Accuracy of Elastic Tissue Stain in Detecting Venous Invasion in Colorectal Cancer

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Abstract

Objective: To determine the accuracy of Elastic tissue stains in detecting venous invasion in Colorectal cancer (CRC).

Methods: Hematoxylin and Eosin (H&E) stained section from 100 cases of colorectal carcinoma were assessed for presence of venous invasion and compared with sections stained with Elastic Van Gieson (EVG) stain. Total number of cases showing evidence of venous invasion with H&E and EVG were calculated.

Results: In total of 100 patients, 52 (52%) were males and 48 (48%) were females. The mean age of patients in the study sample was 59.80±19 SD years. A total of 32 cases initially assessed on H&E stained sections showed evidence of VI while the remaining 68 cases were negative for VI. When EVG stained sections were assessed, VI was seen in 59 (59%) cases while 41 cases did not show any evidence of VI on EVG. Among these 59 cases, 32 cases which were initially positive on H&E were also positive on EVG (True positive). The remaining 27 cases did not show any evidence of VI on H&E but showed VI on EVG stained sections (false negative). A total of 41(41%) cases did not show venous invasion with any of the two stains (True negative). The overall diagnostic accuracy of EVG in detection of venous invasion (VI) in CRC was 59 % whereas the sensitivity, specificity, positive predictive value, and negative predictive value were 54.24%, 100%, 100%, and 60.29%.

Conclusion: Venous invasion was seen in more cases with EVG as compared to H&E alone. This stain is cheap and less time consuming, so it should be incorporated into routine reporting of colorectal carcinoma.

Key words: Colorectal carcinoma, Tissue stain, Venous invasion.

Introduction

Colorectal carcinoma is the fourth most common form of cancer occurring worldwide. Tumor extent, lymph node status, tumour grade and the assessment of lymphatic and venous invasion are still the most important morphological prognostic factor. Venous invasion is an important independent prognostic indicator of both prognosis and risk of development of distant metastases in colorectal carcinoma. Several researchers have emphasized upon the importance of VI in CRC, in addition to lymph node metastasis, for considering systemic neoadjuvant therapy. Routine reporting of VI in CRC specimens is included in data set and checklists laid down by Royal college of Pathologist, the College of American Pathologists (CAP), American joint committee on cancer (AJCC) and the Japanese Society for Cancer of the Colon and Rectum. Brown and Warren were the first to describe significance of VI in CRC. Despite its prognostic importance, VI in colorectal cancer is an under recognized feature with a prevalence in published studies ranging from 10% to 90%. This wide difference in observed incidence of VI is due to difference in processing of sections, inter observer variability and the grade and stage of resected tumour, failure to recognize invaded vein due to destruction by the tumour, errors in sampling technique and area of the tumour to be sampled, number of blocks submitted for processing and lastly the technique and quality of staining. These factors are responsible for an inherent false negative rate of 10.5%-29.6% in detection of VI in colorectal carcinoma. This false negative rate can be minimized by increasing the number of blocks and slides examined for presence of VI, addition of Elastic stain to routine staining of sections from CRC and taking tangential sections from outer perimeter of tumour and mesenteric border of bowel wall thereby increasing the number of sampled veins and so increasing the chances of detection of VI in these veins. Detection of venous invasion in the primary tumor is currently assessed using sections stained with Hematoxylin and Eosin, which has some disadvantages. Vessels are crushed by tumor and at the periphery of tumour are difficult to be picked up, and so is the observed incidence of venous invasion, when assessing sections stained with conventional stains. A number of markers and stains have been used to improve detection of vascular invasion. Elastic stains are cost effective, less time consuming and more sensitive in detecting venous invasion in colorectal carcinoma as compared to various endothelial markers like CD31 and CD34 which are both expensive and time consuming. Elastic stains highlight the elastic layer of...
vessel wall which significantly increases the observed incidence of venous invasion in colorectal cancer. Various studies have shown that introduction of Elastic stains into routine practice considerably increases the sensitivity of detection of VI as compared to H&E alone.3,15-17

The aim of this study was to assess the accuracy of Elastic tissue stain in detecting venous invasion in colorectal carcinoma by comparing it with Hematoxylin and Eosin.

