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Microsatellite instability in Colombian patients with colorectal adenocarcinoma

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Abstract

Introduction: The microsatellite instability (MSI) pathway is involved in the carcinogenesis of 15% of colorectal carcinomas (CRC). The detection of this alteration is relevant for the prognosis and treatment of CRC patients. **Objective:** The aim of this study is to determine the prevalence of MSI in CRC in a cohort of patients in Bogotá, Colombia. **Materials and methods:** The presence of MLH1, PMS2, MSH2, and MSH6 was evaluated by immunohistochemistry in CRC samples collected during colectomy. Clinicopathological variables were analyzed as well. Cases with loss of MLH1 and PMS2 were evaluated for BRAF gene mutation. **Results:** A total of 86 cases were included. The median age was 69 years, 52.3% were male. 12 (13.9%) patients had IMS, 10 (83.3%) had absence of MLH1/PMS2 expression and 2 (16.7%) absence of MSH2/MSH6 expression. The median age of patients with IMS was 52 years (45-76.5), of which 9 were male. 66.7% of carcinomas were located in the right colon and the most frequent histological type was moderately differentiated adenocarcinoma (67%). Tumor infiltrating lymphocytes were observed in 83% of the cases, while the presence of Crohn's-like infiltrate was present in 42%. BRAF mutation was observed in 30% of patients with loss of MLH1 and PMS2. **Conclusion:** The prevalence of IMS in our population was 14%, similar to the data observed in the North American and European populations. However, we observed that 83% had loss of expression of the MLH1/PMS2 complex, a higher prevalence compared to other populations.

Keywords

Colon; Microsatellite instability; Colombia.

INTRODUCTION

Colorectal carcinoma (CRC) ranks fourth globally for cancer incidence (6.1%) and second (9.2%) for cancer deaths in 2018 in both sexes. The incidence of CRC in Latin America is low (7.5% of the total)[(1)]. In Colombia, CRC is the third leading cause of cancer mortality, followed by prostate and lung carcinomas [(2)].

The development of CRC occurs most often sporadically, between 70% and 80%, followed by the hereditary form, between 15% and 30%[(3)]. Less than 10% corresponds to the inherited variant, which is divided into two groups: the non-polyposis form and the polyposis. Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is associated with deoxyribonucleic acid (DNA) repair mechanisms, such as microsatellite instability (MSI), which is the leading cause of Lynch syndrome (LS) (3%-4%). The other variant (less than 1%) refers to familial adenomatous polyposis (FAP), which is characterized by the formation of multiple potentially malignant polyps. A small subset of 1% to

2% of CRC cases arise as a result of chronic inflammation (inflammatory bowel diseases) [4]. It is important to note that sporadic CRC cases do not have identified genetic risk factors; the development of the disease is due to dietary factors, lifestyle, environmental factors or acquired somatic mutations [5]; while hereditary and family cases have an earlier clinical onset. Mutations are acquired throughout life in family and sporadic cases, unlike hereditary cases, whose germline mutation is acquired at birth [6]. The identification of sporadic and hereditary tumors is relevant for the follow-up and prognosis of patients. It is known that hereditary tumors have an increased risk of developing other tumors; therefore, monitoring should be stricter [7].

MSI is caused by a defect in the proteins responsible for the repair of bad mating *(mismatch* repair [MMR]: homologue MutL 1 [*MLH1*], increased postmeiotic segregation 2 [*PMS2*], homologous mutS 2 [*MSH2*] and homologous mutS 6 [*MSH6*] homologue MutL 3 [*MLH3*], homologue mutS 3 [*MSH3*], increased postmeiotic segregation 1 [*PMS1*] and exonuclease 1[Exo1]). These proteins form heterodimers to correct alterations in the mating of nucleotides generated by the cleavage of abnormal DNA helping in the cleavage of mismating errors and in the formation of new corrected DNA strands. Alterations of MLH1 / PMS2 and MSH2 / MSH6 heterodimers are associated with different colon, endometrial, stomach, ovarian, prostate tumors among others (8). At the clinical level, it is important to identify patients with MSI because they have a better prognosis and reduced recurrence rate when treatment has been linked to the use of immunotherapy, which has been shown to be superior to management with conventional chemotherapy [9,10].

