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Microsatellite instability in endometrial polyps

Salete S. Rios^{a,1,*}, Rosângela V. Andrade^b, Rinaldo W. Pereira^b, Nathan R. Wall^c, Khaled Bahjri^d, Érica Caldas^b, Larissa Cavalcante^b, and Florêncio Figueiredo^{a,b}

^aMolecular Pathology Post-graduated Program, University of Brasilia, Brasilia, DF, Brazil

^bGenomic Science and Biotechnology Graduate Program, Catholic University of Brasilia, Brasilia, DF, Brazil

^cDepartment of Biochemistry and Microbiology, Loma Linda, CA, USA

^dDepartment of Epidemiology and Biostatistics, Loma Linda University, Loma Linda, CA, USA

Abstract

Objective(s)—To investigate the prevalence of microsatellite instability (MSI) in endometrial polyps and to evaluate whether there are clinical and histopathological parameters associated with this kind of instability.

Study design—Between September 2008 and April 2009, endometrial polyps were collected from 109 patients. MSI was evaluated using the NCI recommended markers BAT25, BAT26, D2S123, D5S346 and D17S250. Histopathological analysis was performed, and clinical information was obtained from patients' records.

Result(s)—MSI low was detected in 6.4% of the validated samples (7/109). Of the seven MSI that were detected, six were positive for instability at D17S250 and one at D5S346. There were no significant differences between polyps with or without MSI with regard to age, BMI, menarche, parity, miscarriage or menopause; however, MSI was more frequent in polyps with simple hyperplasia without atypia (3/20; 15%). Furthermore, patients with multiple polyps had a marginally but statistically insignificant increase in the frequency of MSI ($p < 0.07$).

Conclusion(s)—This is the first prospective study of MSI in endometrial polyps using hysteroscopically obtained samples. In a population of 109 patients, MSI was infrequent in endometrial polyps. Although MSI appears to be more frequent in multiple polyps and polyps with simple hyperplasia without atypia, this was not statistically significant.

Keywords

Microsatellite instability; Endometrial polyp; Associated factors

1. Introduction

Endometrial polyps are a localized overgrowth of endometrial tissue composed of glands, stroma and blood vessels that are covered by epithelium [1]. The incidence of malignancy among endometrial polyps varies between 0.8 and 13% [2,3] depending on the selection of patients. Polyps are believed to be a risk factor for endometrial cancer due to their association with hyperplastic and neoplastic lesions; however, their role as a precursor for

endometrial cancer has not been established. Opinions differ on whether endometrial polyps are an intrinsic marker for concurrent or subsequent malignancy [3].

Genetic alterations can facilitate neoplastic transformation and could be a step within a multistep model for endometrial oncogenesis. Microsatellite instability (MSI) has been shown to be important in the molecular pathogenesis and prognosis of endometrial carcinoma [4,5]. MSI is caused by defects in the DNA mismatch repair system (MMR) and is defined as electrophoretic shifts in the allele sizes of microsatellite DNA sequences, a finding first described in Hereditary Non Polyposis Colorectal Carcinoma (HNPCC) [6]. Although endometrial polyps have been associated with endometrial carcinoma [2,3], it is still unclear whether MSI plays a role in this disease, as the few published reports have very small sample sizes [7,8]. To the best of our knowledge, there is still no prospective molecular study with polyp samples obtained by hysteroscopy, although endometrial polyps are known to be common premalignant lesions. The objective of this study was to estimate the prevalence of MSI in endometrial polyps and to evaluate whether clinical and histopathological parameters are associated with this type of instability.

