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Mismatch repair protein expression and colorectal cancer in Hispanics from Puerto Rico

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Abstract

Colorectal cancer (CRC) is a leading cause of morbidity and mortality and alterations in mismatch repair (MMR) genes, leading to absent protein (negative) expression, are responsible for approximately 20% of CRC cases. Immunohistochemistry is a tool for prescreening of MMR protein expression in CRC but the literature on its use on Hispanics is scarce. However, Hispanics represent the second leading ethnicity in the United States (US) and CRC is a public health burden in this group. Our objectives were to determine the frequency of MMR protein-negative CRC and to evaluate its association with clinical and pathological characteristics among Hispanics from Puerto Rico, for the first time to our knowledge. A retrospective observational study of unselected CRC patients from the Puerto Rico Medical Center from 2001 to 2005 was done. MLH1 and MSH2, the most commonly altered MMR genes, protein expression was evaluated using immunohistochemistry, with microsatellite instability (MSI) and *BRAF* gene analyses in the absence of MLH1 protein expression. One-hundred sixty-four CRC patients were evaluated: the overall MMR protein-negative

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frequency was 4.3%, with 0.6% frequency of co-occurrence of MLH1-protein negative expression, MSI-high, and normal *BRAF* gene. MMR protein-negative expression was associated with proximal colon location ($p = 0.02$) and poor histological tumor differentiation ($p = 0.001$), but not with other characteristics. The frequency of MMR protein-negative CRC in Hispanics from Puerto Rico was lower than reported in other populations. This finding may explain the lower CRC incidence rate among US Hispanics as compared to US non-Hispanic whites and blacks.

Keywords

colorectal cancer; genetics; Hispanics; immunohistochemistry; mismatch repair

Introduction

Colorectal cancer (CRC) is a preventable and highly curable disease if detected in the early stages of tumorigenesis. However, it is the third most common cancer and the fourth most frequent cause of cancer death worldwide [1]. Furthermore, CRC will be the third most common cancer in incidence and cause of death for both sexes in the United States (US) in 2009 [2]. CRC arising as a result of microsatellite instability (MSI) have distinct clinical and pathological features that distinguish them from those with microsatellite stability [3]. MSI occurs when a germline repeated nucleotide sequence (microsatellite) allele has gained or lost repeated units, and has, thus, undergone a somatic change in length [4]. MSI is the result of inactivation of both alleles of a DNA nucleotide mismatch repair (MMR) gene: *MLH1*, *MSH2*, *MSH6*, *PMS1*, or *PMS2*. These genes are implicated in post-replication repair, DNA damage signaling, and apoptosis when repair is overwhelmed by DNA damage [5–7].

A germline mutation in any of the MMR genes is associated with hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome [8–13]. HNPCC comprises 2–4% of all CRC [8,14,15], is the most common form of hereditary CRC [16], and probably the most frequent cause of hereditary cancer [17]. More than 90% of patients with HNPCC have MMR defects causing MSI [18,19], with *MLH1* and *MSH2* germline mutations accounting for more than 90% of [20,21] or most [14,15,22] HNPCC cases.

MSI associated with MMR gene alterations account for 20% of all CRC cases [23], 15–20% of sporadic CRC [15,19,24], and 85% of hereditary CRC [25]. Specifically, MMR germline mutation is associated with a 70–80% lifetime risk of developing CRC [26,27], compared to 5–6% in the general population [20]. Tumors with a MMR gene defect show absence of MMR protein expression [28], which may be secondary to either a germline mutation (3–5% of all CRC cases, expressed as HNPCC) or a somatic mutation, usually hypermethylation of *MLH1* promoter (15–20% of all CRC cases) [29]. Additionally, *BRAF* (gene involved in growth factor signaling [30]) V600E activating ($BRAF^{V600E}$) mutation is evaluated in MLH1-protein negative expression because it is often present when the *MLH1* promoter is methylated [14].