MSI can be identified by immunohistochemistry (IHC) or by polymerase chain reaction (PCR). Based on its result it can be classified into 4 categories:

1. Sporadic MMR defects (dMMR): hypermethylation of the MLH1 promoter region.
2. Lynch syndrome due to germline mutation in one of the MMRs *(MLH1,* PMS2, *MSH2, MSH6)* or alterations in the *EpCAM* gene (epithelial cell adhesion molecule); tumor-associated calcium signal transducer 1*(TACSTD1)* that causes epigenetic silencing of *MSH2.*
3. Colon cancer with unexplained dMMR (cases with no identified germline MMR mutation or hypermethylation of the *MLH1*promoter region): These cases have unexplained dMMR and have been classified as Lynch type.
4. Constitutional MMR deficiency syndrome (biallelic MMR mutations in the germ line): In these cases, normal adjacent tissue also has dMMR[8].

In Latin America, the prevalence of MSI in different populations has been evaluated [11-15]. In Colombia there is little data about this condition in patients with CRC; for this reason, the objectives of this study are to know the prevalence of MSI in CRC treated patients in two highly complex hospitals in Colombia and, additionally, to describe the clinical-pathological characteristics. Generally, tumors with MSI are in the right colon of high histological grades or with mucinous differentiation, and associated with abundant tumor-infiltrating lymphocytes (TIL) and a Crohn's-like host immune response*(Crohn-like)* [5,16,17].

MATERIALS AND METHODS

Study Population

A cross-sectional study was carried out in which the presence of MSI in CRC was determined through IHC, evaluating the loss of expression of MMR proteins (MLH1, PMS2, MSH2 and MSH6). The databases were reviewed and a filter was made of all patients diagnosed and treated with CRC by colectomy at the *Hospital de San José* and the *Hospital Infantil Universitario de San José in Bogotá*, Colombia during January 2012 and December 2017. CRC biopsies were not included considering that several patients are diagnosed in these hospitals; however, follow-up and clinical treatment are performed in other hospitals; therefore, the tumor stage cannot be known with certainty. The exclusion criterion was those patients for whom the pathology material was not suitable for processing by IHC. These two hospitals are of high complexity and are located in two areas of the Colombian capital, which allows having heterogeneous populations of different ethnicities and socioeconomic strata.

The following clinical and histological variables were evaluated: age, tumor size, tumor location, histological grade (well differentiated [G1], moderately differentiated [G2] and poorly differentiated [G3]). When the mucosecretion exceeded 50% of the tumor surface, it was considered a mucinous tumor. The presence of intratumoral lymphocytes (ITL) was measured in 5 fields of great increase (40x) on the tumor invasion front. It was classified into 0 (absence of lymphocytes), 1 (between 2 and 5 lymphocytes) and 3 (greater than 5 lymphocytes); *Crohn's-like* inflammation was defined as the accumulation of lymphocytes with or without germinal center formation on the front of tumor infiltration, positivity and number of nodes involved. The histological type and the TNM classification (tumor, nodes and metastases) was determined according to the criteria of the World Health Organization (WHO) [18]. The complete medical records of patients with loss of MMR protein expression were reviewed to find out if they

met the criteria for Lynch syndrome based on the Bethesda guideline [19].

IHC Study

All cases selected for the study were cut at 3 μM for immunological marking with the following primary antibodies: MLH1 (clone: BS29, LabVision Autostainer, source and isotype of immunoglobulin [Ig]: immunoglobulin G1 [IgG1], dilution 1:100, presentation: MAD-000726Q); MSH2 (clone: FE11, LabVision Autostainer, Ig source and isotype: IgG1/kg, dilution 1:50, presentation: MAD-000677Q); PMS2 (clone: EP51, LabVision Autostainer, source and isotype of Ig: Rabbit IgG, dilution 1:50, presentation: MAD-000681Q) and MSH6 (clone: EP49, LabVision Autostainer, source and isotype of Ig: Rabbit IgG, dilution 1:50, presentation: MAD-000635Q).

Sections of the tumor were kept at 60 °C for 2 hours, deparaffinized with xylene for 10 minutes, and rehydrated with ethanol. Heat-mediated antigen recovery was carried out using a 1/10 EDTA 10X solution (LabVision™) in a «vaporizer» for 50 minutes. Then, the paraffin blocks were immersed in a solution of hydrogen peroxide 1/10*(Hydrogen Peroxide Block UltraVision)* for 10 minutes at room temperature and incubated with a primary antibody solution for 2 hours at room temperature in a wet chamber. After two washes in TBS 1/10 (saline buffered with Dako Tris, pH: 7,6), tissues were incubated with biotinylated secondary antibody (primary antibody amplifier) for 10 minutes at room temperature. Color development was performed with a DBA buffer and slides were kept with hematoxylin for two minutes. A section was used in which an immunohistochemical procedure was performed without adding the primary antibody as a negative control. The positive control slides included CRC samples.