2. Materials and methods

2.1. Patients and samples

The Institutional Review Board of Human Studies Committee at the Catholic University approved this study. Tissue samples were collected from study participants who gave informed consent. We analyzed the endometrial polyps obtained from 119 consecutive patients diagnosed by transvaginal ultrasonography and who subsequently underwent polyp removal by hysteroscopic surgery at the Brasilia University Hospital and Verhum Institute. Resections were carried out under mild sedation in the operating room. Polyps and fragments of the adjacent endometrial mucosa were removed using a monopolar resectoscope (Karl Storz, Tuttlingen, Germany). All patients were admitted between September 2008 and April 2009. Polyps and adjacent endometrium were collected in order to accurately analyze the polyp [2]. Small fragments of the endometrial polyps and of the adjacent endometrium were placed in a separate container with RNAlater RNA stabilization reagent (Qiagen Inc., Valencia, CA) for genetic analysis. A separate polyp sample with its adjacent endometrium were fixed in 10% formalin and sent for pathological analyses. Only those patients who had a surgical polypectomy and whose histopathology results were available for polyp and normal adjacent endometrial tissue confirmation were included. Patient clinical data were obtained from the medical records. Evaluated factors included patient clinical characteristics and possible risk factors for polyp malignancy; such as age, body mass index (BMI), mean age at menarche, parity, miscarriage, menopause status (women were considered postmenopausal if they reported at least 12 months of amenorrhea or if they had surgical menopause), use of hormone replacement therapy (HRT), and number of polyps. Women were considered to have multiple polyps in the presence of two or more polyps at the hysteroscopic visualization and after histopathological confirmation.

2.2. Histopathology

Pathologists from the Division of Pathology at the University of Brasilia performed the histopathological analyses. Haematoxylin and eosin stained sections were prepared from formalin-fixed, paraffin-embedded tissues. Diagnosis distinguished between benign endometrial polyps (endometrial polyps, endometrial polyps with simple hyperplasia without atypia, endometrial polyps with complex hyperplasia without atypia) or premalignant/malignant polyps (endometrial polyps with simple hyperplasia and atypia, endometrial polyps with complex hyperplasia and atypia or invasive endometrial carcinoma) [9]. The histopathologic definitions are as follows. Endometrial simple hyperplasia is

defined by moderately distorted endometrial architecture with glands that are minimally dilated but focally crowded. The lining epithelium of the glands is pseudostratified and shows variable mitotic activity. Endometrial complex hyperplasia is defined by crowded, branched complex glands with little intervening stroma and lined by stratified and mitotic cigar-shaped cells. Atypical simple hyperplasia is defined by an architecture similar to simple hyperplasia but with more irregular glands. The cells of atypical simple hyperplasia are stratified with a loss of polarity, and nuclei are rounded with granular chromatin. In atypical complex hyperplasia, the architecture is similar to endometrial complex hyperplasia, but the glands are crowded and lined by atypical cells with pleomorphic nuclei. Endometrial carcinoma is defined by crowded, malignant tubular glands that vary in size and invade the stroma with glands lined by cells showing marked atypia and mitotic activity [10].

2.3. Genetic analysis

2.3.1. DNA extraction—DNA from normal and polyp endometrial tissue was extracted and purified by a standard salting-out procedure using the Invisorb Spin Micro DNA kit (Invitek, Berlin, Germany) according to the manufacturer's instructions.

