Importance of MutL homologue MLH1 and MutS homologue MSH2 expression in Turkish patients with sporadic colorectal cancer

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Supported by the Research Foundation of Istanbul University, No T-493/25062004

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Received: 2007-05-23 Accepted: 2007-06-09

Abstract

AIM: To assess the incidence of MLH1 (the human MutL homologue) and MSH2 (the human MutS homologue) protein expression in Turkish patients with sporadic colorectal cancers and to compare their survival and clinicopathological features.

METHODS: We validated the tissue microarray technology in 77 colorectal carcinomas by analyzing the immunohistochemical expression of proteins involved in two main pathways of colorectal carcinogenesis: p53 protein for loss of heterozygosity tumors; MLH1 and MSH2 proteins for microsatellite instability (MSI).

RESULTS: Our analysis showed that 29 (39.2%) had loss of MLH1 expression, 5 (6.8%) had loss of MSH2 expression and 2 cases had loss of expression of both proteins. We found that 60% of MSH2-negative tumors were located in the right side of the colon; all MSH2-negative cases were women. In addition, the loss of MSH2 expression was correlated with low p53 expression. Neither MLH1 nor MSH2 expressions were associated with prognosis, although there seemed a tendency of longer survival (71.7 ± 8.65 mo vs 47.08 ± 5.26 mo) for the patients with MLH1-negative versus MLH1-positive carcinomas. There were not significant differences in overall and recurrence-free survival among MLH1/MSH2-positive and -negative cases.

CONCLUSION: Our data supports that Turkish patients with MLH1- and MSH2-defective tumors have some distinct features from each other. Although prognostic importance remains controversial, immunohistochemical analysis of mismatch repair genes may be used as a routine histopathological examination of sporadic colorectal carcinomas.

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Key words: Colorectal carcinoma; MLH1; MSH2; Immunohistochemistry; Prognosis

INTRODUCTION

In humans, mismatch repair (MMR) system is mediated by at least six genes, including human MutL homologue (hMLH1), human MutS homologue (hMSH2), hMSH3, hMSH6, hPMS1, and hPMS2[1]. MMR deficiency leads to the accumulation of base-base mismatches and short insertion/deletion mispairs, generated as a consequence of DNA replication errors and homologous recombinations. Most cell deficient in hMLH1 and hMSH2 genes often display a high level of genomic instability, characterized by changes in repeated numbers of simple repetitive sequences, microsatellite instability (MSI)[2-3]. The detection of MSI status is based on molecular analysis. According to this, three tumor phenotypes have been defined: Microsatellite stable (MSS), low-frequency MSI (MSI-L) and high-frequency MSI (MSI-H). A germline mutation in one of MMR genes, accompanied by somatic inactivation of the other allele, may result in high level of microsatellite instability (MSI-H)[4-6]. In patients with hereditary nonpolyposis colon carcinoma (HNPPC), MSI was detected in 80% of the tumor samples[7]. It has been reported that MMR genes...
are involved in the 10%-15% of sporadic colorectal carcinomas[13]. In sporadic MSI-H colorectal carcinomas, the mechanism of development of mutant phenotype is inactivation of MLH1 by promoter hypermethylation. Sporadic MSI-H tumors tend to be poorly differentiated and/or mucinous subtype[9-11]. They are usually diploid; p53 mutations, loss of heterozygosity at 18q, APC mutations and K-ras mutations are found less frequently than in MSS tumors[12,13]. In literature, there are controversial results about the prognostic importance of MSI status in sporadic colorectal carcinoma.

Establishing the presence of MSI requires polymerase chain reaction-based technology, examining DNA sequences of intact and tumor tissue. This is an expensive and time-consuming procedure that is not readily available in all pathology laboratories. Immunohistochemically identifying MSI tumors is a less costly alternative procedure. There are some studies which have shown a high correlation of MLH1 and MSH2 immunohistochemical patterns of expression with the DNA analyses. MSI-H correlates with loss of immunohistochemical staining of either MLH1 or MSH-2 in the tumor nuclei[14-17]. The sensitivity of the immunohistochemical technique for detecting MSI-H tumors is about 80% to 100%[15-17].