The IHC study was carried out and its result was reviewed by two pathologists (PL, FP) by light microscopy, categorizing reactivity or non-reactivity of each of the markers, in case of finding loss of expression (non-reactivity) of the markers MLH1, MSH2, MSH6 and PMS2. The positive control was nuclear staining in the normal mucosa or lymphocytic infiltration.

BRAF V600 Mutation Analysis

Based on the algorithms to determine somatic and hereditary CRCs [20], PCR and sequencing were performed to detect V600 mutation (p. Val600Glu, p. Val600Asp, p. Val600Lys and p. Val600Arg) in the *BRAF* gene (NM_004333.4, chr. 7) to the cases with loss of MLH1 evaluated by IHC.

Statistical Analysis

The statistical analysis was performed by summarizing the quantitative variables with medians and interquartile ranges (IQR). Associations between the presence of MSI and clinical-pathological features were assessed using Fisher's exact test. Statistical significance was assumed with p-values < 0.05.

Ethical Considerations

The study was approved by the human research ethics committees of the *Fundación Universitaria de Ciencias de la Salud* and was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

Population with CRC

A total of 86 patients were included, of which 52.3% were men. The median age at the time of the surgical procedure was 69 years old (IQR: 59-77). 39 cases (45.35 %) presented the tumor in the right colon, 33 (38.4 %) between the sigmoid and the rectum, 8 (9.3 %) in the left colon, 7 (8.14 %) in the transverse and one case (1.2 %) presented synchronous tumors of location in the right colon and transverse **(Table 1).**

The histological subtypes observed were 71 (82.5%) patients with conventional adenocarcinomas, 12 (13.9%) presented mucinous adenocarcinoma, 2 showed the seal ring variant (2.3%) and 1 was associated with the adenosquamous and mucinous subtype (1.2%), previously published [21]. Regarding the degree of differentiation of conventional adenocarcinomas, 59 (83.1%) were classified as moderately differentiated tumors, 7 (9.8%) poorly differentiated and 5 (7%) well differentiated. It is important to highlight that 32.6% had metastases to lymph nodes. Tumor deposits were found at 3.49% **(Table 1).**

Population with CRC and MSI

In total, 12 (13.9%) patients presented MSI, all with high MSI (defined by the absence of expression of more than two MMR proteins) **(Table 2).** 10 (83.3%) patients had no MLH1 and PMS2, and 2 (16.7%) MSH2 and MSH6. There were 9 cases in men and 3 in women, and the median age was 52 years old (IQR: 45-76.5 *BRAF* mutation was observed in 30% of patients with loss of MLH1 and PMS2.

The most frequent location was in the right colon (8/12: 66.7 %), followed by sigmoid and rectum (2/12: 16,7 %).

The most frequent histological type was moderately differentiated adenocarcinoma (8/12), followed by mucinous adenocarcinoma (3/12) and an adenosquamous and mucinous case. 10/12 cases were recognized (83.3%) of accompanying ITL, 8 mild to moderate and 2 marked. In addition, 5/12 cases (41.7%) presented Crohn's-like infiltration. 6/12 (50%) patients had infiltration to the muscularis propria (pT2NM), followed by infiltration to the serosa 4/12 (33.3%) (pT4aNM), 1/12 (8.3%) to the lamina propria (pTisNM) and 1/12 (8.3%) with infiltration to other organs (stomach). Only 1/12 had lymph node involvement (pTN1bM) (**Tables 1** and **2**). 5 patients were

