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Original Article

Microsatellite Instability in Young Women with Endometrioid type Endometrial Cancer

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Abstract

Background: This study was designed to determine the frequency of Microsatellite Instability (MSI) in young Iranian patients with endometrial carcinoma and to evaluate its association with histopathologic and clinical features of disease.

Methods: Microsatellite status was analyzed in 23 patients with endometrioid type endometrial cancer who were less than 55 years. Clinicopathologic characteristics such as age, International Federation of Gynecology and Obstetric (FIGO) grading and staging of tumor, family history of Hereditary Non-polyposis Colorectal Cancer (HNPCC), oral conception (OC) consumption, number of pregnancies, fertility, menstrual cycles and underlying disease were considered. Chi-square and Fisher exact tests were used to find the significant relationships.

Results: MSI analysis showed 8 patients (34.8%) were MSS (Microsatellite Stable), 15 patients (62.5%) were MSI positive. Among cases with MSI phenotype, 4 cases (17.4%) had low instability (MSI-L) and 11 cases (47.8%) had high instability (MSI-H). Three cases with MSI-H had family history of HNPCC related cancers. Five cases (21.7%) had infertility in which 4 of them (80%) had MSI phenotype. There was no statistically significant relationship between MSI phenotype and tumor grade and stage.

Conclusion: Few studies reported high frequency of MSI among young patients. Some studies mentioned similar results in endometrioid type of tumor. This study showed even higher frequency (65%) when MSI analyzed in young endometrioid type endometrial patients. Most cases with infertility had MSI-H phenotype. It may suggest that beside women with family history of HNPCC, EC screening using MSI would be beneficial in infertile women too.

Keywords: *Microsatellite instability, Endometrial Cancer, Young patients, Endometrioid, Iran*

Introduction

Endometrial cancer (EC) is the most common malignancy of genital tract in United States and the second most common gynecological malignancy in United Kingdom (1, 2). There are many known risk factors for the disease among them genetic susceptibility is considered. It is proven that some women with EC have a hereditary predisposition due to defects in mismatch repair genes (MMR) which are involved in recognition and repair of DNA damage occurs during mitosis or by exogenous mutagens (3, 4). Impairment in function of MMR genes typically presents with microsatellite instability (MSI). MSI seems to be important for the development of various gynecologic cancers and has both clinical and prognostic values (5). MSI has been reported in 9% to 43% of sporadic endometrial carcinoma (6). This wide range of frequency reports may be caused by the age and tumor pathology influence, as most past studies have included varied age groups and histological subtypes. EC has many histological subtypes; endometrioid type is responsible for 60% to 80% of cases. Recent studies proved that MSI is present in up to 25% of unselected EC and up to 40% of endometrioid type EC (6-8). Few studies showed higher rate of 35-40% in frequency of MSI in young endometrial cancer patients (9-

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12). EC is the second cancer associated with hereditary non-polyposis colorectal cancer (HNPCC) syndrome. MSI can be detected in 40 to 75% of HNPCC-related EC (13, 14).

After the identification of MSI in endometrial cancer, several researchers tried to determine its clinical and pathological significance in the disease; however, the results were contradictory. Many studies have reported a correlation between high histological grade and stage and MSI phenotype (10, 15, 16) whereas other studies declared no relation (11, 17, 18). Conflicting effects on prognosis and patient survival have also been reported. Several studies have reported a correlation between poor prognosis and MSI phenotype (10, 15), few studies mentioned MSI as a better survival indicator (18) and some studies resulted in no association with prognosis in large population-based series (6, 12). The association of MSI phenotype in endometrial cancer with high grade and stage and adverse outcome contrasts with low stage and favorable survival in colorectal cancers with MSI phenotype.

This study was designed to determine the frequency of MSI in young Iranian patients under age 55 with endometrioid type endometrial carcinoma and to evaluate its association with histopathologic and clinical features of disease. The efficiency of MSI screening was evaluated in HNPCC-associated endometrial cancer, as the age at diagnosis of this group was approximately 15 yr younger than for sporadic endometrial cancer. This is the first study of this kind among Iranian patients where the relation between MSI and EC risk factors such as oral contraception (OC) consumption, number of pregnancies, fertility and menstrual cycles was evaluated.