MSI or immunohistochemical testing (with or without $BRAF^{V600E}$ mutation testing) of the tumor tissue are strategies to select patients for subsequent diagnostic testing [14,31–33]. Immunohistochemistry is a simple, accessible, rapid, and relatively inexpensive prescreening method [22,34–36] to evaluate tumor tissue for MMR protein expression as compared to polymerase chain reaction (PCR)-based MSI assays. Absence of protein expression also indicates the MMR gene most appropriate for DNA analysis [15,34,35,37,38]. For screening of HNPCC among patients with CRC, immunohistochemistry is almost equally sensitive as MSI [34,39]. If restricted to experienced pathologists [35], immunohistochemistry is a valid tool to identify patients at risk for HNPCC and patients with sporadic microsatellite instable CRC [40]. For these reasons, immunohistochemistry of these colorectal tumors has gained

popularity as the first step in checkingfor MMR gene mutations, even in community hospitals [15].

Hispanics or Latinos are individuals of Mexican, Puerto Rican, Cuban, Central or South American, or other Spanish culture or origin, regardless of race [41]. Hispanics represent a notable fraction of the world population [42], the second leading ethnicity in the US [43], and almost all the population of Puerto Rico [44]. Indeed, CRC basically has the second highest incidence rate and the third highest mortality rate among cancer sites in Hispanics [2,45,46] (Figure 1). These are reasons why it is important to study this highly preventable but deadly disease in this ethnicity.

Studies of MMR protein expression in unselected CRC patients have been performed in various countries or ethnicities [37,47–58]. However, literature in Hispanic patients is scarce, showing a frequency of MMR protein-negative expression of approximately 7% with no documentation on its association with clinical and pathological characteristics [57,58]. Furthermore, most of the literature about MMR protein expression in CRC is from selected high risk cancer clinics and registries, including HNPCC cohorts [59–66], or sporadic colorectal tumors excluding HNPCC patients [67]. Hence, we designed a study of unselected patients with the objective of evaluating the frequency of MMR protein-negative CRC and the association with clinical and pathological characteristics in Hispanics from Puerto Rico. Moreover, to our knowledge and considering that immunohistochemistry is a relatively inexpensive prescreening method, this is the first study evaluating MMR protein expression by immunohistochemistry in Hispanic CRC patients from Puerto Rico.

Material and methods

Study design and population

A retrospective observational study of unselected patients that visited the Puerto Rico Medical Center (PRMC, San Juan, Puerto Rico), which includes the Hospital Oncologico Isaac Gonzalez-Martinez (HOIGM) between 2001 and 2005 was conducted. These hospitals provide tertiary and supra-tertiary medical care and are the main referral centers for patients in Puerto Rico in need of specialized health care. Hispanic patients with the diagnosis of CRC who were 21 years of age or older and who had pathological tissue from biopsy or surgery at the PRMC or HOIGM were included. Individuals with familial adenomatous polyposis phenotype based on surgical or pathological report were excluded because it is a genetically different disease [68–70]. Patients were identified originally by evaluation of pathology reports and the HOIGM cancer registry for the diagnosis of colorectal carcinoma in situ or adenocarcinoma. The study was approved by the University of Puerto Rico Medical Sciences Campus and the HOIGM institutional review boards.

Hispanics comprised 98.5% of the population in Puerto Rico for 2005 [71]. CRC was diagnosed in approximately 7000 patients in Puerto Rico in 1999–2003: 53% men and 47% women, with more than 90% of patients being older than 50 years old. Additionally, CRC was the cause of death in 2907 patients in Puerto Rico in 2000–2004: 54% men and 46% women [46].

Tumor MLH1 and MSH2 protein expression

Tumor tissue blocks were retrieved from the departments of pathology of both institutions. Hematoxylin and eosin slides from each block were evaluated by a pathologist (C.G.K.) to assess adequacy of the sample and confirm the diagnosis. Coded slides were prepared from each block for blind assessment.