2.3.2. Polymerase chain reaction (PCR) and microsatellite analysis—

Microsatellite analysis was performed using five National Cancer Institute Consensus designated markers for MSI detection in colorectal cancer, which is widely used in endometrial cancer and other tumours [11]. These markers included mononucleotide markers BAT 25 and BAT 26, and dinucleotide markers D2S123, D5S346 and D17S250. PCR was performed on 12.5 µl of reaction solution containing 1.25 µl 10× PCR buffer (Invitrogen), 1.25 µl bovine serum albumin (BSA; 2.5 mg/mL), 0.625 µl (50 mM) MgCl₂, 1.25 µl (25 mM) deoxynucleotide triphosphates (dNTP), 0.2 µl platinum Taq polymerase (5 U/µL; Invitrogen), 2 µl DNA (10 ng) and 0.5 µl each of the forward and reverse primers (0.4 µM). Amplified PCR products were run using a Veriti 96 Well Thermocycler (Applied Biosystems, Foster City, CA, USA). Samples were denatured at 95 °C for 2 min, followed by 20 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 30 s with a final extension at 72 °C for 20 min. After PCR, 1 µl of the product was mixed with 8.85 µl of Hi-Di formamide (Applied Biosystems) and 0.15 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems). Each endometrial polyp specimen and its corresponding normal tissue were analyzed for the five microsatellite markers by singleplex PCR. First, a PCR program was designed to amplify all loci individually under the same conditions; the primers were then combined in various mixtures to amplify all loci simultaneously. The D17S250 marker was not successfully amplified under these conditions however, so we performed a singleplex PCR reaction for this marker and a multiplex reaction for all other markers. The mixture was denatured at 95 °C for 2 min and cooled on ice before loading onto a DNA ABI PRISM 3130XL Genetic Sequencer (Applied Biosystems). Sequencing results were analyzed using GeneMapper software 4.0 (Applied Biosystems). To evaluate the MSI results, a five-marker panel was used to classify tumours. Tumours were categorized as MSI-High if two or more of the five markers showed instability, MSI-low if only one of the five markers showed instability and MSS if the sample showed no differences between the polyp and the normal tissue. Healthy tissue of the surrounding polyp endometrium from the same patient served as a negative control for MSI [11]. The validation of study with the adequate DNA control from the normal tissue (MSI negative) and the neoplastic tissue (MSI-positive) was provided by Dr. Theodoro Ostrowski Micra's Laboratory. MSI status was confirmed in all cases via replication; furthermore, MSI was matched for histological and clinical parameters.

2.4. Statistical analyses

Analyses were performed using frequencies, means and standard deviations. Fisher's exact test analyzed quantitative variables with regard the presence or absence of MSI. We performed multivariable binary logistic regression to determine the odds ratios (OR) for independent associated variables. Beta (β) indicated the probability of making a Type II error. The significance level was established as $p < 0.05$. Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows version 17.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

MSI was not successful for ten polyps samples due to technical difficulties related to DNA; thus, of 119 patients, only 109 subjects were eligible for our study. Of the endometrial polyps included in this study, we found seven cases of MSI (6.4%). The correlations between clinical parameters and MSI are presented in Table 1. There were no significant differences between polyps with and without MSI in regard to age, mean BMI, mean menarche, mean parity, mean miscarriage or menopause status; however, patients with multiple polyps had a marginal but statistically insignificant increase in frequency of MSI when compared to patients with solitary polyps.

The histopathological evaluation of the 109 polyps is shown in Table 2. MSI was detected more frequently in endometrial polyps with simple hyperplasia without atypia (3/20, 15%) than in endometrial polyps (4/86, 4.6%). An unadjusted univariate analysis indicated that endometrial polyps with simple hyperplasia without atypia were associated with having MSI (OR = 3.6; 95% CI: 0.74, 17.66; Table 2). Moreover, there was no association between MSI and pre-malignant or malignant lesions.

We evaluated MSI at five loci, but only D17S250 (six samples) and D5S346 (one sample) were involved (Table 3). All MSI cases had low instability.

A multivariate binary logistic regression (Table 4) included simple hyperplastic polyps without atypia and the number of polyps while age was controlled as variables in the model. After adjusting for age and polyp number, simple hyperplastic polyps without atypia were more likely to have MSI (OR = 2.59; 95% CI: 0.48, 13.88). In addition, multiple polyps were more likely to present MSI (OR = 3.29; 95% CI: 0.61, 17.84). These findings were not statistically significant.

4. Comments

Microsatellite instability has been systematically studied in many conditions characterized by polyps, including intestinal, nasal and gastric polyps [12–14]. Although the prevalence of endometrial polyps in the female population is thought to be 25% [10], and MSI has been found in 20–34% of sporadic endometrial carcinomas [5,15], the prevalence of MSI in endometrial polyps is still unclear because this estimate is based on few publications, small sample sizes and samples obtained by endometrial biopsy and curettage – methods known to be inferior for extracting the entire polyp [7,8,16]. To our knowledge there are no published studies that have focused on the relationship between MSI and endometrial polyps as the main target of the investigation. Most reports in the literature show the endometrial polyps as only one component of the study [7,8].