Materials and Methods

Sample collection

Among registered cases of endometrial cancers in provincial center for health and human services during 2001-2006, 23 consented individuals below 55 yr entered the study. For each patient, paraffin-embedded tissue samples of both tumor and its corresponding tumor-free tissue were obtained from reference diagnostic pathology laboratory. Clinicopathologic characteristics such as age, International Federation of Obstetrics and Gynecology (FIGO) grading and staging of tumor (19), in addition to family history of HNPCC (According to Amsterdam criteria (20), OC consumption, number of pregnancies, fertility, menstrual cycles and underlying disease were considered. This study was approved by the Mashhad University of Medical Sciences Ethics Committee.

DNA extraction

DNA was extracted from paraffin-embedded tissues using Proteinase K digestion method. Three to 5 sections of paraffin-embedded tissues in 5 μm diameter, placed in 1.5 mL microtube, were deparaffinized 2 times by 1 mL of xylene. After centrifugation, it was washed twice by 500μL ethanol 96%. The tissue pellet then digested using 100-200 μL of digestion buffer (50 mM Tris, pH= 8.5; 1 mM EDTA and 0.5% tween 20) containing 200-400 μg/mL Proteinase K (20 mg/ml, Fermentas, Lithuania). After incubating at 65 °C for 3 h followed by overnight incubation at 37 °C, Proteinase K was inactivated by heating at 95 °C for 10 min. Following the centrifugation the supernatant used for PCR amplification. The concentration of extracted DNA was measured using a UV spectrophotometer (UV 1101, Biotech Photometer). In cases where normal tissue was not available peripheral blood was used for obtaining normal DNA.

Microsatellite studies

MSI analysis was performed by conventional polymerase chain reaction using National Cancer institute recommended 5 microsatellite markers followed by denaturating gel electrophoresis and silver nitrate staining as described below (21). The tissue DNA samples were subjected to PCR amplification of the above microsatellite sequence in 20 μl reaction mixture containing 200 ng of extracted DNA, 1X PCR buffer (GeNet Bio, Korea) containing 2 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 10 pmol of each sequence-specific primer, and 1 U of Taq DNA polymerase (GeNet Bio, Korea).

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The primers include two mononucleotide repeats (BAT 25 and BAT 26) and three dinucleotide repeats (D2S123, D5S346, D17S250) as defined at international workshop on HNPCC in Bethesda (National Cancer Institute, Bethesda, MD) (Table 1) (21). All primer sequences and marker localizations were taken from the Genome Database (http://www.gdb.org; Table 1). Amplifications were conducted in a Techgene thermocycler. After an initial denaturation at 94 °C for 5 min, PCR amplification was performed for 35 cycles, each consisting of 45 s at 94 °C, 1 min at 54 °C for all primers, and 1 min at 72 °C, followed by a final extension of 30 min at 72 °C. Two µl PCR products was mixed with a formamide loading buffer (usually 2 µl PCR-products with 18 µl formamide) and denaturated by boiling for 5 min at 95 °C and immediately chilled on ice at least 5 min. The mixture was loaded onto 6% polyacrylamide gel containing 7 mol/L urea. Following the denaturating gel electrophoresis at 60 W (50 mA; 1200 V) for 60min in 0.5X TBE (45mmol/L Tris; 1 mmol/L EDTA; 45 mmol/L Boric acid), the gels were stained with silver nitrate as described previously (26). Microsatellite-associated instability was identified based on comparison between electrophoretic patterns of tumor and their corresponding tumor-free samples. Microsatellite status in tumors were classified into 3 groups: microsatellite stable (MSS) with no MSI at any of the loci examined. MSI-L means one instable marker and MSI-H means two or more markers being instable. This classification is the same that has been used for CRC (20).

Statistical analysis

The relationship between microsatellite instability and clinicopathological parameters was analyzed using SPSS version 11.5 and the *P* value was calculated using Chi-square and Fisher exact tests to find the significant relationships.

Results

MSI analysis

Microsatellite instability was detected in 15 of 23 cases (65.2%) with endometrioid endometrial cancer. Eleven cases (47.8%) exhibited MSI-H phenotype while 4 cases (17.4%) were MSI-L. The most unstable microsatellite marker was D5S346 as its instability detected in 10 cases (43.4%). Other markers had same frequency. Fig. 1 Illustrates complete microsatellite analysis in one patient. The patient has instability in BAT-25 and BAT-26 markers (Arrows) and stable for other markers.

Clinicopathological Data

The mean age of the patients was 48.6 yr, ranged between 22.9 to 54 yr. The mean age in MSS group was 51.5 yr while 47.3 yr in MSI group. The mean age of MSI-H group was 48 yr whereas 45.5 yr in MSH-L group; however, the youngest patients had instability in all 5 markers.