Formalin-fixed, paraffin-embedded tumor tissues were sectioned at 4 μm and affixed to Fisherbrand Colorfrost / PLUS microscope slides (No. 12-550-19, Fisher Scientific, Pittsburgh,

PA) and heated at 60°C for at least 1 hour. The slides were deparaffinized and rehydrated with xylene, descending graded alcohols, and distilled water. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide in methanol solution for 5 minutes. The slides underwent antigen retrieval with 10X Tris-ethylenediamine tetraacetic acid buffer, pH 7.5 (Code MB-006, Rockland Immunochemicals, Inc., Gilbertsville, PA) for 45 minutes on boiling water in a Flavor Scenter Steamer Plus (SKU HS900, Black and Decker, Hunt Valley, MD). The sections were cooled for 20 minutes and transferred to DakoCytomation wash buffer (Product S3006, DakoCytomation, Inc., Carpinteria, CA). The subsequent immunohistochemical staining protocol was performed on an automated immunostainer (Dako Autostainer Plus Universal Staining System, Dako North America, Inc., Carpinteria, CA). The sections were incubated with mouse monoclonal antibodies against MLH1 (Clone G168-728, 1:60; Catalog No. 554073, BD Pharmingen, San Jose, CA) or MSH2 (Clone FE11, 1:30; Catalog No. NA27, Calbiochem, La Jolla, CA) for 60 minutes. The antibodies were diluted with antibody diluent with background reducing components (Product S3022, DakoCytomation, Inc., Carpinteria, CA). Wash buffer (Product S3006, DakoCytomation, Inc., Carpinteria, CA) was used for rinsing. Immunoreactivity was detected using the DakoCytomation EnVision+ System-HRP kit (Code K4007, DakoCytomation, Inc., Carpinteria, CA). Mayer's hematoxylin was used as counterstain for 8 minutes. The sections were dehydrated through ascending graded alcohols, cleared in xylene, and mounted.

Two independent pathologists (C.G.K. and S.R.H.) evaluated the tissue slide staining. Protein expression was scored as positive if both the tumor and internal control (normal germinal centers and basal crypt epithelium) showed nuclear staining; negative if the tissue lacked staining in tumor while the internal control was stained, or uninterpretable if no immunostaining of tumor or internal control could be shown.

Microsatellite instability (MSI)

To evaluate for suggestion of germline mutation or hypermethylation of the *MLH1* promoter, any patient with MLH1-protein negative expression was evaluated for MSI by fluorescently labeled PCR reaction amplification with five microsatellite markers from the panel described by the National Cancer Institute conference on MSI: *BAT-26*, *BAT-40*, *D2S123*, *D5S346*, and *D17S250* [72]. MSI-high (MSI-H) was defined by shifts of bands compared with control DNA in $\geq$ 30% of evaluable markers and / or in a mononucleotide repeat, MSI-low was defined by shifts in one dinucleotide marker representing < 30% of evaluable markers, and microsatellite-stable was defined by absence of shifts in any marker.

Sequencing of *BRAF* gene

In the case of MLH1-protein negative expression, $BRAF^{V600E}$ mutation was evaluated by amplification of *BRAF* gene exon 15 by genomic PCR using intronic primers and a commercial DNA sequencing kit according to the manufacturer's instructions (BigDye Terminator v1.1 Cycle Sequencing kit, Part 4337449, Applied Biosystems, Foster City, CA). The PCR products were analyzed with an automated sequencer (3730 DNA Analyzer, Applied Biosystems, Foster City, CA) using forward and reverse primers. All mutations were confirmed by an independent PCR amplification and sequencing.

Medical record review and independent covariates

Medical records were examined for the following clinical and pathological variables: sex, age at diagnosis, follow up time, status on follow up (alive no vs. yes), personal history of any other cancer, family history of any cancer, tumor location, tumor size (greatest dimension), history of surgery for the primary tumor, history of chemotherapy, history of radiotherapy, and tumor recurrence. The tumor location was classified as proximal (from cecum to transverse

colon, also known as right-sided) or distal (from splenic flexure to rectum, also known as left-sided).

Tumor histological differentiation was classified as well / moderately differentiated (more than 50% gland formation) or poorly differentiated (less than 50% gland formation). The tumor was classified according to the American Joint Committee on Cancer staging (I vs. II vs. III vs. IV vs. not available or not applies) [73]. Stages were I (tumor confined within the muscularis propria of the wall of the large bowel), II (tumor penetrates through muscularis propria of the bowel wall), III (tumor has lymph node involvement), and IV (tumor spread to systemic organs).

