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EXPRESSION OF MSH-6 IMMUNOHISTOCHEMISTRY MARKER IN COLORECTAL CANCER

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Abstract – *Objective:* *Defects in mismatch repair genes or microsatellite instability (MSI) are seen in colorectal cancers (CRCs) both in sporadic and more predominantly in hereditary cases. Loss of MutS homolog (MSH)-6 as a mismatch repair gene may be seen in the CRC. We aimed at evaluating the expression of MSH-6 as a marker of MSI in a common tumor with hereditary and familial features for preventive, diagnostic, and therapeutic purposes.*

Patients and Methods: *Paraffin blocks of 103 patients who underwent colonoscopy or excisional biopsy with a pathologic diagnosis of CRC were selected and immunohistochemistry (IHC) with MSH-6 marker was done.*

Results: *Of all patients, 53 (51.45%) were males and the age range was 29-87 years. Of these, 96 (93.2%) were positive for the MSH-6 marker and 7 (6.8%) were negative. Of the 103 patients, 96 had adenocarcinoma. No significant relationship was observed between MSH-6 marker and gender, age group, cancer location in the colon, and cancerous type (p-values: 0.261, 0.343, 0.75, and 0.401, respectively). Out of 7 cases with MSH-6 loss, female gender, early-onset (≤50 years), mucinous or poorly differentiated, proximal location, and absence of lymph node involvement were seen in 5, 4, 3, 3, and 5 cases, respectively.*

Conclusions: *According to the results and literature, MSH-6 IHC on CRC specimens is recommended especially in young age females, with right-sided tumors, poor differentiation, and mucinous component. First-degree relatives of the patients with MSH-6 loss may be trained for strictly following the guidelines of colorectal cancer screening.*

KEYWORDS: *Immunohistochemistry, Microsatellite instability, MSH-6, Colorectal Cancer.*

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INTRODUCTION

Microsatellites as repeated sequences of DNA are prone to mutations during replication[1-3]. Four genes with a role in correcting errors in replication or mismatch repair are MutL homolog (MLH)-1, MutS homolog (MSH)-2, MSH-6, and PMS-2. Defect in these genes culminates in microsatellite instability (MSI). This alteration may be seen in some tumors, including colorectal cancers (CRCs) especially hereditary nonpolyposis colon cancers or Lynch syndrome (LS). MSI is seen in about 15-20% of sporadic cases of CRC and more than 90% of cases with LS[1]. LS is often underestimated[4-6]. In this syndrome especially with a mutation in MSH-6 colonoscopy is suggested to begin at the age of 30 years and repeated every 1-2 years[7-9]. The detection of mutation is important not only in LS but also in sporadic CRCs. In the later finding, the mutation can impact on prognosis and treatment method[9,10]. Microsatellite Path Score is used by the pathologists considering age, anatomical site, degree of differentiation, and tumor-infiltrating lymphocytes, and other pathological features for prediction of MSI and had a good correlation with mutations in MLH-1 and MSH-2 genes, but MSH-6 needs further research[11,12]. Also, MSH-6 mutation carriers often do not fulfill Amsterdam's criteria for LS[13-15]. MSI is seen in cancer of the stomach, endometrium, and other cancers along with CRC[15,16-21]. CRC is the third most common cancer in the world with an increase of incidence in Iran[22-25]. CRC in Iran is higher in younger age groups with positive family history than in western countries[26-28]. The prevalence of LS in Iran is an estimated 5.5% of CRCs[29]. We aimed to evaluate the expression of MSH-6 as a marker of MSI in a common tumor with hereditary and familial features for preventive, diagnostic, and therapeutic purposes.