Histopathologic features of cancer and its association with MSI are illustrated in Table 2. Accordingly most of the cases were in grade I of the disease. All the patients with MSI-L had grade I of tumor. However there was no significant relationship between MSI and tumor grade. This data was the same for surgical staging of tumor and no significant relationship was obtained.

Three patients (13%) mentioned HNPCC associated cancer in family members compatible with Amsterdam criteria (20) whereas one person had a history of cancer not associated with HNPCC. All three patients with positive history had MSI-H phenotype with the mean age of 49.7 yr.

Evaluating the relationship of MSI phenotype and contraception method, pregnancies, menstrual disturbance and underlying disease revealed no significant association. However, the youngest ECs were detected in patients with infertility (mean of 41 yr) especially in primary type (mean of 33.6 yr). Most cases (80%, 4/5 cases) with infertility had MSI phenotype whereas 61% (11/18 cases) of fertile patients carried this phenotype, however this was not statistically significant. The frequency of oligomenorrhea/amenorrhea among the patients was high (21.7%) in comparison with general population (15%) however no significant relation found with MSI phenotype. All the cases with oligomenorrhea/Amenorrhea also had a history of infertility.

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Table 1: Primer sequence and Tm of primers

MSI marker	GDB Accession ID	location	Sequence	Amplimer
BAT 25	9834508	4q12	F: 5' TCG CCT CCA AGA ATG TAA GT 3' R: 5' TCT GCA TTT TAA CTA TGG CTC 3'	125bp (contains approx. 25bp pA tract)
D2S123	187953	2p16	F: 5' AAA CAG GAT GCC TGC CTT TA 3' R: 5' GGA CTT TCC ACC TAT GGG AC 3'	197-227bp
D5S346	181171	5q21-q22	F: 5' ACT CAC TCT AGT GAT AAA TCG GG 3' R: 5' AGC AGA TAA GAC AGT ATT ACT AGT T 3'	96-122bp
D17S250	177030	17q11.2-17q12	F: 5' GGA AGA ATC AAA TAG ACA AT 3' R: 5' GCT GGC CAT ATA TAT ATT TAA ACC 3'	151-169bp
BAT 26	9834505	2p16	F: 5' TGA CTA CTT TTG ACT TCA GCC 3' R: 5' AAC CAT TCA ACA TTT TTA ACC C 3'	125bp (contains approx. 26bp pA tract)

Table 2: Histopathological features of tumors

Histopathology		No. of patients (%)	MSS	MSI-L	MSI-H
Grade	1	17 (73.9)	6 (75)	4 (100)	7 (63.6)
	2	4 (17.4)	2 (25)	-	2 (18.2)
	3	2 (8.7)	-	-	2 (18.2)
Stage	I	17 (73.9)	6 (75)	4 (100)	7 (63.6)
	II	4 (17.4)	2 (25)	-	2 (18.2)
	III	2 (8.7)	-	-	2 (18.2)
	IV	-	-	-	-
Family History					
	Negative	19 (82.6)	7 (88.9)	3 (75)	9 (81.8)
	Associated Cancer	3 (13)	-	1 (25)	2 (18.2)
	Non associated Cancer	1 (4.3)	1 (11.1)	-	-
Contraception					
	None	10 (43.5)	N/A	N/A	N/A
	LD	6 (26.1)			
	IUD	1 (4.3)			
	Condom	3 (13)			
	Withdrawal	3 (13)			
Pregnancies	Nulipara	3 (13)	N/A	N/A	N/A
	Primipara	1 (4.3)			
	Multipara	19 (82.6)			
Fertility	Fertile	18 (78.3)	7 (88.9)		
	Primary infertility	3 (13)	-	1	2
	Secondary infertility	2 (8.7)	1 (11.1)	-	1
Menstrual	Normal cycle	16 (69.6)	6 (75)	3 (75)	7
	Oligo/Amenorrhea	5 (21.7)	1 (12.5)	1 (25)	3
	Polymenorrhea	2 (8.7)	1 (12.5)	-	1
Underlying disease					
	Negative	19 (82.6)	6 (75)	4 (100)	9 (81.8)
	Hypertension	4 (17.4)	2 (25)	-	2 (18.2)