Statistics

The primary outcome variable for MMR protein expression was either positive or negative. Hypothesis testing for the association of MMR protein-negative expression with clinical and pathological characteristics was done using Pearson X^2 test, two-sample t test with equal variances, or Fisher's exact test as appropriate for small cell sizes. The follow up time was determined from date of diagnosis until May 31, 2007, when patients' survival was sought in the Social Security Death Index at http://www.rootsweb.com if unknown otherwise. Statistical analysis was performed using STATA 9.0 software (Stata Corporation, College Station, TX). A significance value less than 0.05 was considered statistically significant.

Results

Patients

Two-hundred twenty-six potential patients were identified during the study period. Of these, 39 patients were excluded because the block did not include tumor tissue, 20 due to lack of availability of tissue blocks, and 3 due to failure to meet eligibility criteria. Thus, 164 patients were eligible and were included in the study. Table 1 shows the clinical and pathological features of these patients. Our study population included 94 (57.3%) women, had a mean age at diagnosis of 62.72 years, and a mean tumor size of 4.4 cm. At a mean follow up time of 39.2 months, 73.8% of patients were alive. Most of the patients had no personal history of any other cancer (90.2%), had tumor localized in distal colon (70.4%), had well / moderate tumor histological differentiation (81.1%), were stage II (40.2%), had surgery (92.1%), received chemotherapy (56.1%), did not receive radiotherapy (70.7%), and did not have tumor recurrence (90.0%). There were no statistically significant differences between the final cohort evaluated (n = 164) and those excluded (n = 62) with regards to basic demographic and clinical and pathological characteristics (data not shown).

Tumor MLH1 and MSH2 protein expression

Figure 2 shows representative examples of immunohistochemistry for MLH1 and MSH2 protein expression. MLH1 protein was expressed in all but one tumor (0.6%). MSH2 protein was expressed in all but 6 tumors (3.7%). One patient had uninterpretable results. Overall, 7 of 164 tumors evaluated for MMR protein expression showed absence of expression (4.3%).

MLH1 evaluation for MSI and sequencing for *BRAF*^{V600E} mutation

The only patient who showed absence of MLH1 protein expression was evaluated for both MSI and *BRAF*^{V600E} mutation. Two of 5 (40%) microsatellite markers were altered in the tumor, thus classifying the tumor as MSI-H. *BRAF* exon 15 sequencing did not reveal V600E activating mutation. Thus, the *BRAF* gene was considered to be wild type or normal.

Clinical and pathological characteristics

Tumor location and histological differentiation were the only statistically significant clinical and pathological differences observed between colorectal tumors based on MMR protein expression (Table 2). Colorectal tumors with absent MMR protein expression were more commonly located in the proximal colon compared to tumors that expressed both MMR proteins, which were more commonly located in the distal colon (Fisher's exact $p = 0.024$). Colorectal tumors with MMR protein expression were more commonly well or moderately differentiated compared to those with absence of protein expression, which more commonly were poorly differentiated (Pearson X^2 $p = 0.001$). There was no significant difference according to MMR protein status with regards to sex, age at diagnosis of cancer, personal history of any other cancer, family history of any cancer, mean tumor size, tumor stage or tumor recurrence.

Discussion

CRC is a deadly illness of great concern in Hispanic populations and the island of Puerto Rico is a great site to study this ethnicity. During 1998–2002, in both sexes, Puerto Ricans had CRC incidence and mortality rates similar to those for US Hispanics, but their rates were lower than those for non-Hispanic whites and non-Hispanic blacks. However, Puerto Rican men and women with ages 40–59 years had a greater risk of incidence and mortality than their US Hispanic counterparts [74].

Evaluation of colon cancers for MMR defects may have significant therapeutic implications. In patients with stage II or III CRC, adjuvant 5-fluorouracil chemotherapy provides survival improvement in patients with MMR-competent tumors while the survival of patients with MMR-defective tumors does not improve with the adjuvant chemotherapy [75–77]. Also, loss of tumor MMR function may predict improved outcome in stage III colon cancer patients postoperatively treated with a weekly bolus irinotecan, fluorouracil, and leucovorin regimen as compared with those receiving weekly bolus of fluorouracil and leucovorin [78].

