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ORIGINAL ARTICLE

Microsatellite instability in Bulgarian patients with endometrial cancer

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Summary

Purpose: *Microsatellite instability (MSI) is a frequent event in different types of cancer. In several studies MSI was shown to have both clinical and prognostic value. The aim of our study was to determine the frequency of MSI in Bulgarian patients with endometrial cancer (EC) and the possible relation of this phenomenon to their clinicopathological characteristics.*

Patients and methods: *A total of 33 histologically confirmed EC patients were analyzed for tumor MSI using a panel of 6 microsatellite markers.*

Results: *We identified MSI in 30% of endometrial cancer cases. Six of them had high degree of MSI (MSI-H), and 4 displayed low degree of MSI (MSI-L).*

Conclusion: *The frequency of MSI in Bulgarian EC patients does not differ significantly from that reported in other European studies.*

Key words: endometrial cancer, microsatellite instability

Introduction

Endometrial cancer is one of the most common malignant tumors of the female genital tract in the Western world, showing highest incidence rates in North America and Western Europe [1].

Well-established risk factors for developing EC include environmental or endogenous factors associated with unopposed estrogen as well as inherited genetic predisposition to the disease.

The Lynch syndrome (or HNPCC - hereditary nonpolyposis colorectal cancer) is an autosomal dominantly inherited cancer-susceptibility syndrome. It is associated with an early onset of diverse types of cancer, including cancer of the colon and rectum, endometrium, ovary, small bowel, ureter, and renal pelvis. The lifetime risk of EC for women with Lynch syndrome is 40-60%, which equals or exceeds their risk of colorectal cancer [2].

The molecular genetic basis of the HNPCC syndrome is an inherited mutation in one of the DNA-mismatch repair genes (MMR genes) [3-6]. Somatic inactivation of the homologous wild-type allele results in defect in postreplicative DNA repair, leading to MSI - a phenomenon believed to drive the process of tumorigenesis in the affected tissue [7,8].

Microsatellites are repetitive DNA sequences, widely dispersed throughout the genome. During replication they are particularly susceptible to slippage errors, leading to insertion or deletion mutations i.e. widening or shortening of the repeat. Mismatch repair proteins normally recognize and repair these mutations. Altered or inactivated MMR genes render MMR proteins unable to fulfill the process of mismatch repair, leading to accumulation of mutations at microsat-

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ellite loci (designated as MSI) as well as elsewhere in the genome [9,10].

Though a typical feature of the Lynch syndrome tumors, MSI can also be found in unselected cases: approximately 20% of EC and 17% of colorectal cancers [11].

Most sporadic tumors with MSI do not have mutations in MMR repair genes; rather MSI in them is often a consequence of promoter hypermethylation of the MLH1 or MSH2 genes, leading to the absence of expression of these DNA repair proteins.

Many studies have shown presence of MSI ranging from 17 to 38% in EC [12-14].

The aim of our study was to determine the frequency of MSI in Bulgarian patients with EC and its relation to the patients' clinicopathological characteristics.

Patients and methods

A total of 33 patients with EC were included in this study. Patients were referred by the University Hospital of Obstetrics and Gynecology, Sofia, and the National Cancer Institute in the period 2005-2006. All patients included had histologically confirmed EC. Family information was obtained and analyzed from 26 of them. An accurate genealogical tree was prepared in which the main causes of morbidity and mortality of first- and second-degree relatives were recorded. The mean age in the patient group was 61.9 years (range 47-78).

All patients involved in the study gave written informed consent for DNA analysis. They were further inquired about risk factors that could have contributed to developing the disease - hormonal therapy, concurrent disease, body weight, lifestyle, as well as eating, drinking and smoking habits.

Blood and fresh surgically removed tumors along with corresponding normal tissue (where available) were collected from 26 patients and genomic DNA was isolated using standard procedures [15].

Following an erythrocyte lysis step, blood leukocytes were separated and treated with proteinase K overnight. DNA was then purified by either adding phenol-isoamyl alcohol or by addition of salt. At this point, a centrifugation of 15 min was performed. The supernatant was further mixed with ice-cold ethanol to precipitate DNA which was then dissolved and the solution was stored at 4°C.

In parallel a retrospective analysis was performed in 7 patients whose DNA was extracted from paraffin embedded tissue samples using Nucleospin Tissue kit following the manufacturer's protocol (Macherey & Nagel, Germany).

The assessment of MSI was based on the use of a panel of 6 microsatellite markers (BAT26, D2S123, D5S346, D18S35, FGA, and TP53), which includes one mono-, four di- and one tetra-nucleotide repeat markers. These markers were amplified from both normal and tumor DNA samples and independently electrophoresed on an automated fluorescence sequencer (ALF Express, Pharmacia). MSI was defined by the appearance of different alleles in the tumor DNA when compared to the corresponding normal DNA.

Tumors that displayed MSI at 3 or more loci were classified as MSI-H, those showing MSI at 1 or 2 loci as MSI-L, and all the rest as MSI-stable (MSI-S).

Associations between MSI status and patient characteristics were evaluated using Fisher's exact test and x^2 test as appropriate.