To our knowledge, this is the first published study in Turkey concerning investigation of MSI status in sporadic colorectal carcinomas. Therefore, it is important to find out prognostic importance of MSI status in patients with colorectal carcinoma in Turkish population to perform their appropriate treatment and follow-up. In this study, we determined (1) the frequency of MLH1- and MSH2-deficient colorectal carcinomas in Turkish patients, (2) the relationship between MLH1/MSH2 expression and clinicopathological features, and (3) the predictive and prognostic relevance of loss of MLH1 and/or MSH2 expression in recurrence-free and overall survival.

MATERIALS AND METHODS

Tissue specimens
A total of 77 colorectal carcinoma specimens were obtained from the archives of the Department of Pathology of Cerrahpasa Medical College, Istanbul University. Patients with familial adenomatosis polyposis or inflammatory bowel disease were excluded. Tumors were staged according to the TNM staging system[18]. Tumor type and grade of differentiation were determined by criteria of the World Health Organization[19]. Peritumoral Crohn’s-like reaction, pattern of growth and lymphocytic infiltration were also evaluated according to literature[20]. Tissue microarray (TMA) was applied to study normal colorectal mucosa and colorectal carcinomas. The patients had received neither chemotherapy nor radiation therapy before tumor resection. Their pathological specimens and slides were revised. After revision, two slides and corresponding two paraffin blocks were chosen, one for normal colorectal mucosa and one for colorectal carcinomas tissues for each case. Corresponding areas for normal mucosa and carcinomas were marked on chosen paraffin blocks. In TMA, 2-mm cores were taken from these selected areas, two for normal mucosa and two for cancer areas, and then they were embedded into microarray block. Each block containing 60 cores also contained other tissues (for example, sausage blocks included tissues from breast, thyroid, prostate, skin, gastric mucosa, lymph node, etc) for negative and positive controls.

Immunohistochemistry
Formalin-fixed paraffin-embedded TMA blocks were cut into 3-μm thick sections and mounted on polarized glass slides. After mounting, they were kept in an oven at 56°C overnight. Sections were deparaffinized in xylene and rehydrated. They were incubated in a microwave containing 10 g/L EDTA solution 3 times for 5 min each, and then kept in room temperature for 20 min and placed in 10 mL/L hydrogen peroxide for 10 min. After being washed with distilled water and phosphate-buffered saline (PBS), sections were incubated overnight at 4°C with primary antibodies of MSH2 (Ab-1, Clone 2MSH01, NeoMarkers; 1:25) and MLH1 (Clone 14, Zymed Lab; 1:50) with pre-antibody blocking solution (Immunovision-Sitogen). Primary antibody was replaced with PBS for a negative control. After being washed with PBS, the sections were incubated with primary antibody enhancer for 30 min and then with HRP polymer for 30 min. After being washed thrice with PBS for 10 min each, the sections were stained with a streptavidin-peroxidase detection system.

Immunostaining for p53 (p53 AB-5 clone DO-7, NeoMarkers; 1:100) was almost the same as above-mentioned, except using citrate buffer solution instead of 10 g/L EDTA solution in microwave.

Immunohistochemical evaluation
Stained tissues in tissue microarray slides (TMS) were scored under the light microscope and the extent and intensity of staining with MLH1, MSH2 and p53 antibodies were evaluated independently by two pathologists (SE and EU) without knowledge of clinicopathological data. Tumors showing loss of nuclear MLH1 or MSH2 expressions were classified as MLH1- or MSH2-negative, respectively. Nuclear immunostaining of normal epithelial cells, lymphocytes and stromal cells served as internal positive controls. For p53, tumors showing a proportion of stained nuclei of >10% were classified as p53-positive.

Statistical analysis
Spearman rank, Kendall correlation test, Cox regression and Kaplan-Meier test were used, when appropriate. P < 0.05 was considered statistically significant.