MATERIALS AND METHODS

This cross-sectional and analytical study was approved by the Local Ethics Committee (Code: IR.KUMS.1397.534). Paraffin blocks of patients who underwent colonoscopy or excisional biopsy with a pathologic diagnosis of CRCs were selected from archives of the Pathology Department from 2014 to 2018. In this study, 111 blocks of colorectal specimens from 111 patients were used. Eight specimens were removed due to improper slide or lack of tumor in the slide and finally, 103 specimens were evaluated. The hematoxylin and eosin (H&E) stained slides were reviewed by a pathologist for re-confirmation of diagnosis. Then 4-micron cuts were prepared from paraffin blocks and IHC staining with MSH-6 marker was done. The IHC process and staining were according to the brochure of the kit. Rabbit Anti-human MSH-6 Monoclonal Antibody (Clone EP49) of master diagnostic, Granada (Spain) was used (ready-to-use MAD-000635QD-3). Nuclear cell staining was evaluated and divided into two groups: MSH-6 positive and MSH-6 negative. Quantitative evaluation of positive cells for MSH-6 was performed by the two observers, the pathologist and his assistant (Figure 1). The intensity of staining was not assessed. Normal colon tissue was examined as an internal control for nuclear staining and then the staining of the tumor tissue nuclei was compared. All the slides were examined by light microscopy with X400 magnification and a lack of nuclear staining was considered as a mutation.

Statistical analysis

The data were analyzed using Pearson's chi-square and Fisher's exact test and in SPSS version 22 software (IBM Corp., Armonk, NY, USA).

RESULTS

In this study, 103 CRCs from 103 patients were evaluated. Of all patients, 53 (51.45%) were male. The age range was 29-87 years. As we know, the MSH-6 gene is a repair gene, and nuclear staining implies that the repair process is performed by this gene. Lack of staining of nuclei means non-function gene and presence of a mutation or misregulated epigenetic mechanisms. Of these, 96 (93.2%) were positive for the MSH-6 marker and 7 (6.8%) were negative. Based on the Fisher Exact Test result, no significant relationship was observed between the MSH-6 marker and gender in Table 1 *($p = 0.261$)*. Patients were grouped into 3 age groups considering the risk of CRC. These age groups consisted $\leq$ 45 years, 46-65 years, and $\geq$ 66 years. Based on the Fisher Exact Test result, no significant relationship was found between MSH-6 marker expression and age in Table 2 *($p = 0.343$)*. According to the site of involvement in pathology report the patients categorized in sigmoid, rectum, cecum, rectosigmoid, sigmoid, and colon, and the last group with no further designation as colon NOS (Not Otherwise Specified). So, the most common site of involvement was colon NOS with 49 cases reported. Based on the Fisher Exact Test result, no significant relationship was observed between MSH-6 marker expression and cancer location in

