Original Article

Comparing the efficacy of routine H&E staining and cytokeratin immunohistochemical staining in detection of micro-metastasis on serial sections of dye-mapped sentinel lymph nodes in colorectal carcinoma

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Abstract

Background: The significance of techniques used for detecting micro-metastasis (MM) or isolated tumor cells (ITCs) is a controversial issue among investigators. We evaluated the different techniques used on sentinel lymph node (SLN) to detect MM/ITCs.

Materials and Methods: Ninety-one SLNs of 15 patients underwent serial section with 100 μm interval. In each level, two sections were prepared. One section was stained with H&E and another with anti-cytokeratin antibody (immunohistochemistry). Then the sections were evaluated for detecting MM/ITCs. Results were analyzed by chi-square test.

Results: 1656 sections of 91 SLNs of 15 patients were evaluated by a pathologist; MM was found in 1 and ITCs in 1 case. Overall, 2 out of 15 cases (13.3% of the patients) showed MM/ITCs by IHC staining. So, serial section along with using IHC was superior than serial section and routine H&E staining. But it did not affect the 5-year survival of the patients ($P = 0.47$).

Conclusion: Using the combined techniques of serial section and IHC staining could up-stage 13.3% of colon cancer patients who were lymph node negative. In other studies with different combination of serial section, IHC staining, and PCR, investigators were able to find MM/ITCs in 3-39% of the cases. In our study, although serial section and IHC staining could up-stage 13.3% of patients, it could not affect the 5-year survival of the patients.

Key Words: Colorectal cancer, cytokeratin, immunohistochemistry, micro-metastasis, sentinel lymph node

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INTRODUCTION

Colon cancer is considered to be the third leading cause of cancer mortality. In a study conducted in 1995, it was found that 334,000 new cases of colorectal cancer were reported in Europe area; in the same year, 189,000 deaths were reported from this kind

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of cancer.[1] Despite the progress made in early detection and surgery of these kinds of cancer, their mortality rate has not changed in recent decades. The main reason behind the latent spreading of cancer cells in various stages of tumorigenesis is that in other places it cannot be detected by the common pathologic methods. In order to detect these micro-metastases (MMs), serial section method and H&E staining or immunohistochemistry (IHC) with anti-cytokeratin antibody can be used. Sentinel lymph node (SLN) assessment was used initially to evaluate melanoma and breast cancer,[2] but recently its use in gastrointestinal tract cancers, particularly colorectal cancer, has been considered by the investigators.[3] Isolated tumor cells (ITCs) (as individual cells) or their MMs (cell cluster less than 200 μm) may be overlooked in H&E staining and, therefore, to detect them definitely, anti-cytokeratin antibody can be used. Given the fact that performing serial section and IHC is time consuming and expensive, to reduce the costs and get the maximum results, it is better to do these actions on SLN which is more likely to find micro-metastasis. To detect this lymph node, various methods such as injection of lymphozurin 1% are used. Studies that have been done in this area can be divided into two groups. The first group involves the studies in which IHC method has been performed on all lymph nodes and MM rate is found to vary from 2 to 15.78%.[4,5] The second group involves the studies in which IHC method has been performed on SLNs and MM rate is found to vary from 6.7 to 32%.[6-8]

While in some of these studies the difference in the rate of MM detection has been statistically significant, in others it has been not significant. However, this method is not yet accepted as a standard method and the need for more research in this area is felt. Regarding the assessment of lymph nodes, two issues have been considered. One is the evaluation of SLNs versus all lymph nodes that is based upon statistical evidences. The chance of finding MM/ITCs in SLNs is significantly higher than in non-SLNs (22% vs. 4%).[9] The other issue is SLN evaluation techniques. Three techniques of serial section, IHC staining, and polymerase chain reaction (PCR) have been used in different studies. In the current study, we compared the efficacy of H&E and IHC staining in detection of MM/ITC on serial sections of SLNs.

MATERIALS AND METHODS

This study was done in Alzahra Hospital from 2006 till 2008. It was a cross-sectional and prospective one, and had two phases. In the first phase, all the patients with colorectal cancer who had volunteered for surgery were selected; then, during surgery, 1-2 cc lymphozurin 1% was injected in the tumor's surrounding serosa, and 10 min after injection, colorectal surgery was performed. Then the samples were sent to the laboratory and all colon lymph nodes were isolated. After sectioning, the blue lymph nodes were considered to be sentinel. At the end of this phase, the percentage of metastasis was evaluated and compared both in SLN and non-SLNs. In the second phase of the study, the patients who were diagnosed with lymph node-negative metastasis (the patients with stage II or less) using the routine methods accepted in all laboratories and the patients without distant metastasis were studied; these patients' SLNs were selected and they underwent serial section in different levels and with 100 μm interval. If the average diameter of each node was considered as 1 cm, each patient underwent serial section in 10 levels and two slides were made up for each level. At first, one of the slides was stained, in all levels, by H&E; if no metastasis was found, the other slide was stained by anti-cytokeratin antibody (DAKO, clone: AE1⁄AE3, code 3515, USA) using IHC. Finally, using SPSS software and K2 test, the percentage of MM was statistically analyzed in both methods.