RESULTS
There were positive staining of normal control mucosa in the lower third of the epithelium and nuclear staining of lymphocytes from a control germinal centre (Figure 1A and B). Three MLH1/MSH2-stained cases were excluded from the study because the quality of immunostaining was unsatisfactory. Of the remaining 74 colorectal
adenocarcinomas, 42 (56.7%) cases demonstrated normal expression of both MLH1 and MSH2 gene products (MLH1+/MSH2+) (Figure 2A and B). Loss of MLH1 or MSH2 expression was detected in 32 (43.2%) of all cases examined. Complete loss of MLH1 expression (Figure 3A) and normal immunoreactivity for MSH2 were observed in 27 (36.48%) cases of adenocarcinoma, while 5 (6.8%) cases displayed complete loss of MSH2 expression (Figure 3B) and normal immunoreactivity for MLH1. Two (2.07%) adenocarcinoma cases showed lack of both MLH1 and MSH2 expressions.

Immunohistochemical pattern of MLH1/MSH2 expressions was found to be related to some clinical and pathological variables (Tables 1 and 2). MSH2-negative carcinomas occurred only in women ($P = 0.049$). In addition, MSH2-negative carcinomas developed more frequently in patients $\geq 50$ years than did MSH2-positive ($P \geq 0.05$). Majority of MSH2-negative tumors (60%) were located in the right colon ($P > 0.05$). However, no any preferential location for MLH1-negative cases was observed. There was no significant correlation between MLH1/MSH2 expression and tumor size and tumor type, while a significant relation was detected between tumor invasion and MSH2 expression (Table 2).

All MSH2-negative tumors had no or low p53 expression, while MSH2-positive cases had p53 expression $\geq 10\%$ ($P < 0.05$). MLH1/MSH2 expressions were not significantly associated with other histopathological variables, such as perineural invasion, lymphatic/blood vessel invasion, peritumoral Crohn’s-like lymphoid reaction. On the other hand, 80% of cases with loss of MSH2 expression had no perineural, lymphatic and blood vessel invasion (Table 2).

Our study included 32 (41.6%) females and 45 (58.4%)
males, with average age of 56 (range, 23-77) years. The mean follow-up in surviving patients was 34 (range, 3-97) mo. The patients with MSH2-negative/MLH-positive carcinomas more frequently died of disease (average 12.5 ± 1.06 mo post-operatively) than the patients with MLH1-positive/MSH2-positive carcinomas (47.08 ± 5.26 mo post-operatively) (Table 3). However, overall and disease-free survival analysis did not show significant differences among the four groups of patients.

In COX regression analysis, only lymph node metastasis and stage were found as independent prognostic factors in all clinicopathological features. But loss of MLH1 and MSH2 expressions did not show prognostic significance (Table 4).

**DISCUSSION**

One of the two different pathogenetic pathways in colorectal carcinogenesis is mutator pathway which is characterized by explicit microsatellite instability (MSI). Mutator pathway covers DNA mismatch repair genes like MLH1 and MSH2. Identification of MSI status of large bowel adenocarcinomas is clinically important, since patients with MSI carcinomas demonstrated several distinct features compared to microsatellite stability (MSS) carcinomas [22-25]. It has also been suggested that MSI carcinomas might be particularly sensitive to 5-fluorouracil-based adjuvant chemotherapy [26,27]. Besides, patients with MSI tumors are considered to be at risk of developing metachronous colorectal cancers and need long-term colonoscopic surveillance [28].

As mentioned in previous studies, immunohistochemical analysis of MLH1 and MSH2 protein expression represents a rapid, easier and less costly alternative method for detection of colorectal tumors of the mutator phenotype [11,13,16,20-32]. This analysis could be performed in the histopathology laboratories as routine immunohistochemical staining, while genetic analysis of MSI status is time-consuming and expensive and requires specialized equipment [3,28]. This difference is important for developing countries like Turkey. Lindor et al [11] showed that absence of expression of MLH1 or MSH2 had a 100% specificity and 92.3% sensitivity for predicting a tumor with MSI-H phenotype. So, immunohistochemical