Table 1. Clinical-pathological characteristics of patients with CRC

Characteristics	Total (%) n = 86	MSS (%) n = 74	MSI (%) n = 12	p-value
Age, Median (IQR)	69 (59-77)	69 (60-78)	52 (45-76.5)	0.0606
Sex				0.090
- Male	45 (52.3)	36 (48.6)	9 (75)	
- Female	41 (47.7)	38 (51.4)	3 (25)	
Tumor size (IQR)	5 (3-7)	4.25 (3-7)	6 (4.75-8.5)	0.1330
Location				0.180
- Ascending colon (right and transverse)	46 (52.8)	37 (42.5)	8 (10)	
- Descending colon (left and sigmoid) and rectum	41 (47.2)	37 (49)	2 (3)	
Histological type				0.516
- Conventional adenocarcinoma	71 (82.6)	62 (83.8)	8 (72.7)	
- Mucinous	12 (13)	10 (12.2)	3 (25)	
- Seal ring	2 (2.3)	2 (2.7)	0	
- Adenosquamous	1 (1.2)	1 (1.3)	1 (8.3)	
Histological grade of conventional adenocarcinomas				0.735
- Well differentiated	5 (7)	5 (7)	0	
- Moderate	59 (83.1)	51 (71.8)	8 (11.2)	
- Poorly differentiated	7 (9.8)	6 (8.4)	1 (1.4)	
Multifocality	9 (10.5)	8 (10.8)	1 (8.3)	0.795
pT				0.130
- Lamina propria/muscularis mucosa	3 (3.4)	3 (4)	0	
- Submucosa	6 (6.9)	5 (6.8)	1 (8.3)	
- Muscularis propria	19 (22.1)	13 (17.6)	6 (50)	
- Up to the subserosa	2 (2.3)	2 (2.7)	0	
- Serosa	53 (61.6)	49 (66.2)	4 (33.3)	
- Other organs	3 (3.5)	2 (2.7)	1 (8.3)	
pN	28 (32.6)	27 (36.5)	1 (8.3)	0.054
- N0	58 (67.4)	47 (63.5)	11 (91.7)	
- N1a	8 (9.3)	8 (10.8)	0	
- N1b	10 (11.6)	9 (12.1)	1 (8.3)	
- N2a	4 (4.6)	4 (5.4)	0	
- N2b	0	0	0	
Tumor deposits	3 (3.5)	3 (3.5)	0	0.478
Crohn's-like infiltration	18 (20.9)	13 (17.6)	5 (41.7)	0.057
Tumor-infiltrating lymphocytes				
- Absent	22 (25.6)	20 (27.0)	2 (16.7)	0.680
- Mild-moderate	54 (62.8)	46 (62.2)	8 (66.7)	
- Marked	10 (11.6)	8 (10.8)	2 (16.7)	

MSS: microsatellite stability.

Table 2. Clinical and pathological characteristics of patients with MSI

Nº of cases	Age	Sex	Right colon	Mucinous differentiation	Tumor-infiltrating lymphocytes	Crohn's-like lymphoid reaction	Abnormal expression				BRAF V600 gene mutation	Does the patient meet the criteria for Lynch syndrome?	Clinical stage
							MLH1	MSH2	MSH6	PMS2			
1	21	Male	No	Yes	Yes	No	Yes	No	No	Yes	No	No	T3N0M0 Stage IIa
2	36	Female	No	No	Yes	No	Yes	No	No	Yes	No	No	T3N0M0 Stage IIa
3	43	Male	Yes	Yes	No	No	Yes	No	No	Yes	Yes	No	T2N0M0 Stage I
4	48	Male	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	T3N0M0 Stage IIa
5	50	Male	Yes	No	Yes	No	Yes	No	No	Yes	No	No	T4aN0M0 Stage IIb
6	51	Male	Yes	No	Yes	Yes	Yes	No	No	Yes	No	No	T4BN0M0 Stage IIc
7	53	Male	No	No	Yes	No	Yes	No	No	Yes	No	No	T2N0M0 Stage I
8	61	Female	Yes	No	Yes	No	Yes	No	No	Yes	Yes	No	T3N0M0 Stage IIa
9	76	Male	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	T2N0M0 Stage I
10	77	Female	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes	No	T2N0M0 Stage I
11	82	Male	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes	No	T2N0M0 Stage I
12	82	Male	No	No	Yes	No	Yes	No	No	Yes	No	No	T3N0M0 Stage IIa

categorized as stage IIA, 5 patients in stage I, 1 patient in stage IIB and 1 patient in stage IIC, based on the classification of the American Joint Committee on Cancer (AJCC), in its eighth edition. According to The Bethesda guideline [19], two patients met the criteria for Lynch syndrome.

DISCUSSION

The prevalence of MSI in patients with CRC may vary between populations, which may be due to environmental characteristics or genetic factors. Data reported in different multicenter studies report that approximately 10% to 15% of patients are in stages II-III and 5% in stage IV [22-26]; we found a high prevalence of MSI in 14% of CRCs.