HNPCC is characterized by young onset CRC [8,9,15], an increased risk for gynecologic, urinary tract, and gastrointestinal cancers [9,27,79–88], and most commonly occurs in men [89]. In HNPCC, a single mutation is inherited in the germline, and MSI occurs only after inactivation of the other allele [90]. Most sporadic MSI-H cancers are caused by methylation and silencing of the *MLH1* gene [91,92], most probably develop in women, and have their origin within serrated polyps [89]. Colorectal tumors with MSI are associated with location proximal to the splenic flexure and poor histological differentiation [67,93,94].

MSI occurs in approximately 20% of CRC cases [23]. These tumors typically fail to express MMR protein as seen on immunohistochemistry [28]. In the present study, only 4.3% of 164 patients lacked protein MMR expression. Lack of protein expression as a surrogate for MSI is clinically important to patients. A patient with absence of MMR protein expression may be a member of a Lynch syndrome kindred, hence results may have implications for themselves and other family members. Furthermore, individuals belonging to a HNPCC-kindred have a higher risk of developing other non-colorectal cancers such as endometrial, ovarian, and gastric [9,27,79–88]. Of the seven tumors in our study that lacked MMR protein expression, six tumors lacked expression of MSH2, which usually indicates a germline mutation in the *MSH2* gene, and one patient had absence of MLH1 with MSI-H.

The tumor with absence of MLH1 protein expression with MSI-H may have a either a germline mutation or hypermethylation of the *MLH1* promoter. Additionally, this patient had normal *BRAF* gene, which may be suggestive of HNPCC-associated *MLH1* germline mutation. Virtually 100% of individuals with HNPCC do not carry the $BRAF^{V600E}$ mutation [14,15],

whereas 68% of those without HNPCC do [14]. Moreover, *BRAF*^{V600E} mutation is associated with sporadic CRC with MSI [30,95]. *MLH1* promoter hypermethylation leads to gene inactivation [96] and, if present, may be secondary to CpG island methylation phenotype (CIMP), progressing via the MSI-H pathway. CpG islands are regions rich in cytosine-guanosine dinucleotide repeats at the 5′ region of approximately half of all human genes. Methylation of cytosine residues within CpG islands of promoters and proximal exons is associated with loss of gene expression [97]. CIMP tumors have similarities to tumors with MSI-H, such as right-sided location and poor histological differentiation [98]. However, *BRAF* mutation is frequently seen in sporadic MSI-H CRC, associated with DNA methylation secondary to CIMP-high (3–4 methylated in tumor markers are methylated) status [99]. For these reasons, it is unlikely that a *MLH1* promoter hypermethylation secondary to CIMP may have occurred. However, none of the patients underwent germline genetic testing.

In our study of Hispanics from Puerto Rico, the frequency of MMR protein-negative tumors was far lower than in other studies of unselected CRC patients in other countries or ethnicities, especially regarding MLH1-protein negative expression (Table 3) [37,47–58]. Moreover, the frequency of MMR protein-negative tumors in our study was lower than in Spain, including MLH1-protein negative expression [57,58].

As seen in other studies [4,15,37,56,67,93,94,100–102], colorectal tumors with lack of MMR protein expression, suggesting MMR deficiency, were associated with a proximal location in the colon and poor histological differentiation. However, opposing previous publications [4, 17,19,37,55,72,79,101–104], we did not observe associations of MMR protein-negative tumors with gender, earlier age at diagnosis of cancer, positive personal and family history of cancer, or earlier tumor stage, when compared to tumors with MMR protein-positive expression.