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**Table 1 Clinicopathological parameters and MLH1 and MSH2 expressions n (%)**

| Gender | MLH1 (+) | MLH1 (-) | P | MSH2 (+) | MSH2 (-) | P |
|--------|----------|----------|---|----------|----------|---|
| Male   | 26 (57.8)| 17 (38.6)| 0.943 | 38 (55.1)| 0 (0)    | 0.049 |
| Female | 19 (42.2)| 12 (4.4) | 0.602 | 31 (44.9)| 5 (100)  | 0.579 |
| Age (yr) |         |          |      |          |          |    |
| < 50   | 15 (33.3)| 8 (27.6) | 0.630 | 22 (31.9)| 1 (20)   | 0.244 |
| ≥ 50   | 30 (66.7)| 21 (72.4)|       | 47 (68.1)| 4 (80)   |      |
| Localization | |         |      |          |          |    |
| Right colon | 10 (22.2)| 9 (31) | 0.020 | 16 (23.2)| 3 (60)   |    |
| Left colon  | 11 (24.4)| 9 (31) |       | 20 (29) | 0 (0)    |    |
| Rectum     | 16 (35.6)| 7 (24.1)|       | 22 (31.9)| 1 (20)   |    |
| Colon (NOS) | 8 (17.8)| 4 (13.8)|       | 11 (15.9)| 1 (20)   |    |
| Tumor size |          |          |      |          |          |    |
| < 5 cm    | 17 (37.8)| 12 (41.4)| 0.757 | 27 (39.1)| 2 (40)   | 0.969 |
| > 5 cm    | 28 (62.2)| 17 (58.6)|       | 42 (60.9)| 3 (60)   |    |
| Macroscopic type | |         |      |          |          |    |
| Ulcerofungating | 22 (48.8)| 17 (57.1)| 0.479 | 36 (52.1)| 3 (60)   | 0.144 |
| Ulceroinfiltrative | 22 (48.8)| 12 (42.9)|       | 33 (47.9)| 1 (20)   |    |
| Polypoid | 1 (2.4)| 0 (0) | 0.206 | 0 (0) | 1 (20) |    |
| Tumor type |          |          |      |          |          |    |
| Adenocarcinoma | 35 (77.7)| 27 (85.1)| 0.805 | 58 (84) | 4 (80) |    |
| Mucinous AdenoCarcinoma | 8 (17.9)| 2 (14.9)| 0.060 | 10 (14)| 0 (0) |    |
| Adeno+neuroendocrine | 1 (2.2)| 0 (0)| 0.057 | 0 (0) | 1 (20) |    |
| Undifferentiated carcinoma | 1 (2.2)| 0 (0)| 0.0 (0) | 1 (2) | 0 (0) |    |
| Grade |          |          |      |          |          |    |
| 1 | 4 (10.5)| 4 (14.8)| 0.451 | 7 (11.7)| 1 (20) | 0.743 |
| 2 | 32 (84.2)| 21 (77.8)| 0.537 | 49 (61.7)| 4 (80) |    |
| 3 | 2 (5.3)| 2 (7.4) | 0.0 (0) | 4 (6.7) | 0 (0) |    |
| Tumor necrosis |          |          |      |          |          |    |
| - | 5 (11.1)| 5 (17.2)| 0.451 | 9 (13) | 1 (20) |    |
| + | 40 (88.9)| 24 (82.8)| 0.0 (0) | 60 (87) | 4 (80) |    |
| Stromal desmoplasia |          |          |      |          |          |    |
| Mild | 12 (26.7)| 5 (17.2)| 0.258 | 15 (21.7)| 2 (40) | 0.556 |
| Moderate | 28 (62.2)| 23 (79.3)| 0.0 (0) | 48 (69.6)| 3 (60) |    |
| Severe | 5 (11.1)| 1 (3.4)| 0.0 (0) | 6 (8.7) | 0 (0) |    |
| Stromal inflammatory reaction |          |          |      |          |          |    |
| Mild | 9 (20)| 9 (31)| 0.512 | 18 (26.1)| 0 (80) | 0.352 |
| Moderate | 31 (68.9)| 18 (62.1)| 0.0 (0) | 45 (65.2)| 4 (80) |    |
| Intense | 5 (11.1)| 2 (6.9)| 0.0 (0) | 6 (8.7) | 1 (20) |    |

MLH: MutL homologue; MSH: MutS homologue; NOS: Not otherwise specified.
analysis of MLH1/MSH2 proteins can be used as a prescreening method for mutation analysis of mismatch repair genes\(^3\). The inactivation of MLH1 and MSH2 genes is resulted in loss of expression of these proteins by immunohistochemistry.