N/A: data not available

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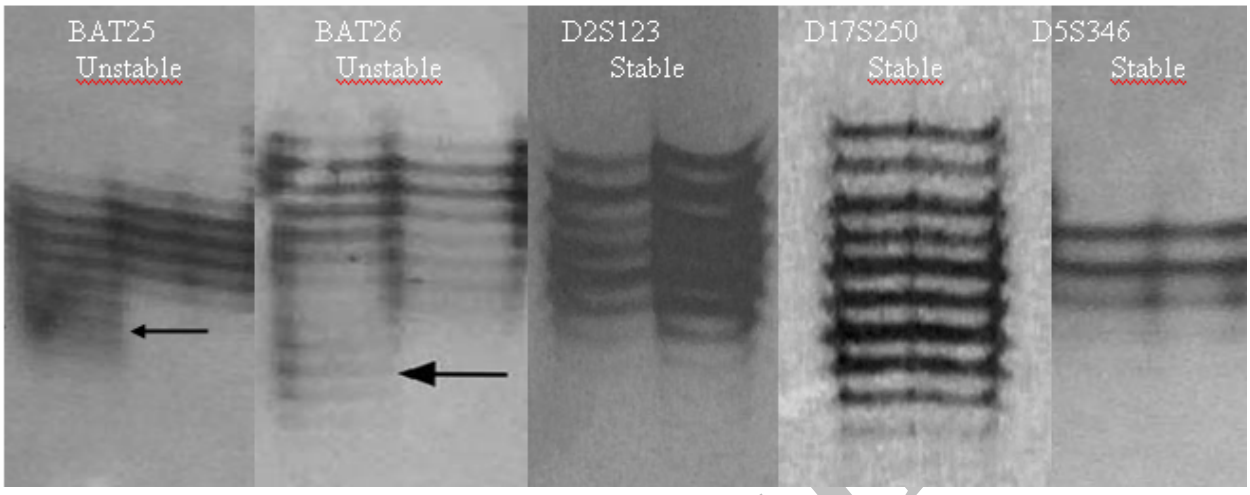

Fig. 1: Illustration of complete microsatellite analysis in one patient. The patient has instability in BAT-25 and BAT-26 markers (Arrows) and stable for other markers

Discussion

Five to ten percent of endometrial cancers have genetic basis mainly due to HNPCC syndrome, as the EC is the second common syndrome-associated tumor. It is now accepted that immunostaining for mismatch repair genes and microsatellite instability is important in EC patients with some features including young age, pre-menopausal status, coexistence with colonic or other HNPCC associated tumors and family history (22). The main genetic dysfunction in HNPCC is MMR function impairment. The mechanism of MMR gene silencing in EC was shown to be predominantly an epigenetic alternations rather than caused by mutations, mainly due to hypermethylation of promoter of these genes (23). Kawaguchi et al., showed that aberrant hMLH1 hypermethylation can be found in 58.8% (10/17) of MSI-positive cases, with a significant positive correlation between aberrant hMLH1 hypermethylation and MSI-positive cases of sporadic endometrial cancer (P= 0.02). Based on this result, they suggested that aberrant hMLH1 hypermethylation causes MSI in endometrial cancer (24).

MSI has been reported in wide range of sporadic EC between 9% to 43% (6), however it may differ according to age and tumor histology. Recent studies proved that MSI is present in up to 40% of endometrioid type EC and higher rate of 35-40% in frequency of MSI in young endometrial cancer patients (6-12). These two groups of study just considered one parameter of age or tumor histology. Our study revealed when age and tumor histology considered together the MSI frequency is even higher up to 65%. MMR impairment is the main genetic cause of young age endometrioid type EC. Similar tumorogenesis pathways of endometrioid type EC and HNPCC as progression through a hyperplasia-carcinoma may explain this high frequency of MSI.

There was no significant relationship between MSI and tumor grade and surgical staging. Similar result has been reported before (11, 17, 18). There was also no relationship between MSI phenotype and other clinical parameters except for infertility. Most cases with infertility had MSI-H phenotype and were at young age. However, exact relationship between MSI and infertility has not been reported yet.

This high incidence rate of MSI in studied group especially in infertile cases may suggest the efficacy of screening for EC at younger age group. Various methods have been introduced, including annual transvaginal ultrasound and endometrial biopsy (25) to screen for EC in HNPCC. A possible adjunct to the current screening modalities is

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MSI analysis in menstrual effluent. In support of this, DNA analysis of vaginal secretions in sporadic EC has been recently reported that EC was detected with a sensitivity and specificity of 100% and 92.7%, respectively (26). This modality followed by other routine workups could be suitable for screening of EC in infertile women as infertility is the main risk factor for developing endometrial cancer.

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