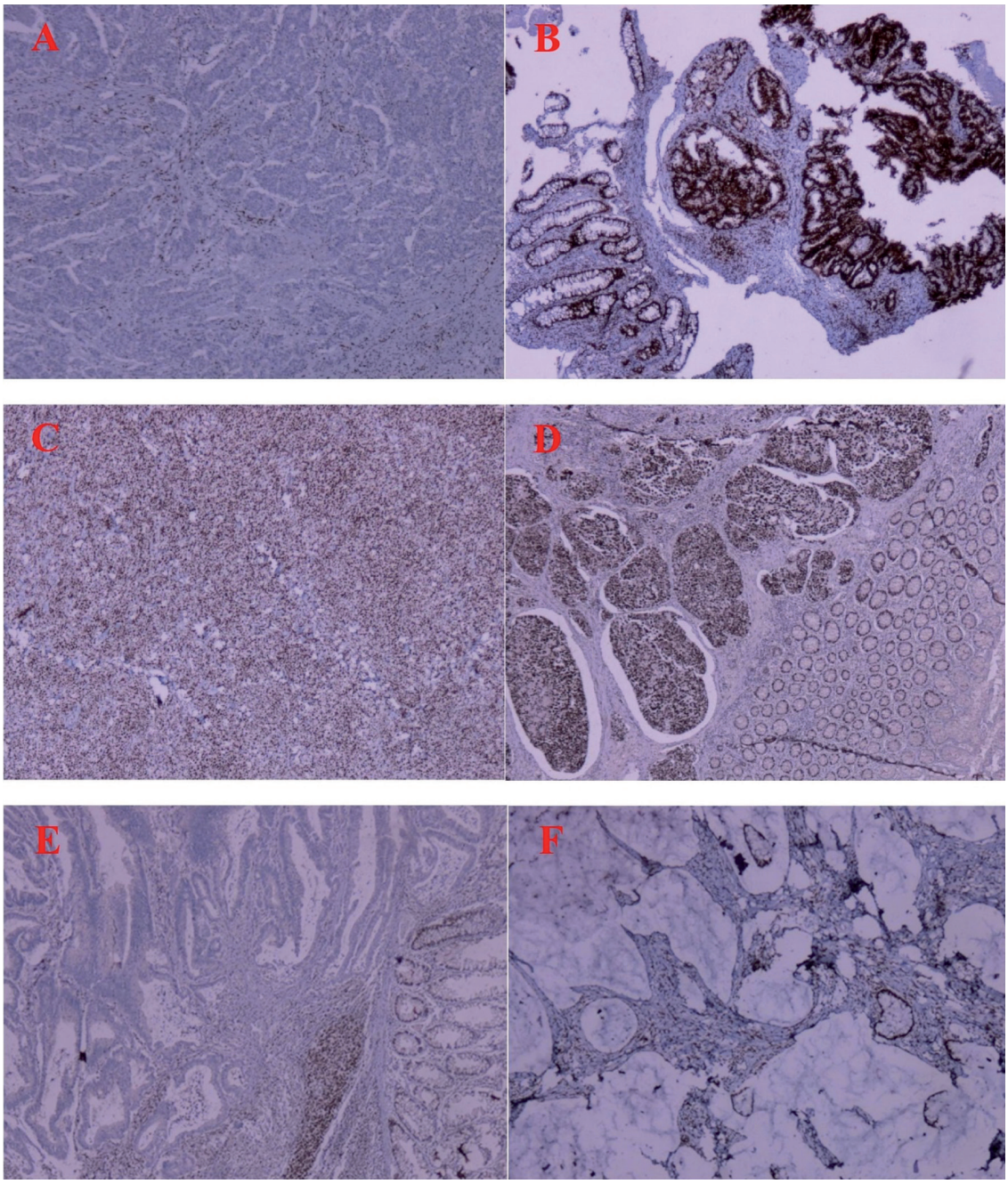

Fig. 1. Immunohistochemistry staining of MSH-6. *A*, Negative, Adenocarcinoma; *B*, Positive, Adenocarcinoma; *C*, Positive, Malignant lymphoma; *D*, Positive, Adenocarcinoma with neuroendocrine features; *E*, Negative, Adenocarcinoma with mucinous features; *F*, Positive, Adenocarcinoma with mucinous features. All X40 magnification.
Abbreviations: M, methylated; U, unmethylated; NBM, normal bone marrow.

Table 3 *(p = 0.750).* Of the 103 patients, 96 had adenocarcinoma. Based on the Fisher Exact Test result, no significant relationship was observed between MSH-6 marker expression and cancerous type in Table 4 *(p = 0.401).* Seven cases with loss of MSH-6 described with details as follows: 1) Female, 40-year-old, poorly differentiated adenocarcinoma of the colon, 6 cm without lymph node involvement. 2) Male, 37-year-old, moderately differentiated neuroendocrine carcinoma of the right colon, 5 cm without lymph node involvement. 3) Female, 68-year-old, moderately differentiated adenocarcinoma of the cecum, 5 + 3 cm with half of lymph node involvement. 4) Female, 50-year-old, moderately differentiated adenocarcinoma of sigmoid, 3.5 cm without lymph node involvement. 5) Female, 72-year-old, adenocarcinoma of the colon with no further

TABLE 1. Relationship between gender and MSH-6 staining *(p=0.261)*.