We report here our first results of MMR (mismatch repair) analysis in Turkish sporadic colorectal carcinomas. To our knowledge, this TMA-based study about MSI status was completely performed for the first time in Turkey. We found loss of MLH1 expression in 29 (39.2%) and loss of MSH2 expression in 5 (6.8%) of cases. In addition, loss of either MLH1 or MSH2 expression was seen in 32 (43.2%) of cases, while loss of expression of both MLH1 and MSH2 was detected in 2 (3.1%) of cases.

### Table 2 MLH1/MSH2 expression and other clinicopathological parameters

|                | MLH1 (+) | MLH1 (-) | P   | MSH2 (+) | MSH2 (-) | P   |
|----------------|----------|----------|-----|----------|----------|-----|
| PT Crohn\(^1\) | 42 (93.3)| 26 (89.7)| 0.571| 63 (91.3)| 3 (60) | 0.492|
| -              | 3 (6.7)  | 3 (10.3) | 0.953| 6 (8.7)  | 2 (40) | 0.172|
| PT Lymph\(^2\) | 37 (82.2)| 24 (82.8)| 0.747| 58 (84.1)| 3 (60) | 0.184|
| -              | 8 (17.8) | 5 (17.2) |       | 11 (15.9)| 2 (40) |       |
| Tumor border   | 7 (15.6) | 3 (10.3) | 0.363| 8 (11.6) | 2 (40) | 0.394|
| Expansive      | 17 (37.8)| 13 (44.8)| 0.865| 29 (42)  | 1 (20) |       |
| Infiltrating   | 21 (46.7)| 13 (44.8)|       | 32 (46.4)| 2 (40) |       |
| Both           |          |          |       |          |        |       |
| Invasion level |          |          |       |          |        |       |
| Submucosa      | 1 (2.2)  | 0 (0)    | 0.363| 42 (60.9)| 4 (80) | 0.660|
| Muscularis     | 7 (15.6) | 4 (13.8) | 0.522| 3 (4.5)  | 1 (20) | 0.927|
| Subserosa      | 33 (73.3)| 22 (75.9)|       | 52 (75.4)| 3 (60) |       |
| Serosa         | 4 (8.9)  | 3 (10.3) | 0.014| 7 (10.3) | 0 (0)  | 0.002|
| PNI\(^3\)      |          |          |       |          |        |       |
| -              | 27 (60)  | 19 (65.5)|       | 42 (60.9)| 4 (80) | 0.816|
| +              | 18 (40)  | 10 (34.5)|       | 27 (39.1)| 1 (20) |       |
| LVI\(^4\)      |          |          |       |          |        |       |
| -              | 7 (15.6) | 3 (10.3) | 0.114| 9 (13)   | 4 (80) | 0.562|
| +              | 38 (84.4)| 26 (87.7)|       | 60 (87)  | 1 (20) |       |
| BVI\(^5\)      |          |          |       |          |        |       |
| -              | 38 (84.4)| 20 (69)  | 0.035| 54 (78.3)| 4 (80) |       |
| +              | 7 (15.6) | 9 (31)   |       | 15 (21.7)| 1 (20) |       |
| LN metastasis  |          |          |       |          |        |       |
| -              | 25 (58.1)| 21 (75)  | 0.146| 43 (65.2)| 3 (60) |       |
| +              | 18 (41.9)| 7 (25)   |       | 23 (34.8)| 2 (20) |       |
| Survival       |          |          |       |          |        |       |
| Disease-free   | 19 (45.2)| 13 (50)  | 0.035| 31 (48.4)| 2 (40) |       |
| Recurrence/metastasis |   2 (4.8)|   6 (23.1)| 0.119| 7 (10.9) | 1 (20) | 0.520|
| Extend         | 21 (50)  | 26 (79.6)|       | 26 (40.6)| 2 (40) |       |
| Stage (AJCC-2002)\(^6\) |          |          |       |          |        |       |
| 1              | 5 (11.6) | 3 (10.7) |       | 6 (9.1)  | 2 (40) |       |
| 2              | 18 (41.8)| 17 (50.7)|       | 31 (48.4)| 2 (40) |       |
| 3              | 16 (37.2)| 5 (17.6) |       | 34 (51.5)| 1 (20) |       |
| 4              | 10 (22.7)| 3 (10.7) |       | 20 (30.3)| 1 (20) |       |
| p53 expression |          |          |       |          |        |       |
| ≤ 10%          | 27 (60)  | 16 (57.1)|       | 0 (0)    | 5 (100)|       |
| > 10%          | 18 (40)  | 12 (42.9)|       | 74 (100) | 0 (0)  |       |

