsterdam Criteria (10) and Bethesda Criteria (11) were used for genetic test and HNPCC was diagnosed in the patients with previously diagnosed sporadic colorectal cancer Therefore, complete study made according to Bethesda and Amsterdam Criteria and 9 of 54 patients are belong to HNPCC group. Our study was based on samples of tumor and surrounding healthy tissue of patients with colorectal cancer. Samples were collected from Gastroenterological and Surgical Clinic of University Clinical Center in Tuzla (Bosnia and Herzegovina). Both tumor and healthy surroundings tissue was formalin fixed and thereafter that embedded in paraffin blocks. Methods of genomic DNA isolation are made on de-paraffinization of tissue sections as on cell proteolyses with proteinase K Fluoroscent chain synthesis of DNA is a method which has very broad application in tumour detection, and it is especially important in determination of microsatellite instability (MSI) and loss of heterozygosity (LOH) of tumour suppressor gene. We used mononucleotide and dinucleotide microsatellite markers in detection of microsatellite instability. In the group of mononucleotide markers the following were used: BAT25, BAT26 and BAT40, but in the group of dinucleotide markers DS123 and TP 53. for detection of LOH were used, we used intragene markers for following tumour suppressor genes: NM23, p53, APC, RB1, DCC1 and DCC2 (9). Amplification reaction contained: 2,5 µl 10 x Pufer; 1µl

DNA (5-10 ng); 2 µl (25mM) MgCL2; per 0,5 µl (2,5 mM) of each dNTP-a; 0,1µl (0,5 U) AmpliTaq Gold polymerase (Perkin-Elmer); 1µl forward primer (12,5 pmol) and 1µl reverse primer (12,5 pmol) by each pair of initial oligonucleotide for each microsatellite marker, and sterile water until final volumen of 25µl.. PCR was performed in a PCR Thermocycler 9600 (Perkin-Elmer). PCR conditions included an initial denaturation step at 94°C for 2 min, 30 cycles denaturation at 94°C for 10 s, a 30 annealing at 55°C, and for 30 s and elongation at 72°C for 30 s and final extension at 72°C for 7 min. Amplified PCR products were separated in an automated sequencer 310 ABI PRISM, Genetic Analyser 310 (Perkin Elmer) which enabled separation and quantification of DNA fragments according to the principle of capillary electrophoresis. Microsatellite analysis comprises of comparation between healthy and tumour tissue of the same patient by using of Genescan program package for analysis. Software detects fluorescent peaks and shows them on electropherograme. Each fluorescent peak is automatically quantified in base size, height and peah field. All samples were tested twice for result confirmation. Loss of heterozygosity was calculated by matematics (9) for heterozygosity cases, allele ratio was calculated for each pair of normal and tumour tissue according to formula T1:T2/N1:N2 , where T1 and N1 are valuable fields of shorter allele length; T2 and N2 are fields of value of longer allele for tumour and healthy samples. The result had rank from 0.00-1.00 if the result was lower or equal to 0, 50. Then the significant loss of heterozigosity was shown and on the longer allele. Homozygosity cases cannot be included in calculation. We used $\chi 2$ test and Fisfer's exact test (Arcus Quickstat biomedical for Windows) in statistic data proccessing.

Results

Analysis of genetic instability of microsatellite loci and RER phenotype of hereditary non-polyposis colorectal cancers
In total sample of 54 patients, 9 or 16,67% belong to hereditary non-polyposis colorectal cancer, according to Amsterdam Criteria and Bethesda Criteria. Analysis of microsatellite instability showed that mononucleotide marker Bat 40 was presented in 7/9 (77,78%) of tumour samples, than Bat 26 and Bat 25 had in 5/9 (55,56%) (Figures 1 and 2). From dinucleotide markers, microsatellite instability showed TP 53 in 5/9 (55,56%) and DS 123 in 4/9 (44,44%) of tumour samples (Table 1).