RESULTS

Twenty-nine cases of colorectal carcinoma were enrolled in the study; 12 patients (41.4%) were female and 17 patients (58.6%) were male. The mean length of removed colon was 33.3 ± 3.75 cm, the mean diameter of the tumor was 4.43 ± 0.3 cm, and the mean thickness of the tumor was 1.9 ± 0.12 cm The total number of lymph nodes was 511 (151 SLNs and 360 non-SLNs); the mean number of lymph nodes was 17.6, the mean number of SLNs was 5.2, and the mean number of non-SLNs was 12.4. Ninety-one of SLNs of 15 patients with no lymph node involvement were included in the study. From the total of 1656 lymph node sections, 828 sections were stained with H&E and 828 sections with IHC, and then were evaluated. None of the H&E sections had MM or ITCs, while in three IHC sections (of one case) MM was observed [Figure 1] and in another section (of the second case) ITCs were observed [Figure 2]. In 3-6 years follow-up of these 15 patients, 9 were alive, 3 were dead, and the data of 3 others were not available. Detecting MM/ITCs by IHC was not significantly associated with the 5-year survival of the patients (P = 0.47). Therefore, IHC performed on serial sections of SLNs could upstage in 13.3% of node-negative patients on routine examination of the lymph nodes; however, this detection of MM/ITCs could not affect the 5-year survival of the patients and, therefore, was clinically not significant (P = 0.47).

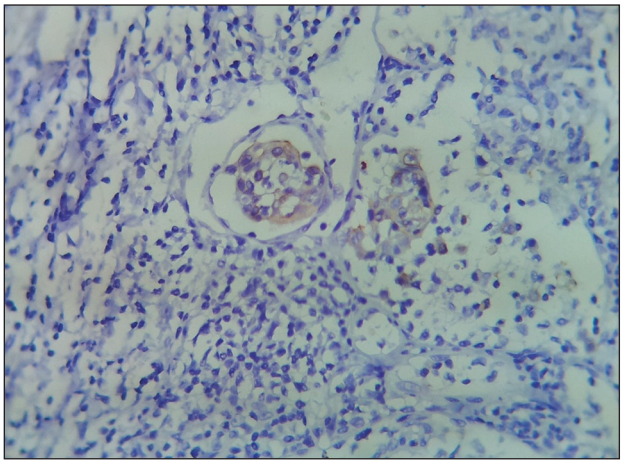

Figure 1: Micro-metastases: Concentration of tumor cells in the lymph node, cytokeratin staining

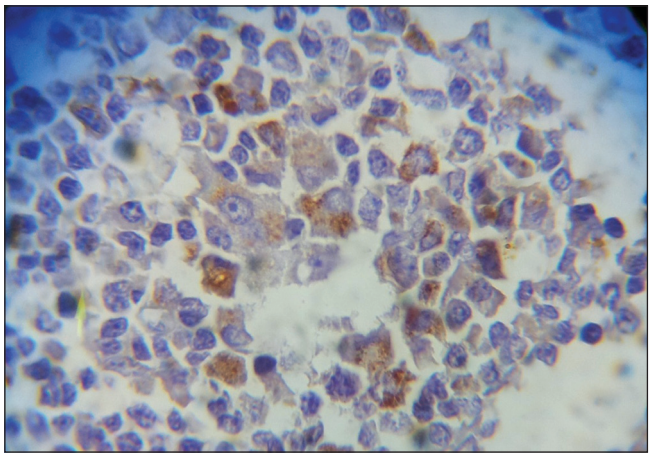

Figure 2: Isolated tumor cells with macrophages containing cytokeratin in lymph nodes, cytokeratin staining

DISCUSSION

In the current investigation, 91 SLNs from 15 patients with negative lymph nodes on routine examination were studied. Of the total 1656 lymph node sections, 828 sections were stained with H&E and 828 sections with IHC, and then were evaluated. IHC was able to upstage 13.3% of the patients who were lymph node negative.

Dagan *et al*. detected MM/ITCs in 26.9% of the SLNs using IHC method.[10] In Wasif *et al*.'s study in which IHC was performed randomly on the lymph nodes with no involvement, MM/ITC rate was obtained as 4.23%.[11] D'Armento *et al*. evaluated 100 random lymph nodes using three methods of H&E, IHC (CK), and reverse transcriptase-polymerase chain reaction (RT-PCR). In H&E method, MM was detected in four nodes; in IHC (CK) method, ITCs were reported in 14 nodes; and in RT-PCR method, CEA(Carcinoembriogenic Antigen) positivity was reported in 50 nodes.[12] Likewise, Lim, using IHC (CK) method, could detect ITCs in 7% of the SLNs.[13] Kelder, by using IHC method, found 18% of MM/ITCs in SLNs.[14] Bembenek detected 21% of MM/ITCs in SLNs by using IHC method.[15] In another study, by using IHC and PCR, Kelder could detect 33% of MM/ITCs in SLNs.[16] Using IHC (CK-CEA), Thomas was able to detect MM/ITCs in 3% of the SLNs.[17] In a study conducted by Bembenek *et al*., both SLNs and non-SLNs were evaluated by using serial section and IHC methods and 39% of MM/ITCs were detected in 33 patients with lymph node negative involvement.[9] Park, by doing anti-cytokeratin on SLNs, could upstage 20.0% cases.[18]

In this study and others, the ability of IHC method in finding MM/ITCs has been demonstrated. However, as this method is more expensive and time consuming, the clinical significance of finding MM/ITCs has to be evaluated. In this study (P = 0.47) and other similar studies, no significant association has been observed between the disease-free survival of the patients and MM/ITC finding.[13] In study of Lips's patients with ITC/MM-positive SLNs who were candidates for adjuvant chemotherapy, they had 3-year disease-free survival (DFS).[19] Braat showed that overall 5-year-survival was statistically significant in the SN group (P = 0.04).[20]

In most studies, for detecting MM/ITCs, only IHC method has been used, while in this study serial sections stained with routine H&E and IHC were simultaneously used, and all SLNs were evaluated.

Therefore, according to the findings of the study, using this method is not scientifically justifiable and depends on further investigations in the future.

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