\(^1\)Peritumoral Crohn-like inflammation; \(^2\)Peritumoral lymphocytic inflammation; \(^3\)Perineural invasion; \(^4\)Lymph vessel invasion; \(^5\)Blood vessel invasion; \(^6\)American Joint Committee of Cancer.

### Table 3 Relation of MLH1/MSH2 expression and survival time (mean ± SD, mo)

|        | Survival time | P     |
|--------|---------------|-------|
| MLH1 (+)/MSH2 (+) | 47.08 ± 5.26 | 0.065 |
| MLH1 (+)/MSH2 (-) | 12.50 ± 1.06 |       |
| MLH1 (-)/MSH2 (+) | 71.71 ± 8.65 |       |
| MLH1 (-)/MSH2 (-) | 51.00 ± 16.26|       |

### Table 4 Results of COX regression analysis

| Predictors | P   |
|------------|-----|
| Loss of MLH1 expression | 0.127 |
| Loss of MSH2 expression | 0.325 |
| p53 over-expression | 0.417 |
| Stromal reaction | 0.124 |
| Stromal inflammatory reaction | 0.407 |
| Level of invasion | 0.572 |
| Perineural invasion | 0.267 |
| Lymphatic invasion | 0.085 |
| Blood vessel invasion | 0.769 |
| Peritumoral Crohn’s-like inflammation | 0.247 |
| Peritumoral lymphocytic inflammation | 0.952 |
| Stage | 0.000 |
| Lymph node metastasis | 0.000 |

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expressed either one or both of MLH1 and MSH2; MLH1 and MSH2 were not expressed in 35 (8.7%) and 19 (4.7%) cases, respectively; and 3 cases showed neither MLH1 nor MSH2 expression.

Lanza et al. found that 106 (80.3%) MSI-H carcinomas showed complete loss of MLH1 expression, 14 (10.6%) displayed complete loss of MSH2 expression, 12 (9.1%) MSI-H carcinomas demonstrated normal expression of both MLH1 and MSH2, but no MSI-H tumors showed lack of both MLH1 and MSH2 expression. In contrast, nuclear immunoreactivity for MLH1 and MSH2 proteins was observed in all MSS and MSI-L tumors analyzed. The common finding of all these studies is that MLH1 extinction is more frequent than MSH2 extinction. This supports the hypothesis that involvement of the MLH1 gene is prevalent in the development of sporadic large bowel MSI cancers.

In our study, unlike previous studies, MLH1-negative cases had no gender, age or tumor localization predominance. On the other hand, loss of MSH2 seemed to be related to some parameters more than loss of MLH1; for example, all MSH2-negative cases were women, 60% of them were located in the right colon and no serosal involvement was detected in MSH2-negative cases. Other interesting but statistically insignificant findings were both MLH1-negative/MSH-negative cases had peritumoral Crohn’s-like lymphoid infiltration and prominent intratumoral neutrophilic infiltration.

Although there was no statistical significance, 80% of MSH2-negative cases did not show perineural invasion, lymphatic invasion and blood vessel invasion. Bernardo et al. found that only vascular invasion was significantly correlated with MSH2 expression. Similarly, Wright et al. found that in MLH1- and MSH2-negative carcinomas, extravascular, lymphatic and perineural invasion were all significantly less than the others.