The research showed that 6/9 (66,67%) of tumour samples belong to RER positive phenotype, and 3/9 (33,33%) belong to RER negative phenotype. From RER positive phenotype 4/6 (66,67%) of tumours showed instability in three loci, and 2/6 (33,33%) in four loci. There was a significant difference between RER+ and RER- tumour phenotype in occurrence of number of microsatellite instability loci ($p<0,01$). In the tumour group of RER+ phenotype, it was detected that mononucleotide marker Bat 40 showed microsatellite instability in 5/6 (83,33%). In the tumour group of RER- phenotype, it was detected that mononucleotide markers Bat 40 and Bat 26 showed microsatellite instability in 2/3 (66,67%) tumor tissues. There was no significant difference between mononucleotide and dinucleotide markers in regard to RER status ($p>0,05$). Analysis of seperate clinico-pathological parameters (sex, age, tumour localization, histopathological type) showed that there were more males 7/9 (77,78%); furthermore that the age category was over 50 in 6/9 (66,67%); then that cancers were located on left side (region of rectum and sigma) 7/9 (77,78%) and that according to histopathological finding they were in adenocarcinomas 8/9 (88,89%). Analysis of microsatelite instability of separate markers and clinicopathological characteristics showed that there was no significant difference ($p>0,05$). Analysis RER phenotype and clinicopathological characteristics showed that RER + phenotype was presented at males in 5/7 (71, 43%). In the age group over 50, there was in 5/6 (83, 33%) and in tumour localization RER+ phenotype was presented at left side tumours in 5/7 (71, 43%) and adenocarcinomas were in 5/8 (62,5%). RER- phenotype was most presented at females in ½ (50%); in age group below 50 in 2/3 (66,67%); and belong to the group of right side tumours 1 /2 (50%) and adenocarcinomas in 3/8 (37,5%) (Table 2)

Microsatellite instability	Mononucleotide markers						Dinucleotide markers			
	Bat 25		Bat 26		Bat 40		TP 53		DS 123	
	No.	%	No.	%	No.	%	No.	%	No.	%
MSS	4	44,44	4	44,44	2	22,22	4	44,44	5	55,56
MSI	5	55,56	5	55,56	7	77,78	5	55,56	4	44,44
Total	9	100,00	9	100,00	9	100,00	9	100,00	9	100,00

TABLE 1. Microsatellite instability of mononucleotide and dinucleotide markers

Clinopatrhological characteristics	N	Tumor RER + phenotype		Tumor RER – phenotype	
		No.	%	No.	%
Sex					
males	7	5	71,43	2	28,57
females	2	1	50,00	1	50,00
Age category (years)					
>50	6	5	83,33	1	16,67
<50	3	1	33,33	2	66,67
Tumor localization					
Left-side	7	5	71,43	2	28,57
Right-side	2	1	50,00	1	50,00
Histopathological classification					
muciounus	1	1	100,00	0	0
adenocarcinoma	8	5	62,50	3	37,5

TABLE 2. Relation between RER status and clinicopathological forms

There was no significant difference between RER status of tumour and clinicopathological characteristics (p>0,05).

Analysis of genetic alterations of tumour suppressor in HNPCC

Genetic alteration of tumour suppressor genes APC appears in 8/9 (88, 89%) samples, then NM 23 tumour suppressor gene in 5/9 (55, 56%) (Figures 3 and 4), DCC 2 in 4/9 (44,44%), tumour suppressor gene DCC1, p53 and RB1 in 2/9 (22,22%) of tumour tissues The highest frequency of homozogosity in locus is present in DCC1 tumour suppressor gene with 4/9 (44, 44%), in DCC 2 with 3/9 (33,33%) (Table 3).

There is a significant difference between tumours with allele loss and without allele loss (p<0,05). Microsatellite instability of marker Bat 25 was found in tumour tissues with heterozygosity loss of locus p53 and RB1 in 2/2 (100%), Bat 26 existed in tumour tissues with heterozygosity loss of locus DCC2 in ¾ (75%) and marker Bat 40 was existed in tumours with heterozygosity loss of locus DCC1 in 2/2 (100%) as like as DCC2 in 4/4 (100%) of tumour tissues. There is no significant difference (p>0,05) in appearance of microsatellite instability of mononucleotide markers in tumour tissues with loss of heterozygosity. Microsatellite instability of dinucleotide marker TP53 was

Genetic alteration	RER + tumours N=6		RER – tumours N=3	
	No.	%	No.	%
NM23 AI	3	50,00	2	66,67
P53 AI	1	16,67	1	33,33
APC AI	5	83,33	3	100,00
RB1 AI	1	16,67	1	33,33
DCC1 AI	2	33,33	0	0,00
DCC2 AI	2	33,33	2	66,67

TABLE 4. Relations between RER status with genetic alterations of tumour suppressors

presented in tumour tissues with heterozygosity loss in locus p53, RB1 in 2/2 (100%) and DCC2 in 4/4 (100%) and marker DS123 which was appeared in tumours with loss of heterozygosity in locus DCC1 in 2/2 (100%) samples. There is no significant difference (p>0,05) in appearance of microsatellite instability of dinucleotide markers in tumour tissues with heterozygosity loss. Loss of heterozygosity of tumour suppressor gene APC was found in tumours which belong to RER+ phenotype in 5/6 (83, 33%), and low frequency was showed at p53 in 1/6 (16, 67%), in RER+ phenotype LOH APC in 3/3 (100%) samples. Alterations were not seen at DCC1 (0%) in regard to LOH DCC2 in 2/3 (66, 67%) (Table 4).

