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HMSH2 and HMSH6 gene expression profiles in colorectal adenocarcinoma in patients up to 50 years of age

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ABSTRACT

Lynch syndrome, previously called hereditary non-polyposis colorectal cancer (HNPCC), is a major mortality threat. It is an autosomal dominant disease which is caused by a germline mutation in the DNA mismatch repair (MMR), especially in patients aged up to 50 years. Such mutation more frequently occurs in the hMSH2 gene (38–40%) and less frequently in the hMSH6 gene (14–16%). These mutations, when associated with the patient's lifestyle, may reveal a considerable variability in the disease manifestations, such as the degrees of penetrance and clinical aggressiveness. The aim of this study is to analyze the expression of DNA MMR genes, and correlate this expression with the clinical and anatomopathological findings of the neoplasia in patients aged between 39 and 49 years. A total of 45 patients were included: (48.9%) males and (51.1%) females, and they all underwent resection of a colorectal adenocarcinoma. The tissue microarray technique was used to analyze the relative and absolute expression of hMSH2 and hMSH6. Amsterdam II criteria for the diagnosis of HNPCC were obtained from the data provided by medical records and interviews with patients. hMSH2 and hMSH6 was expressed in all patients, which correlated between each other (RHO = 0.669 and $p < 0.001$) but not to age. There is a positive correlation between the expressions of males (RHO = 0.673 and $p = 0.001$) and females (RHO = 0.006 and $p < 0.001$). It is possible to evaluate the expression of MMR genes in embedded anatomopathological samples. Gene expressions correlated between each other and to the sex of the patients, but no difference in relation to age

1. Introduction

Lynch syndrome (LS), previously called hereditary non-polyposis colorectal cancer (HNPCC), represents 1–7% of all cases of colorectal cancer. It is the most common malignant neoplasia of the digestive tract. Nearly 80% of the patients develop colorectal cancer (CRC) sporadically; the remaining 20% of the cases have a hereditary susceptibility to the neoplasia. It is estimated that around half a million people die of this disease every year, a number that tends to drop in the years to come [1]. It is an autosomal dominant disease with high penetrance (around 85%) characterized by accelerating the carcinogenesis process due to a germline mutation in the DNA mismatch repair genes (MMR) [2]. The primary function of the MMR is to eliminate the mismatch of base–base insertions and deletions that appear as a consequence of DNA polymerase errors during deoxyribonucleic acid synthesis [3].

The main genes involved are hMLH1 (MutL Homolog 1), hMSH2 (MutS Homolog 2), hMSH6 (MutS Homolog 6), hMLH3 (MutL Homolog 3), PMS1 (Post-Meiotic Segregation 1) and PMS2 (Post-Meiotic Segregation 2) [4,5]. Mutations in hMLH1 and hMSH2 genes are found in nearly 90% of LS cases. [6]. There are more than 500 types of different mutations distributed as follows: 50% are

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Table 1
Sequence of primers of hMSH2 and hMSH6.

Primer	Sequence (5′ → 3′)
hMSH2	Forward – CCTTGTAAAACCTTCATTTGATCC
	Reverse – ATCCAAACTGTGCACTGGAA
hMSH6	Forward – GAACATTCATCCGCGAGAAA
	Reverse – TGAGGGCTCATCACAAACTG

related to hMLH1; 40% correlated to hMSH2 and 10% to other kinds [7].

Adenomas are relatively unusual before the age of 50 years, both in Lynch syndrome carriers [8,9] and in the general population [10]. It is believed that patients with Lynch syndrome spontaneously develop adenomas like the general population; however, once the tumor is established, such patients are more prone to have malignant transformation [9,11]. Moreover, it is more likely that the microadenomas in patients with Lynch syndrome will not remain latent for many years, as it is probably the case with the general population [9]. Deficient DNA mismatch repair in Lynch syndrome patients with adenomas raises the hypothesis of a pre-malignant phase in the beginning of the development of CRC [12].

The aim of this study is to analyze the expression of DNA mismatch repair genes (MMR), hMSH2 and hMSH6 in paraffin embedded samples through qRT-PCR, and correlate this expression with the clinical and anatomopathological findings of the neoplasia in patients with colorectal adenocarcinoma up to 50 years of age.