Results

All 33 patients were evaluated for MSI. Ten (30%) of them were unstable, 6 (18.2%) showed MSI-H, 4 (12.1%) had MSI-L, and 23 (69.7%) were MSI-S (Table 1). Because of the small size of the study group, we were cautious in correlating MSI status to any of the clinical or histological features of the patients.

Nonetheless, we found that MSI phenotype prevailed in those patients presenting some variables predictive of a better patient outcome such as endometrioid histology and better tumor differentiation. Depth of myometrial invasion did not show such an association (Table 2).

Of the 26 patients characterized for histological type, 24 (92.3%) had endometrioid or mixed type with endometrioid component and only 2 had clear cell or serous papillary histology, none of the latter showing instability. Of the 10 MSI patients 8 (80%) had endometrioid tumors; for the remaining 2 no relevant information was available.

Twenty-one patients were characterized for histological grade: 18 had well or moderately well differentiated tumors, and only 3 showed poor differen-

Table 1. Number of endometrial cancer patients with and without MSI

	MSI-unstable	*MSI-S*	*MSI-L*	*MSI-H*
Patients, n	10	23	4	6
%	30	69.7	12.1	18.2

Table 2. Pathology characteristics and their distribution in relation to MSI cases

Variable	*Patients, n*	*MSI positive, n*	*%*	*p-value*
Histological grade				
well differentiated	9	4	44.4	0.60
moderately differentiated	9	3	33.3	0.70
poorly differentiated	3	1	33.3	0.85
Histological type				
endometrioid or mixed	24	8	33.2	0.32
clear cell / serous papillary	2	0	–	
Myometrial invasion				
none	3	0	–	
< 1/3	11	4	36.3	0.60
> 1/3	5	2	40.0	0.63

tiation. MSI was slightly associated with better differentiation. Seven out of 18 (38.8%) well or moderately well differentiated tumors were unstable, while one of the 3 (33.3%) poorly differentiated showed MSI of any degree.

The depth of myometrial invasion was not associated with MSI; 4 out of 11 patients (36.3%) with less than 1/3 or no myometrial invasion were MSI-H, while 2 out of 5 (40%) with invasion more than 1/3 showed MSI of any degree.

The MSI phenotype was not associated with risk factors such as smoking or drinking habits. In fact, very few of the patients reported such habits. Diabetes mellitus - a well known risk factor for developing endometrial cancer - also showed no association as only 1 of the 6 (16.6%) patients comorbid for diabetes mellitus showed MSI.

Personal history of cancer rather than family history of the disease was more often present in patients with MSI (47.4 and 14.3%, respectively). MSI(+) distribution among family and sporadic cases revealed a prevalence for the sporadic ones. No dependence of MSI frequency with regard to the patients' age at diagnosis was found (Table 3).

Discussion

The frequency of MSI in sporadic endometrial carcinoma ranges from 17 to 45% in European populations [16-19].

Tumor MSI is generally regarded as a good prognostic factor. A plausible explanation of this finding suggests that MSI-positive tumors, which are obviously unable to repair mutations, produce many mutant proteins with high immunogenicity.

Consistent with this, one EC study reported that MSI occurs more often in tumors which show marked infiltration by lymphocytes around the tumor tissue [20].

The established molecular diagnosis of the Lynch syndrome involves MSI testing, followed by hypermethylation analysis of MSI positive cases, and finally mutational screening of hypermethylation negative patients. Apart from HNPCC molecular diagnosis, in the past years MSI testing has become essential for tumor characterization and is now offered to patients worldwide as a routine test in many types of malignancy.

Although the biologic significance of MSI in sporadic tumors is not yet fully understood, it has been demonstrated that this type of genetic instability can be used as a marker for disease prognosis [21-24].

Several studies have shown that MSI confers a survival advantage in both sporadic and hereditary colorectal cancers [25-27]. A survival benefit among MSI positive tumors has also been reported for gastric cancer patients [28].

Table 3. Patient characteristics and their distribution in MSI(+) and MSI(-) cases

	Patients n	*MSI+ n (%)*	*MSI– n (%)*	*p-value*
Age at diagnosis (years)				
<60	7	2 (28.6)	5 (71.4)	0.88
≥60	19	6 (31.6)	13 (68.4)	
Sporadic cancer	19	9 (47.4)	10 (52.6)	0.12
Family history of cancer	7	1 (14.3)	6 (85.7)	

On the basis of MSI as well as other molecular and clinical features, endometrial tumors fall into two major subtypes - endometrioid and nonendometrioid [29,30].

The strong association of MSI with endometrioid histology suggests in itself a favorable outcome because these cases usually have much better survival than those with serous or clear cell histology [31].

Three studies have reported a positive correlation between high histological grade and the MSI phenotype [32-34] and one study also revealed a positive correlation between 5-year survival and 5-year recurrence-free survival and MSI in EC patients [35].

Our results, although based on a small group of patients, are generally consistent with the established overall frequency of MSI for this type of malignancy. In order to correlate MSI status to patient clinical characteristics, a much larger study group will be required.

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