There is no significant difference between RER+ and RER- tumour phenotype in occurrence of loss heterozygosity of tumour suppressor gene (p>0, 05). Analysis of genetic alterations and clinocopathological characteristics showed that loss of heterozygosity in locus' APC, RB1 and NM23 genes was more present at females with 2/2 (100%), and at males allele loss was found in locus APC in 6/7 cases or 85,71%. Age group below 50 had a loss of heterozygosity in locus APC gene in 3/3 (100%) samples, and age group over 50 had loss of heterozygosity APC in 5/6 (83, 33%) samples. Tumour localization had more frequency heterozygosity loss in left side tumours in locus APC in

	Tumor suppressor genes											
Genetic alterations	NM23		P53		APC		RB1		DCC1		DCC2	
	No.	%	No.	%	No.	%	No.	%	No.	%		
AI	5	55,56	2	22,22	8	88,89	2	22,22	2	22,22	4	44,44
NAI	4	44,44	7	77,78	1	11,11	7	77,78	3	33,33	2	22,22
H	0	0	0	0	0	0	0	0	4	44,44	3	33,33
Total	9	100,00	9	100,00	9	100,00	9	100,00	9	100,00	9	100,00

AI allele imbalance
*NA*I without loss of heterozygosity
H homozygous

TABLE 3. Genetic alteration of tumour suppressor gene

Clinicopathological characteristics	N	Genetic alterations of tumour suppressor genes											
		NM23		P53		APC		RB1		DCC1		DCC2	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sex													
m	7	3	42,86	1	14,29	6	85,71	0	0,00	2	28,57	4	57,14
f	2	2	100,00	1	50,00	2	100,00	2	100,00	0	0,00	0	0,00
Age category (years)													
>50	6	3	50,00	1	16,67	5	83,33	1	16,67	2	33,33	3	50,00
<50	3	2	66,67	1	33,33	3	100,00	1	33,33	0	0,00	1	33,33
Tumour localization													
Left-side	7	4	57,14	2	28,57	2	85,71	2	28,57	1	14,29	2	28,57
Right-side	2	1	50,00	0	0,00	6	100,00	0	0,00	2	100,00	2	100,00
Histopathological classification													
muciounus	1	0	0,00	0	0,00	1	100	0	0,00	1	100,00	1	100,00
adenocarcinom	8	5	62,50	2	25,00	7	87,5	2	25,00	1	12,50	3	37,5

TABLE 5. Relation between genetic alterations of tumour suppressor genes and clinicopathological characteristics

6/7 (85, 71%), in right side tumours allele loss in locus APC, DCC1 and DCC2 was 2/2 (100%). According to histopathological classification, LOH APC was found in adenocarcinomas in 7/8 (87, 5%), and at mucinous adenocarcinomas in locus DCC1 and DCC2 with 100% (Table 5). There is no significant difference (p>0,05) in appearance of genetic alterations in tumour tissues with heterozygosity loss and clinico-pathological characteristics.