Variable	Positive n, (%)	Negative n, (%)	Total n, (%)
Male	51 (96.2%)	2 (3.8%)	53 (100.0%)
Female	45 (90.0%)	5 (10.0%)	50 (100.0)
Total	96 (93.2%)	7 (6.8%)	103 (100.0%)

TABLE 2. Evaluation of the association between age group and MSH-6 staining *(p=0.343)*.

Variable	Positive n, (%)	Negative n, (%)	Total n, (%)
≤ 45 years	12 (85.7%)	2 (14.3%)	14 (100.0%)
46-65 years	38 (92.7%)	3 (7.3%)	41 (100.0%)
≥ 66 years	46 (95.8%)	2 (4.2%)	48 (100.0%)
Total	96 (93.2%)	7 (6.8%)	103 (100.0%)

TABLE 3. Evaluation of the association between cancer location and MSH-6 staining *(p=0.750)*.

Variable	Positive	Negative	Total
Colon NOS	44 (89.8%)	5 (10.2%)	49 (100.0%)
Sigmoid	30 (96.8%)	1 (3.2%)	31 (100.0%)
Rectum	7 (100.0%)	0 (0.0%)	7 (100.0%)
Cecum	11 (91.7%)	1 (8.3%)	12 (100.0%)
Sigmoid and Rectum	3 (100.0%)	0 (0.0%)	3 (100.0%)
Sigmoid and Colon	1 (100.0%)	0 (0.0%)	1 (100.0%)
Total	96 (93.2%)	7 (6.8%)	103 (100.0%)

Abbreviation: NOS, Not Otherwise Specified.

TABLE 4. Evaluation of the association between cancer type and MSH-6 staining *(p=0.401)*.

Variable	Positive	Negative	Total
Variable	Positive	Negative	Total
Adenocarcinoma	90 (93.8%)	6 (6.3%)	96 (100.0%)
Signet cell carcinoma	1 (100.0%)	0 (0.0%)	1 (100.0%)
Carcinoma	2 (66.7%)	1 (33.3%)	3 (100.0%)
Malignant lymphoma	2 (100.0%)	0 (0.0%)	2 (100.0%)
Carcinosarcoma	1 (100.0%)	0 (0.0%)	1 (100.0%)
Total	96 (93.2%)	7 (6.8%)	103 (100.0%)

Abbreviation: NOS, Not Otherwise Specified.

information. 6) Female, 46-year-old, mucinous adenocarcinoma of the colon with signet ring cells, 3 cm without lymph node involvement. 7) Male, 58-year-old, mucinous adenocarcinoma in the ascending colon and cecum, 7 cm without lymph node involvement. In summary, out of 7 cases with MSH-6 loss, female gender, early-onset (≤50 years), mucinous or poorly differentiated, proximal location, and absence of lymph node involvement were seen in 5, 4, 3, 3, and 5 cases, respectively. Some data was not available for definite characterization in this case series.

DISCUSSION

This study was conducted on 103 CRC cases in the west of Iran in which 7 cases (6.8%) showed loss of MSH-6. Five of mutant cases (5/7) had no lymph node involvement. Karahan *et al* [30] found a loss of MSH-6 in 7/186 (3.7%) of their cases in Turkey, which was lower than our result of 6.8% in Iran in their neighborhood. They suggested screening for MSI by IHC for finding familial cases and LSs. According to their findings, this screening must be applied at least to right-sided tumors with