We acknowledge that our study has several limitations. First, the study was retrospective, which limited the information to that present in the medical record. This limited our ability to accurately evaluate family history of cancer and other factors, like environmental exposure. Second, we were only able to include patients that had tumor blocks available for analysis. However, the percentage of patients excluded from pathological analysis was small and did not statistically differ with regards to basic demographic characteristics from the patients included in this investigation. As such, we believe our results are representative of the referral population seen at our Center. Third, the sample size was relatively small. The sample size may have limited the power of the study to identify important differences according to MMR protein expression status. This is also a pilot retrospective study and a larger prospective study is undergone to verify our findings. Fourth, only *MLH1* and *MSH2* protein expression were evaluated. However, over 90% [20,21] or most [14,15,22] of genetically characterized HNPCC cases are accounted for by germline mutations in any of them.

Additionally, a fifth limitation is that all patients were not subsequently evaluated for MSI to confirm the suggestive findings from immunohistochemistry. However, for screening of HNPCC among patients with CRC, immunohistochemistry is almost equally sensitive as MSI [33,34,39]. Indeed, MSI testing is not available in the majority of routine pathology service laboratories, and these rely on immunohistochemistry to detect loss of MMR protein expression as a surrogate marker for the presence of MSI [105]. In consequence, immunohistochemistry is a valid tool to identify patients at risk for HNPCC and patients with sporadic microsatellite instable CRC [40] when evaluated by experienced pathologists [35] like the authors. Additionally, many consider immunohistochemistry and MSI testing as complementary. In a sequential approach, immunohistochemistry is done first and, if informative, may result in cost savings. In the other hand, if immunohistochemistry is not informative, MSI testing can then be performed [15]. Nonetheless, this investigation provides insightful information about the

frequency and clinical and pathological characteristics associated with MMR protein deficiency in Hispanics from Puerto Rico, which may be ethnically related.

To our knowledge, the present study is the first one to evaluate the frequency of MMR protein-negative expression in unselected Hispanic CRC patients from Puerto Rico using the relatively inexpensive [35] prescreening method of immunohistochemistry and its association with clinical and pathological characteristics.

In conclusion, this investigation of unselected Hispanic CRC patients from Puerto Rico revealed a low frequency of MMR protein-negative tumors. Similar to other populations, Hispanic MMR protein-negative tumors were proximally located and exhibited poor histological differentiation. The results may be relevant to the evaluation and management of Hispanic patients with CRC from Puerto Rico in immigrant as well as native populations. The relatively low frequency of MMR protein-negative CRC in unselected Hispanic patients from Puerto Rico may be a reflection of, or explain, the lower CRC incidence rate among US Hispanics as compared to non-Hispanic whites and blacks. Our study is significant on shedding light on the scarce literature on MMR protein expression in CRC.in Hispanic populations.

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Abbreviations

CIMP	CpG island methylation phenotype
CRC	Colorectal cancer
HNPCC	Hereditary nonpolyposis colorectal cancer
HOIGM	Hospital Oncologico Isaac Gonzalez-Martinez
MMR	Mismatch repair
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
PCR	Polymerase chain reaction
PRMC	Puerto Rico Medical Center
US	United States

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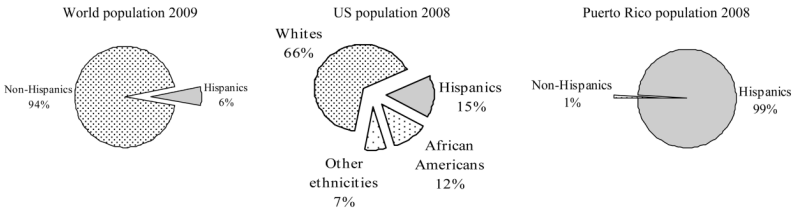

World Hispanics cancer sites 1998-2002		*US Hispanics cancer sites 2001-2005*				*Puerto Rico Hispanics cancer sites*			
Incidence		*Incidence*		*Mortality*		*Incidence 1999-2003*		*Mortality 2000-2004*	
Men	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*
Prostate	Breast	Prostate	Breast	Lung and bronchus	Breast	Prostate	Breast	Prostate	Breast
Lung and bronchus	**Colorectal**	**Colorectal**	**Colorectal**	Prostate	Lung and bronchus	**Colorectal**	**Colorectal**	Lung and bronchus	**Colorectal**
Colorectal	Cervix and uterus	Lung and bronchus	Lung and brochus	**Colorectal**	**Colorectal**	Lung and bronchus	Lung and bronchus	**Colorectal**	Lung and bronchus

Fig. 1.