Discussion

The heterogeneous pattern of tumor mutation suggest that multiple alternative genetic pathway to colorectal cancer exist and accepted genetic model of cancer development is not representative of major tumors.(12). Microsatellite instability was detected in the group of 39 tumours HNPCC with high frequency of 95% (13) . RER+ phenotype showed 95% tumours (14). Authors of the study concluded that RER+ tumour can be found in earlier stages of carcinogenesis in HNPCC, except nonhereditary for which we can suppose that HNPCC tumours can be developed through different genetic changes in the frame of own adenoma-carcinoma sequences . Results of our study showed that RER phenotype was presented in 66,67% of tumour tissues, and RER- phenotype in 33,33% of samples. There is a significant difference between RER+ and RER- phenotype of tumours in appearance of the number of microsatellite locus (p<0,01). However, other study found (15) that MSI was detected in 36% of tumour HNPCC, 27% from that belongs to the group of MSI-L level, but only 9% belongs to MSI-H level. Both tumour groups did not show simple clinico-pathological forms and they showed a significant connection between MSI and mucinous histological tumour type. Furlan et al. (15) concluded that microsatellite screening could be an the most efficient strategy for HNPCC identification. At Slovakia patients, report (16) showed that MSI-H tumour status was noted only at patients younger than 50 and MSI-H was not find at patients older than 50, although they had a positive familiar history for colorectal cancer. Analyses confirmed that 100% patients had instability in 2 locus and 67% had only one instability locus. Between markers used for MSI tumor status, the biggest frequency of microsatellite instability showed mononucleotide marker Bat 26 with frequency of 100%. According to the results of study, Fridrichova et al.(16) concluded hat some patients which with MSI-H tumor status at HNPCC group of ''suspicious'' patients had better expressed clinico-pathological characteristics than patients with positive familial illness history.. Some study (17) found that RER+ phenotype had all patients 18/18 in HNPCC group, in the group of patients with CRC family, but with uncompleted data for HNPCC diagnosis only 9%..

RER + phenotype was noted in families showing clinical HNPCC forms and it was commonly varied from 70-100% (18). Other researched group (19) which was classified as HNPCC ''suspicious'' group (because uncompleted data according to Amsterdam Criteria) had RER+ status in 22% cases. As a consequence, tumours in HNPCC, reveal alterations in the length of simple repetitive genomic sequences like poly A, poly T repeats and least 90% of cases (25). Some report (2) showed that 10-13 families had RER+ phenotype, and analysis confirmed that this group of colorectal patients had a significant difference between RER+ and RER- tumour for each tested marker. Analysis of microsatellite status of some marker

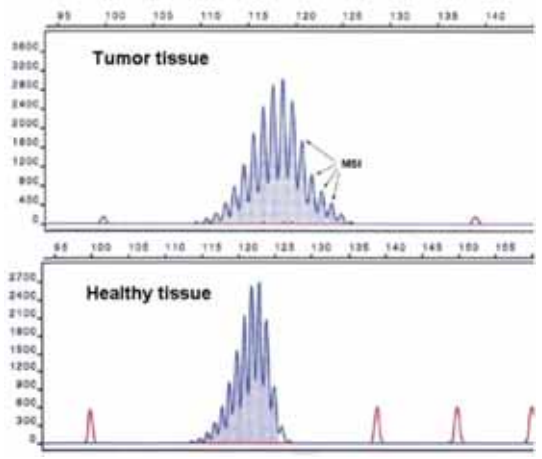

FIGURE 1. Microsatellite instability of the Bat 40 mononucleotide marker

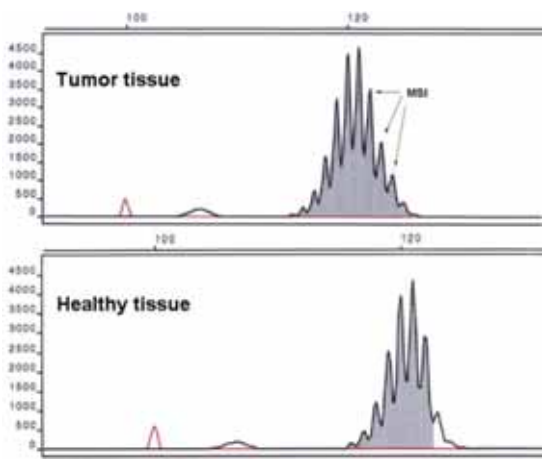

FIGURE 2. Microsatellite instability of the Bat 25 mononuclotide marker

(20) showed that mononucleotide marker Bat 26 was high sensitive marker for screening MLH1/ MSH2 of positive mutation in HNPCC, and Bat 26 was positive at all 27 mutations in these studies. Our study of microsatellite instability showed that mononucleotide marker Bat 40 had frequency 77,78% (Figure 1), of tumour samples, than that Bat 26 and Bat 25 had the same 55,56% (Figure 2).