In our study population, we evaluated 109 polyps from patients with no known history of HNPCC. The prevalence of MSI in endometrial polyps was 6.4% (6/109), and all polyps were MSI-low. The biological significance of MSI-low in colorectal carcinoma has been

conflicting because although MSI-low seemed to be located in the “gray zone” between MSS and MSI-high, the sporadic MSI-low displayed clinicopathological features and might form a distinct subgroup [17]. The prevalence of MSI-low in sporadic endometrial carcinoma varies between 10 and 30%, but its clinical and histopathological significance are not clear [5,15].

MSI was more frequent in endometrial polyps with simple hyperplasia without atypia (15%) than in endometrial polyps (4.6%) (Fig. 1).

Of note, the exact behaviour of pre-malignant lesions as they progress to endometrial carcinoma has not been completely established. To date, nuclear atypia is the most important risk factor for progression to malignancy [18]. According to the Vogelstein model, malignant tumours develop from normal tissue through a series of precursor lesions. This process is accompanied by a stepwise acquisition of genetic alterations, particularly in oncogenes and tumour suppressor genes; thus, there is a subsequent increase in genetic instability [19]. There is epidemiological and molecular evidence that suggests endometrial hyperplasia is a precursor lesion for endometrial neoplasia. Studies have also shown MSI in atypical hyperplasia from patients who are MMR gene mutation carriers and progress to late endometrial carcinoma. Furthermore, studies conclude that MSI may develop before clinical and histopathological findings, thus predating the carcinoma diagnosis by years [8,20,21].

Multiple polyps were found in 28 patients (25.68%). MSI was more frequent among women who had more than one polyp – 4/28 (14%) versus women who had only one polyp 3/81 (3.7%) – a finding that showed a trend towards significance ($p < 0.07$). Multiple polyps have been associated with an increased risk of endometrial carcinoma [22]. Our study indicates that the prevalence of MSI does not differ based on BMI, mean menarche, mean parity, number of miscarriages and menopause status.

One of the limitations of this study is its cross-sectional design, which prevents establishing a causal relationship. Considering that the D17S250 locus required a specific PCR optimization and that it was almost the only locus found to be altered, it will be necessary to evaluate a larger sample with the use of additional MSI markers. On the other hand, our study has the largest sample size with respect to the study of MSI in endometrial polyps in the literature. In addition, the polyps were obtained by operative hysteroscopy, and we specifically evaluated MSI in endometrial polyps.

Improvements in diagnostic methods are crucial for increasing early detection, which is important for positive outcomes in all disease. Although studies demonstrated established association between the increased risk of malignant transformation and clinical parameters such as age, hypertension and menopause status [2,3,9], there is still no marker that can predict if an endometrial polyp is at risk of neoplastic transformation.

We expected to find a larger number of cases of MSI in endometrial polyps; however, heterogeneity in the sample population and the small number of MSI markers tested in this study may have affected the results. In conclusion, our study showed infrequent MSI in endometrial polyps. Given that we found no clear association between MSI in multiple polyps and hyperplastic polyps without atypia, future investigations with a larger sample size are required to determine whether MSI could be a useful marker to predict the endometrial polyps at risk of neoplastic transformation.