Percentage of Hispanics within the world [42], United States [43], and Puerto Rico [44] populations and their corresponding leading cancer sites in incidence and mortality rates, for men and women [2,45,46] in decreasing order. World population 2009 [42]: it is still an underestimation of the actual Hispanic population for not considering those living in non-Hispanic countries, including United States. World Hispanic cancer sites 1998–2002 [45]: based on good quality data provided by United States and Hispanic countries, registries, and populations.

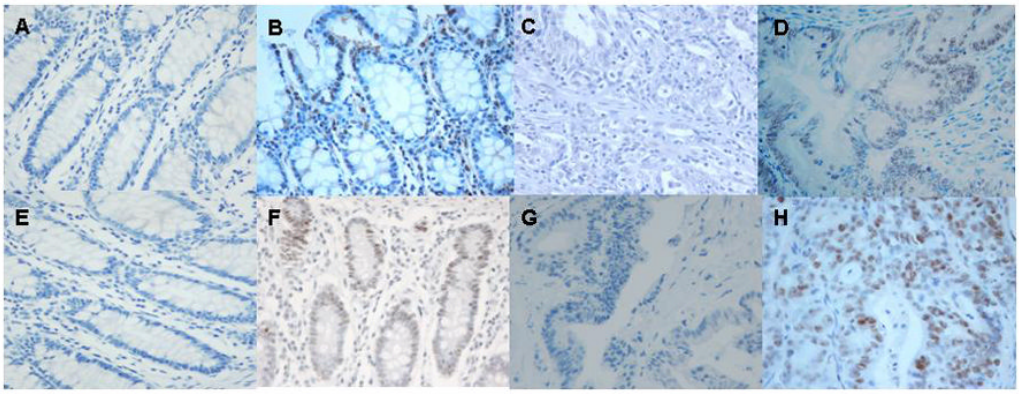

Fig. 2.

Immunohistochemistry for MLH1 and MSH2 protein expression (hematoxylin, 400x). **(a)** Normal colorectal tissue with no antibody staining for MLH1 (negative control). **(b)** Normal colorectal tissue with MLH1 antibody staining (positive control). **(c)** Tumor with absence of MLH1 protein expression. **(d)** Tumor with MLH1 protein expression. **(e)** Normal colorectal tissue with no antibody staining for MSH2 (negative control). **(f)** Normal colorectal tissue with MSH2 antibody staining (positive control). **(g)** Tumor with absence of MSH2 protein expression. **(h)** Tumor with MSH2 protein expression.

Table 1

Clinical and pathological characteristics of study population

Characteristic	
Patients, n	164
Sex, *n* (%)	
Men	70 (42.7)
Women	94 (57.3)
Age at diagnosis (years), mean ± SD (range)	62.72 ± 13.54 (23-9)
Follow up time (months), mean ± SD (range)	39.2 ± 22 (0.67–154.1)
Alive on follow up, n (%)	
No	43 (26.2)
Yes	121 (73.8)
Personal history of any other cancer, *n* (%)	
No	148 (90.2)
Yes	16 (9.8)
Family history of any cancer if available, *n* (%)	
No	51 (41.8)
Yes	71 (58.2)
Tumor location if available, *n* (%)	
Proximal	47 (29.6)
Distal	112 (70.4)
Tumor size if available (cm), mean ± SD (range)	4.42 ± 2.34 (1–13.2)
Tumor histological differentiation, *n* (%)	
Well / moderate	133 (81.1)
Poor	31 (18.9)
Tumor stage, *n* (%)	
I	9 (5.5)
II	66 (40.2)
III	46 (28.1)
IV	14 (8.5)
Not available or not applies	29 (17.7)
Surgery received, *n* (%)	
No	13 (7.9)
Yes	151 (92.1)
Chemotherapy received, *n* (%)	
No	72 (43.9)
Yes	92 (56.1)
Radiotherapy received, *n* (%)	
No	116 (70.7)
Yes	48 (29.3)
Tumor recurrence, *n* (%)	
No	149 (90.9)