From dinucleotide markers, microsatellite instability was showed at TP 53 in 5/9 or 55,56% and DS123 with 44,44% of tumour samples. Some report showed (21) that there is no significant relation between MSI status and sex, age and adenoma size. The biggest number of patients belongs to female sex, and age was about 60. These analysis' showed that tumors belong to MSI-H were usually located in distal part of colon.. High level of microsatellite instability was detected in patients over 35 years old and the majority belonged HNPCC group (22). Our study are agreed with some previous cited analysis of clinico-pathological parameters. Analysis of some clinico-pathological parameters (sex, age, tumour localization, histopathological classification) showed that there were more males (77,78%), that age category was over 50 (66,67%), that cancers were located on left side-rectum and sigma region (77,78%) and according histopathological finding it was adenocarcinoma (88,89%). Analyses (26) showed that there is no significant difference between RER+ and RER- tumours regardless of the age, the average age being 68, 2 at RER- group and 55, 2 at RER+ group. Further study (27) showed that RER phenotype showed connection with histopathological variables at patients from Hong Kong. There is no significant difference between RER+ phenotype and tumour localization which are usually right- sided. There is a significant difference between sex and age. These data showed that RER+ phenotype was more presented at males. Report (27) showed that young patients' age under 50 had RER+ tumour phenotype and that there is important difference between RER+ and RER- tumour phenotype. RER+ tumours were found at patients about 60 years old, while RER- tumours were found at older females about 65 years old. This study (28) showed a significant difference between RER+ tumours and tumour localization in proximal colon. There is no significant difference between these two groups of tumours and RER- tumour localization. Our analysis of RER phenotype and clinico-pathological characteristics showed that RER+ phenotype existed at males 71, 43%. In age group over 50, it was in 5/6 (83,33%) and at tumour localization RER+ phenotype existed in left side tumours in 71,43% and adenocarcinomas were 62,5%. So, RER- phenotype was more present at females in 50%; in the age group under 50 in 66,67%, and they belong to the group of right-side cancers in ½ cases or 50%, and adenocarcenomas was in 37,5% of tumour samples. There is no significant difference between RER tumour status and clinicopathological characteristics ($p>0,05$). Alteration of tumour suppressor gene p53 in HNPCC patients (14) were in 64% cases, and alteration of APC gene in 57% cases. Analyses of loss heterozygosity in APC and p53 of tumour suppressor genes were significantly less than in HNPCC tumours in regard to sporadic tumours. Results showed (24) that 81% of MSI tumours HNPCC did not show a genetic alteration in p53 tumour suppressor gene, while 93% MSS tumours of sporadic tumours had genetic alterations in tumour suppressor gene p53. Our results showed that loss of heterozygosity was