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References

- [1]. Peterson WF, Novak ER. Endometrial polyps. *Obstet Gynecol.* 1956; 8:40–9. [PubMed: 13335063]
- [2]. Savelli L, De Iaco P, Santini D, et al. Histopathologic features and risk factors for benignity, hyperplasia and cancer in endometrial polyps. *Am J Obstet Gynecol.* 2003; 188(4):927–31. [PubMed: 12712087]
- [3]. Hileeto D, Fadare O, Martel M, Zheng W. Age dependent association of endometrial polyps with increased risk of cancer involvement. *World J Surg Oncol.* 2005; 3(1):8. [PubMed: 15703068]
- [4]. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. *Clin Oncol.* 2006; 24(29):4783–91.
- [5]. Tibiletti MG, Furlan D, Taborelli M, et al. Microsatellite instability in endometrial cancer: relation to histological subtypes. *Gynecol Oncol.* 1999; 73(2):247–52. [PubMed: 10329042]
- [6]. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *New Engl J Med.* 2003; 348:919–32. [PubMed: 12621137]
- [7]. Shia J, Black D, Hummer AJ, Boyd J, Soslow RA. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. *Hum Pathol.* 2008; 39(1): 116–25. Erratum in: *Hum Pathol* 2008; 39(7):1122. [PubMed: 17949789]
- [8]. Faquin WC, Fitzgerald JT, Lin MC, Boynton KA, Muto MG, Mutter GL. Sporadic microsatellite instability is specific to neoplastic and preneoplastic endometrial tissues. *Am J Clin Pathol.* 2000; 113(4):576–82. [PubMed: 10787358]
- [9]. Ben-Arie A, Goldchmit C, Laviv Y, et al. The malignant potential of endometrial polyps. *Eur J Obstet Gynecol Reprod Biol.* 2004; 115(2):206–10. [PubMed: 15262357]
- [10]. Kurman, RJ., editor. Blaunstein's pathology of the female genital tract. Springer; New York: 2002.
- [11]. Boland CR, Thibodeau SN, Hamilton SR, et al. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of micro-satellite instability in colorectal cancer. *Cancer Res.* 1998; 58(22):5248–57. [PubMed: 9823339]
- [12]. Sandmeier D, Benhattar J, Martin P, Bouzourene H. Serrated polyps of the large intestine: a molecular study comparing sessile serrated adenomas and hyperplastic polyps. *Histopathology.* 2009; 55(2):206–13. [PubMed: 19694828]
- [13]. Karatzanis AD, Tzortzaki E, Samara KD, et al. Microsatellite DNA instability in nasal polyposis. *Laryngoscope.* 2009; 119(4):751–6. [PubMed: 19205008]
- [14]. Nogueira AM, Carneiro F, Seruca R, et al. Microsatellite instability in hyperplastic and adenomatous polyps of the stomach. *Cancer.* 1999; 86(9):1649–56. [PubMed: 10547536]
- [15]. Wong YF, Ip TY, Chung TK, et al. Clinical and pathologic significance of microsatellite instability in endometrial cancer. *Int J Gynecol Cancer.* 1999; 9(5):406–10. [PubMed: 11240802]
- [16]. Ebstein E, Ramirez A, Skoog L, Valentin L. Dilatation and curettage fails to detect most focal lesions in the uterine cavity in women with postmenopausal bleeding. *Acta Obstet Gynecol Scand.* 2001; 80(12):1131–6. [PubMed: 11846711]
- [17]. Kim YH, Min BH, Choi HK, et al. Sporadic colorectal carcinomas with low-level microsatellite instability in Korea: do they form a distinct subgroup with distinguished clinicopathological features? *J Surg Oncol.* 2009; 99(6):351–5. [PubMed: 19204939]
- [18]. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of “untreated” hyperplasia in 170 patients. *Cancer.* 1985; 56(2):403–12. [PubMed: 4005805]
- [19]. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.* 1990; 61(5):759–67. [PubMed: 2188735]
- [20]. Doll A, Abal M, Rigau M, et al. Novel molecular profiles of endometrial cancer-new light through old windows. *J Steroid Biochem Mol Biol.* 2008; 108(3–5):221–9. [PubMed: 18061438]
- [21]. Ichikawa Y, Tsunoda H, Takano K, Oki A, Yoshikawa H. Microsatellite instability and immunohistochemical analysis of MLH1 and MSH2 in normal endometrium, endometrial

- hyperplasia and endometrial cancer from a hereditary nonpolyposis colorectal cancer patient. *Jpn J Clin Oncol.* 2002; 32(3):110–2. [PubMed: 11956307]
- [22]. Fernández-Parra J, Rodríguez Oliver A, López Criado S, Parrilla Fernández F, Montoya Ventoso F. Hysteroscopic evaluation of endometrial polyps. *Int J Gynaecol Obstet.* 2006; 95(2):144–8. [PubMed: 16920120]

Fig. 1.