Characteristic	
Yes	15 (9.2)

SD = Standard deviation, cm = Centimeters

Table 2

Association of mismatch repair protein expression with clinical and pathological characteristics

Characteristic	Mismatch repair protein expression		*p-value*
	negative	positive	
Sex, *n* (%)			
Men	3 (42.9)	67 (42.7)	0.99[a]
Women	4 (57.1)	90 (57.3)	
Age at diagnosis (years), mean ± SD (95% confidence interval)	59.9 ± 21.7 (39.8–79.9)	62.85 ± 13.2 (60.8–64.9)	0.57[b]
Personal history of any other cancer, *n* (%)			
No	6 (85.7)	142 (90.4)	0.68[a]
Yes	1 (14.3)	15 (9.6)	
Family history of any cancer if available, *n*(%)			
No	1 (25)	50 (42.4)	0.49[a]
Yes	3 (75)	68 (57.6)	
Not available	42		
Tumor location if available, *n* (%)			
Proximal	5 (71.4)	42 (27.6)	0.024[c]
Distal	2 (28.6)	110 (72.4)	
Not available	5		
Tumor size (cm), mean ± SD (95% CI)	6.0 ± 2.0 (3.9–8.1)	4.4 ± 2.33 (4.0–4.8)	0.09[b]
Tumor histological differentiation, *n* (%)			
Well / moderate	2 (28.6)	131 (83.4)	0.001[a]
Poor	5 (71.4)	26 (16.6)	
Tumor stage, *n* (%)			
I	0 (0)	9 (5.7)	0.63[a]
II	3 (42.9)	63 (40.1)	
III	3 (42.9)	43 (27.4)	
IV	1 (14.2)	13 (8.3)	
Not available	0 (0)	29 (18.5)	
Tumor recurrence, *n* (%)			
No	7 (100)	142 (90.4)	0.39[a]
Yes	0 (0)	15 (9.6)	

SD = Standard deviation, cm = Centimeters

p-value determined by

[a] Pearson X^2 test,

[b] two-sample t test with equal variances, and

[c] Fisher's exact test

Table 3

Comparison of our study with others in other countries or ethnicities for mismatch repair protein expression in unselected colorectal cancer patients

Authors	Country or ethnic group	Evaluated patients, *n*	MLH1-protein negative expression, *n* (%)	MSH2-protein negative expression, *n* (%)
Lindor et al [22][a]	Minnesota, US	255	48 (18.8)	3 (1.2)
De Jesus-Monge et al	**Hispanics from Puerto Rico**	**164**	**1 (0.6)**	**6 (3.7)**
Brim et al [47]	Iran	25	10 (40)	1 (4)
Ashktorab et al [48]	African	b	16 of 34 (47.1)[c]	12 of 31 (47.1)[c]
	Americans		3 of 34 (8.8)[d]	1 of 31 (8.8)[d]
Pandey et al [49]	India	46	7 (15.2)	1 (2.2)
Ashktorab et al [50]	Oman	49	5 (10.2)[e]	3 (6.1)[e]
Brim et al [47]	Oman	61	19 (31.1)	5 (8.2)
Erdamar et al [51]	Turkey	74	29 (39.2)	7 (9.5)
Brim et al [47]	African Americans	b	26 of 76 (34.2)	8 of 74 (10.8)
Jin et al [52]	China	146	17 (11.6)	9 (6.2)
Jensen et al [53]	Denmark	262	37 (14.1)	3 (1.1)
Boardman et al [54]	Alaska Natives	329	42 (12.8)	3 (0.9)
Molaei et al [37]	Iran	343	19 (5.5)	24 (7)
Wright et al [55]	New Zealand	458	80 (17.5)	9 (2)
Coggins et al [56]	England	732	52 (7.1)	5 (0.7)
Xicola et al [57]	Spain	1058	59 (5.6)	22 (2.1)
Piñol et al [58]	Spain	1222	60 (4.9)	21 (1.7)

[a] Representative sample for United States

[b] See columns to the right to see the number of evaluated patients for each protein

[c] Partially negative expression

[d] Completely negative expression

[e] Partially or completely negative expression