In Latin America, the prevalence of MSI in CRC has been reported in different populations in Mexico (21.3%), Brazil (23%), Peru (38.4%) and Argentina (45%) [11-15]. Also, another study conducted in the U.S. Latino population found a prevalence of 12.6% (n = 111) [27].

Few studies have been conducted in Colombia. On the one hand, the only study similar to this one, in which the presence of MSI was evaluated through IHC, was the one published by Shamekh et al. [28], who found in 45 patients with CRC from southwestern Colombia the presence of MSI in 11 patients (24%), 5 of them with loss of MLH1/PMS2, 4 with isolated loss of PMS2, 1 with isolated loss of MLH1 and 1 case with loss of MSH6 and PMS2. On the other hand, Montenegro et al. [29] conducted a multicenter study in which they evaluated in peripheral blood the presence of 6 microsatellite markers (BAT-25, BAT-26, BAT-40, D17S250, D2S123 and D5S346). They looked for the presence of these markers in 10 patients with sporadic CRC and in 31 patients with hereditary non-polyposis colon cancer. 34.1% presented MSI, and 76%, high MSI. In

addition, they found a new polymorphism, C399T, in exon 3 of the *MSH2* gene. On the other hand, Cárdenas et al. [30] in the northeast determined the presence of MSI by evaluating the BAT-26 marker in 11 patients with CRC, and this presence was reported in 3 patients (27%). Afanador et al. [31] evaluated the presence of 5 markers (BAT-25, BAT-26, NR21, NR24 and NR27) by PCR in 39 patients with sporadic CRC. In total, a MSI prevalence of 35.9% (14/39) was found: 12.8% (5/39) with high MSI and 23.1% (9/39) with low MSI.

Alterations in the individually assessed MMR gene proteins indicate that MSH2 and MLH1 proteins account for most of the alterations in CRCs with MSI globally, with a frequency of 40-60% and 40-50%, respectively, while lower figures have been found for MSH6 (10 - 20%) and PMS2 (2%) [32]. With respect to the alterations hereby, a higher proportion of cases with loss of expression of MLH1/PMS2 complexes was found compared to MSH2/MSH6 (83% and 17%, respectively). Discordant results than those published in the West Asian population where an equivalent proportion was found between these two MLH1/PMS2 versus MSH2/MSH6 complexes (56.3% and 43.8%, respectively) [25], also with cases reported in Australia (58.3% and 41.7%, respectively)[15] and in Latino individuals in the United States (44.4% and 55.6%,respectively) (27). In Mexico, they performed an individual analysis of the expression of MMR proteins in which they found no expression of MLH1 (72%), PMS2 (62.7%), MSH2 (21%) and MSH6 (23.2%); therefore, the analysis by MSH1/MSH2 and MSH2/MSH6 complexes is unknown [11]. However, our study and the one previously published by Shamekh et al. [28] suggest that MLH1/PMS2 alteration may be the most frequent in our population. The cause of this expression is unclear, but it is associated with the combination of genetic, epigenetic and environmental factors [33].

Over the last few years, the American College of Pathology (ACP) has modified the indications of the MSI study in CRC; in fact, in some centers it is performed on all patients. The ACP recommends conducting the study in patients under 70 years of age, tumors of right location, tumors with increased lymphocytes infiltrating the tumor and with Crohn-like infiltration in medullary subtypes or seal ring, in cases with intratumoral heterogeneity (conventional mixed carcinoma, mucinous and poorly differentiated), high-grade histology and absence of dirty necrosis [34]. Based on these results, we also recommend conducting the MSI study to all cases with CRC, taking into account that in our patients we find cases with MSI in the transverse, descending colon and rectum; and, in addition, with a moderately differentiated histological degree.

CONCLUSION

In conclusion, the prevalence of MSI associated with CRC in our population is 14%, data similar to that reported in the population of North America and Europe; although it is a lower prevalence compared to other Latin American countries. It is important to highlight 83% presented loss of expression of the MLH1/PMS2 complex, a much higher figure compared to other countries. These results should be the beginning to generate studies with a greater number of patients and, in this way, to know the prevalence of this clinical condition in Colombia. This will allow clinical behaviors to be taken in our population, considering that MSI in CRC has an impact on the prognosis, treatment and follow-up of patients.

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