2. Patients and methods

The current study encompassed patients of different genders and ethnicities, who were 50 years old or younger at diagnosis, with histological confirmation of colorectal cancer (CRC). Such patients had undergone curative or palliative surgery, elective or urgent, at Hospital do Servidor Público do Estado (HSPE), under the ethical protocol CAAE #00690338000-11. Exclusion criteria comprised not only patients with diagnosis of familial adenomatous polyposis, intestinal inflammatory disease or colorectal neoplasias other than adenocarcinomas but also the impossibility to obtain proper genetic material for analysis and the non-location of the patient or family members for the acquisition of the necessary data.

CRC tissue samples, obtained from the department of pathological anatomy of HSPE, were fixed in paraffin. The slides were then reviewed by expert pathologists so that the diagnosis of CRC could be confirmed and the biological material would be available for further studies.

A total of 45 patients with CRC who had undergone curative or palliative surgery at HSPE were retrospectively evaluated. From this total, 22 (48.9%) were males and 23 (51.1%) were females. The mean age was 46 years (range 39–49).

CRC was located in the colon in 27 patients (60%) and in the rectum in 18 (40%). The location of CCR in the right colon (up to the splenic flexure) was observed in 9 cases (19%) and in the left colon (past the splenic flexure) in 21 (44%). Total RNA extraction from the paraffin-embedded samples was carried out using the RNeasy FFPE kit (Qiagen) whereas complementary DNA (cDNA) was synthesized using the enzyme SuperScript II RNase reverse transcriptase (Invitrogen).

Table 2
GAPDH primers sequence.

Primer	Sequence (5′ → 3′)
GAPDH	Forward –CTGTGAGGTAGGTGCAAATGC
	Reverse – GCCCACTTCACCGTACTAACCA

Table 3
Clinical laboratory profile of patients with bowel cancer. Santo André, 2014.

Characterization	n	%
Sex		
Male	22	48.9
Female	23	51.1
Medical Care		
Non-urgent	39	86.7
Urgent	6	13.3
Site		
Left colon	19	42.2
Right colon	8	17.8
Rectum	18	40
Neoplasia family history		
Yes	17	37.8
No	28	62.2
Inflammatory infiltrate		
Absent	30	66.7
Mild	6	13.3
Moderate	7	15.6
Severe	2	4.4
Desmoplastic reaction		
Absent	35	77.8
Mild	2	4.4
Moderate	7	15.6
Severe	1	2.2
Differentiation		
Undifferentiated	3	6.7
Differentiated	3	6.7
Moderate	39	86.6
Vascular invasion		
Present	12	26.7
Undetected	33	73.3
Neuro-invasion		
Present	10	22.2
Undetected	35	77.8
Lymphatic invasion		
Present	12	26.7
Undetected	33	73.3
Stage levels		
I	9	20
II	13	28.9
III	13	28.9
IV	10	22.2
	Median	p.25–p.75[a]
Age	46	39–49

[a] 25 and 75 percentiles respectively.

The qRT-PCR technique was applied to the obtained cDNA sequence so that hMSH2 and hMSH6 could be analyzed as follows: 7.5 μl of buffer 2X RT^2 Real-Time SYBR Green PCR Master Mix, 2.0 μl of cDNA and 1.5 μl of RT^2 PCR Primer Assay resulting in 15 μl of the final reaction volume. The volume was completed using deionized water. The reaction was run on a thermal Cycler 7500 (Applied Biosystem) according to the following program: 95°C for 10 min; 40 cycles comprising 95°C for 15 s and 60 °C for 60 s. Table 1 shows the sequence of primers used in genes hMSH2 and hMSH6.

The results of hMSH2 and hMSH6 expression levels were analyzed in relation to their relative expression levels and standardized by GAPDH expression. GAPDH primers are shown in Table 2.

Clinical criteria (Amsterdam II criteria) for the diagnosis of HNPCC were obtained from the data provided by medical records and interviews with patients.

2.1. Statistical analysis

Clinical and laboratory variables were described in absolute and relative frequencies. Age and expressions of hMSH2 and hMSH6