poor differentiation, mucinous component, and prominent tumor infiltrating-lymphocytes in colorectal adenocarcinomas. Tumors with these criteria had a higher probability of MSI. However, it must be noticed that tumors may be heterogeneous for IHC staining and larger surgical specimens are more informative than small colonoscopy biopsies[30]. In another study from Turkey, Ozkara et al[31] evaluated 608 cases of CRCs, 27 of them were high-risk groups of MSI. They demonstrated loss of MSH-6 in 7/20 (25.03%) of the high-risk cases. They proposed an MSI positive group with better prognosis, less lymph node metastasis, but less responsive to adjuvant chemotherapy. In a study[32] in Pakistan, neighboring south-west of Iran, none of the 212 cases of definite or suspected LS were positive for MSH-6 mutation similar to the studies in Slovenia in Europe[13] and Boston in the USA[33]. In China 6/146 of CRCs were mutant for MSH-6[34]. Others confirmed the idea of MSI positive CRCs with different clinicopathological characteristics, including right-sided location, poor differentiation, less lymph node involvement, and metastasis, with a presentation in early stage[35]. This report[35] suggested a 2-antibody panel (PMS-2 and MSH-6) as effective as a four-antibody panel (MLH-1, MSH-2, PMS-2, and MSH-6). Qin Q analysis revealed 90/440 (20.5%) MSI in CRC and suggested MSI as an independent prognostic factor. They also confirmed a higher meaningful loss of repair genes in right-sided, mucinous, poorly differentiated tumors, albeit with higher disease-free survival[36]. Molaei *et al*[37] used a four-antibody panel of MSI in 104 patients with CRC, all of them with early-onset presentation (Age range: 20-50 years). They found 29 MSI-positive patients (27.9%). Loss of MSH-6 was found in 9 patients (8.6%). Meanwhile, the loss of MSH-2 and MHS-6 together was associated with a higher P53 expression that is a marker of chromosomal instability. However, they suggested PMS-2 IHC as a screening tool in early-onset CRC. In another program, this researcher evaluated 343 CRCs and found 14 MSI in a four-antibody panel (4.08%). The loss of MSH2/MSH-6 was found in 12 patients. They confirmed right-sided involvement and more association with positive family history, but non-significant better survival[29].

Goshayeshi *et al*[38] found 33 MSI positive cases in 322 CRCs in which nine of them had MSH-6 loss. They revealed that 29 cases had a positive screen for LS. Finally, they suggested at least a 2-antibody panel (PMS-2 and MSH-6) for screening added to clinical criteria. In another research[4], 321 CRCs were evaluated in two groups of early-onset (≤50 years) and late-onset (>50 years). The difference between groups for MSI positivity was not significant. Authors found that most of the suspected cases of LS in the early-onset group had tumors on the left side of the colon rather than the right side[4]. Our result in cancer location is not statistically significant and this result may be due to the low sample number. Meanwhile, we could not identify the exact site of some cases and put them in the category of colon NOS. Loss of MSH-6 has also been proven in sebaceous neoplasms of Muir-Torre syndrome[39,40]. Despite ideas of higher family history of CRC in Iran[26], Geramizadeh[1] found that the expression of molecular biomarkers of CRCs is similar between Iranian and western populations. Therapeutic use of MSI has been emphasized by Valeri et al[41,42]. They showed that microRNA-21 overexpression downregulated mismatch repair and culminates in a low therapeutic response to 5-fluorouracil (5-FU) chemotherapy[41]. Similar results are obtained by microRNA-155[42]. However, Kawakami et al[43] recommended 5-FU-based chemotherapy regimens only for stage III and not stage II patients. The current study used a small group of samples, with consequential lack of statistical power; however, it may represent a source for scientific community which may employ for future meta-analyses. Although this study has limitations including a few numbers of cases and evaluating only one marker due to limited resources, highlighting a special group (young age females, right-sided tumors with poor differentiation and mucinous component) is a point of strength. Evaluating the first-degree relatives of this special group with a low cost may culminate in early diagnosis of cancer and saving lives.

CONCLUSIONS

According to the findings of this research and literature review, we suggest MSH-6 IHC staining of CRC specimens, especially in young age females, with right-sided tumors with poor differentiation and mucinous component. First-degree relatives of the patients with MSH-6 loss may be trained in strictly following the guidelines of CRC screening.

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Conflict of interest:
There are no conflicts of interest.

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