In our study, 72.4% of MLH1-negative and 80% of MSH2-negative cases were ≥ 50 years. Tumor size was ≥ 5 cm in 58.6% of MLH1-negative cases and 60% of MSH2-negative cases. But there was no significant correlation between these parameters. Lanza et al. showed that MLH1- and MSH2-negative carcinomas were located in the proximal colon, more often of > 7 cm in diameter, poorly-differentiated, and had expanding pattern of growth and intense peritumoral Crohn’s-like lymphoid reaction.

Several studies demonstrated that MSI tumors were correlated with clinicopathological features, such as right colon location, mucinous type, expansive borders, peritumoral Crohn’s-like lymphoid reaction and peritumoral lymphoid response. A Japanese study of a series of colorectal carcinomas did not find any correlation between MSI status and any clinicopathological features except for tumor location in the proximal colon.

The p53 gene is mutated in 70% of colorectal carcinomas. Over-expression of p53 has been used as an indicator of p53 mutational status in many studies. In the present study, there was no significant difference between p53 expression and MLH1 expression, whereas a significant correlation between low p53 expression and loss of MSH2 expression was detected (P < 0.05).

Park et al. demonstrated that there was a significant difference in p53 expression between the MLH1-positive group and MLH1-deficient group, indicating a correlation of loss of MLH1 or MSH2 expression with low p53 expression. We found that all MSH2-negative tumors showed low p53 immunostaining. This finding supports that different carcinogenic pathways, different molecular changes and different genes can be affected. Thus, an inverse correlation between genetic alterations of p53 and the mismatch repair system may simply reflect different carcinogenic pathways.

It has been reported that survival in patients with colorectal cancer with MMR gene defect is better than without one. In our study, loss of MLH1/MSH2 expression had no significant correlation with the survival. It has been clearly elucidated that tumor stage and lymph node metastasis are the independent prognostic factors for colorectal carcinomas. Hameed et al. found similar results in their study. Chapusot et al. found that three independent factors were significantly associated with the loss of expression of MLH1 and MSH2: proximal location, the presence of Crohn’s-like lymphoid reaction and poor differentiation.

Interestingly, in our study overall survival of the cases with loss of only MLH1 or both MLH1 and MSH2 expression was longer than those with both MLH1- and MSH2-positive or with only MSH2-negative expression. Although this finding is not statistically significant (P = 0.065), we think that this analysis should be repeated and combined with the result of genetic analysis which will be planned for near future. This finding showed some similarities with other studies. Lanza et al. found that cases with MLH1-negative carcinomas more often died of disease, but the survival of the cases was not statistically significant. However, Gafa et al. found that cases with both MLH1- and MSH2-negative carcinoma showed a better clinical outcome and survival.

In conclusion, our findings suggest that the assessment of MSI status using immunohistochemistry is important in genetic and biologic characterization of colorectal carcinomas. Turkish patients with colorectal cancers show some similarities with other populations in terms of histopathological features and MSI status. Although prognostic importance remains controversial, immunohistochemical analysis of MSI status may be used as routine histopathological examination of colorectal cancer tissues. However, genetic analysis should be combined with these results.
hereditary polypsis colon cancers. Last studies showed that MSI was found also in sporadic CRC. So, searching MSI status of CRC is clinically important, since patients with carcinomas demonstrated several distinct features compared to MSS carcinomas. In this point immunohistochemistry is useful method since it is easy, cheap and reliable method to detect these patients.

Innovations and breakthroughs

This analysis was performed for the first time in Turkey. Loss of MLH1 and/or MSH2 expression is important finding to predict their different morphology and behaviour in CRC cases.

Applications

Searching MSI status of CRC should be performed more commonly. Immunohistochemical analysis of MSI status of CRC may be used as screening method in developing countries.

Peer review

This is the first study of MMR deficiency and its prognostic importance in sporadic CRCs and the incidence of MLH1 and MSH2 expression losses in Turkish sporadic CRCs and the data was compared with survival and clinicopathological features of the patients.

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