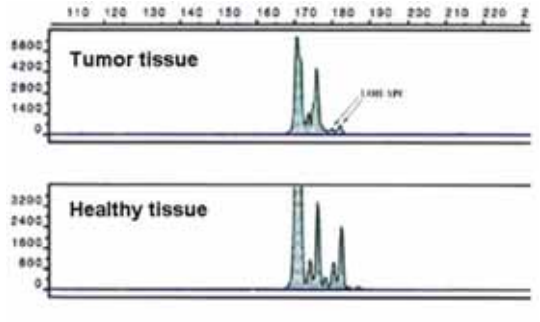

FIGURE 3. Loss of heterozygosity of the APC tumour suppressor gene

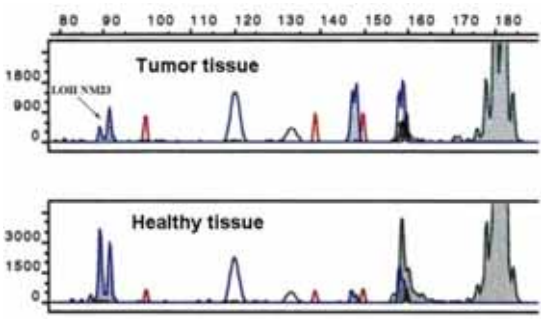

FIGURE 4. Loss of heterozygosity of the NM23 tumour suppressor gene

found at tumour suppressor gene APC in 88,89% (Figure 3), then NM 23 tumour suppressor gene 55,56% (Figure 4), DCC2 44,44% tumour suppressor gene DCC1, p53 and RB1 in 22,22 tumour tissues. Homozygosity of locus was presented at DCC1 tumour suppressor gene in 44, 44%, at DCC2 in 33, 33%. There is a statistic significant difference between tumour with allele and without allele (p<0, 05). Indinnimeo et al. (26) concluded that genetic instability of tumour suppressor genes p53 and NM 23 as clinicopathological forms at rectal cancers were found out that NM 23 showed alteration at all RER+ tumours, while p53 tumour suppressor showed this occurrence only in one case. There is no statistic difference between clinical parameters and RER status. Loss of heterozygosity was confirmed on 18q chromosome in 53,8% samples and RER+ phenotype was found in 17,85% cases.(36) There is no significant difference between LOH of 18q chromosome and clinicopathological characteristics regardless of sex, tumour state and differentiation level. Allele loss (29) which was usually detected at adenocarcinomas in distal part of colon than at tumours in proximal part. Our results of loss of heterozygosity of tumour suppressor gene APC showed in tumours belonging RER+ phenotype in 83,33%, and low frequency showed at p53 in 16,67%, at RER- phenotype LOH APC in 100% samples, and alterations were not find at DCC1 (0%), but LOH DCC2 had it in 66,67%. Watatani et al.(30) found that loss of heterozygosity of 17p chromosome in 61% was presented at right –side tumours and only in 60% cases at left-side tumours. RER+ phenotype had 43% of right-side tumours and 24 % of left-side tumours belong to RER+ phenotype According to Ikenaga et al. (31) the occurrence of microsatellite instability at younger patients with colorectal cancer was 50, 9% and it was significantly higher than at older patients (12%). At 24 patients without MSI, it was established that only one case belongs to HNPCC and two have familial history of illness. Rebishung et al. (32) concluded that RER+ phenotype was rather less present at rectal tumours with prior loss of heterozygosity. Mutation of APC locus had smaller frequency than mutation of p53 locus at colorectal tumours with the same phenotype. Loss of heterozygosity at p53 was presented at 53% samples and at APC in 26% cases. Tumours belonging to RER- phenotype which have no loss of heterozygosity can be developed as a result of carcinogenesis model.. HNPCC is an inherited disease characterized by the development of cancer at a predominance of proximal colon, excess of multiple cancers increased risk for selected extracolonic adenocarcinomas and better prognosis (33). Our analysis of genetic alteration and clinico-pathological characteristics showed that loss of heterozygosity in locus APC,RB1 and Nm23 gene were presented at females in 100%,and at males had allele loss in locus APC in 85,71%. Age group below 50, loss of heterozygosity was presented in locus APC gene in 100% samples, but in age group over 50 this loss was found in locus APC in 83,33%. At tumour localization, loss of heterozygosity was presented at left side tumours in locus APC with 85, 71%, and at right side tumours allele loss in locus APC,DCC1 and DCC2 was 100%. According to histological classification, LOH APC was presented at adenocarcinomas in 7/8 or 87,5%, but at mucinous adenocarcinomas in locus DCC1 and DCC2 with 100% (Table 5). Germ-line mutations in the mismatch-repair genes MLH1, MSH2, MSH6, and PMS2 lead to the development of the Lynch syndrome (hereditary nonpolyposis colorectal cancer), conferring a strong susceptibility to cancer (34,35) The disorder (36) has traditionally been recognized in kindreds with a clustering of related cancers in association with mutations in DNA mismatch repair genes. HNPCC is associated with a substantially increased risk for several forms of malignancy but particularly colorectal and endometrial cancer.

Conclusion

The results of the study show the importance of usage of Amsterdam and Bethesda criterion in detection of HNPCC patients. Unless this is not done, all tumours should be treated as occasional.

The analysis of microsatellite instability showed that mononucleotide marker Bat 40 was present in 77,78% of tumour samples. Bat 26 and Bat 25 were present in 55,56% of the samples. From dinucleotide markers, microsatellite instability was present in TP53 in 55,56% and in DS123 in 44% of tumour samples.

The result show that the analysis of loss of heterozygosity was marked in tumour suppressor gene APC in 88,9%, in NM 23 tumour suppressor gene at 55,6%, DCC 2 at 44,4% tumour suppressor gene DCC, p53 and RB1 in 22,2% of tumour tissues.

The study reveals that mononucleotide marker Bat 40 has significant microsatellite instability and as such it is efficient for the fast microsatellite screening with HNPCC patients.

Tumour suppressor APC gene can be highly sensitive marker of mutations which are only related to HNPCC group of patients.

List of Abbreviations

MMR	-	Mismatch repair genes
MSI	-	Microsatellite instability
RER +, –	-	Replication error positive
hMSH2	-	Human mut S homolog 2
hMSH3	-	Human mutS homolog 3
hPMS1 and hPMS2	-	Human post-meiotic segregation 1 and 2
GTBP	-	G/T mismatch-binding protein
FAP	-	Familial adenomatous polyposis
APC	-	Adenomatous polyposis coli
LOH	-	Loss of heterozygosity
DCC	-	Deleted colorectal carcinoma
MCC	-	Mutated in colorectal cancer

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