(A) Electropherogram of the microsatellite marker D17S250 for MSI analysis. The endometrial polyp sample (P) from this MSI-positive case shows deviations in the electrophoretic patterns when compared to normal (N) tissue. (B) Microscopic examination of a hyperplastic endometrial polyp (Haematoxylin-Eosin, 40 $\times$).

Table 1

Relationship between clinical parameters and microsatellite instability.

Variables	MSI negative (<i>n</i> = 102)	MSI positive (<i>n</i> = 7)	<i>p</i> -Value
Age (years)	50.06 ± 0.55	53.42 ± 12.67	0.421
BMI (kg/M ²)	26.36 ± 6.04	29.41 ± 11.98	0.602
Menarche (years)	13.34 ± 1.92	13.33 ± 1.97	0.99
Parity	2.14 ± 1.37	3 ± 2.61	0.184
Miscarriage	1 ± 1.3	2 ± 1	0.186
Menopause (%)			
Yes	39 (92.8%)	3 (7.2%)	0.54
No	63 (94.0%)	4 (6.0%)	
Polyp number			
Solitary (%)	78 (96.3%)	3 (3.7%)	0.07
Multiple (%)	24 (85.7%)	4 (14.3%)	

Table 2

Relationship between microsatellite instability and histopathological findings.

Histopathological results		MSI		OR (CI)	p-Value		
		Yes				No	
		Number	Percent (%)			Number	Percent (%)
Benign							
Endometrial polyps	4	4.7	82	95.3	1.0		
Endometrial polyps with simple hyperplasia without atypia	3	15	17	85.0	3.6 (0.74–17.66)		
Endometrial polyps with complex hyperplasia without atypia ^b	0	0.0	1	100.0	N/A ^a		
Pre-malignant or malignant							
Endometrial polyps with simple atypical hyperplasia ^b	0	0.0	1	100.0	N/A ^a		
Endometrial carcinoma ^b	0	0.0	1	100.0	N/A ^a		

^a N/A OR cannot be generated.

^b This row was not considered in the calculation of the p-value due to its low frequency.

Table 3

Allele size instability in endometrial polyps and its relationship with histopathological findings, patient age and number of polyps.

Patient number	Histopathological findings	Patient age (years)	Number of polyps		MSI markers				
			Solitary	Multiple	BAT25	BAT26	D2S123	D5S346	DI7S250
18	Endometrial polyps	66		×	—	—	—	—	+
42	Endometrial polyps with simple hyperplasia without atypia	65	×		—	—	—	—	+
47	Endometrial polyps with simple hyperplasia without atypia	68		×	—	—	—	—	+
69	Endometrial polyps	46		×	—	—	—	—	+
84	Endometrial polyps	38		×	—	—	—	—	+
105	Endometrial polyps with simple hyperplasia without atypia	41	×		—	—	—	+	—
112	Endometrial polyps	50	×		—	—	—	—	+

Table 4

Multivariate binary logistic regression.

Dependent variable = microsatellite instability (yes/no)			
Variables	Beta	Odds ratio (OR)	95% Confidence interval
Constant	-4.67		
Age (≥ 50 years)	0.12	1.13	(0.22, 5.81)
Endometrial polyps with simple hyperplasia without atypia	0.95	2.59	(0.48, 13.88)
Multiple polyps	1.19	3.29	(0.61, 17.84)