Materials and Methods

This cross sectional study was conducted in Histopathology Department (FGPC), Islamabad. The study got approval from ethical committee of the hospital. Paraffin blocks of 100 cases of colorectal carcinoma received in Histopathology Department (FGPC), Islamabad from February 2010 till October 2013 were retrieved. Three to five micrometer thick sections were cut for each block from the representative area by using Accu-Cut SRM. All the sections were initially stained with Hematoxylin and Eosin stain. These sections were independently observed under microscope by the two authors for the presence or absence of VI and the findings were recorded on a performa. EVG stained sections of the same blocks were assessed by the same observers for the presence or absence of VI. These assessments were done on different days to minimize opinion bias. The observers were completely blinded to the findings of each other on H&E and EVG stained sections.

VI in H&E stained sections was defined as the presence of tumour cells in a space which is encased by smooth muscles and/or containing RBCs and is usually lying close to an artery. For EVG, VI was said to be present when tumour cells were seen in a space surrounded by elastic fibers in a vicinity of an artery. VI was assessed irrespective of its intramural or extramural location. Multifield microscope was used and a joint consensus was developed in cases where there was disagreement of opinion between the two observers. χ² test was used to compare the incidence of observed VI in CRC using H&E and EVG stains.

Results

The mean age of the study sample was 59.80±19 SD years. Majority of the patients i.e. 53 (53%) were in the range 51–60 years. 52 (52%) patients were male and 48 (48%) were females. Majority of the cases involved ascending colon followed by rectum. Both the observers recorded VI in 32 (32%) cases on H & E staining. The remaining 68 (68%) cases did not show any evidence of VI on H & E stained sections. Sections from all the cases included in the study were than stained with EVG. In EVG stained sections VI was seen in 59 (59%) cases while the remaining 41 (41%) did not show any evidence of VI. All 32 cases showing evidence of VI on H&E also showed VI on EVG stained sections (True positive). Furthermore 27 (39.7%) cases which were initially negative for VI on H&E stained sections also showed evidence of VI in sections stained with EVG (False negative). All the 41 cases which were negative for VI in EVG stained sections were also negative for VI in H & E stained sections (True negative) as shown in table 1. The overall diagnostic accuracy of venous invasion Elastic Stain was 59 % whereas the sensitivity, specificity, positive predictive value, and negative predictive value were 54.24%, 100%, 100%, and 60.29% respectively. These results show that EVG showed evidence of VI in a significant percentage of cases which were reported as negative on H&E stained sections.

<table>
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<tr>
<th>Table 1: Venous Invasion seen on Elastic Stain and H&amp;E</th>
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<tr>
<td>Venous Invasion seen on H&amp;E</td>
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<tr>
<td>Venous Invasion seen on Elastic Stain</td>
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<td>32</td>
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<td>Total</td>
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Sensitivity= 54.24%, Specificity= 100%, PPV= 100%, NPV=60.29%, Accuracy= 59%

Discussion

Detection of VI in CRC is an important negative prognostic factor and its detection can significantly change the management of patients. Several studies including the present study have emphasized upon the role of Elastic stains in detection of VI in CRC.15-17 However there is a marked variation in the reported incidence of VI in CRC.18-21 Moreover the current guidelines do not recommend the routine use of Elastic stains to facilitate the detection of VI in CRC.3

The importance of elastic tissue stains for the detection of VI in CRC has been endorsed by several authors.3 Inoue et al compared elastic stained sections with adjacent H&E sections and found that elastic (EVG) tissue stain significantly increased the rate of detection of observed venous invasion especially in patients with hematogenous metastases.25 In these cases, the incidence of VI detected by H&E was 31% which significantly increased to 81% when EVG stained sections were examined. Vascular invasion and the development of subsequent haematogenous metastases were not significantly correlated when cases were stained only by H&E were analyzed. However, analysis of results of elastic stained sections revealed a significant correlation.

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between them. The results of two previous studies have also shown that elastic stains are superior to H&E in detection of VI in CRC. In the first study Sternberg and colleagues found 19% increase in detection of VI in stage IV colorectal cancers when elastic (Weigert’s) stained sections were examined as compared to H&E stained ones. In the second study Vass et al reported a 30% overall increase in detection of VI in CRC when elastic (Van Gieson) stained sections were examined and compared with H&E stained sections. They also reported an increase of 29% and 19% in detection of intramural and extramural VI respectively with the use of elastic tissue stain. In a study done by M Abdulkader et al the sensitivity of detection of VI in CRC increased from 35.5% (with H&E) to 46.2% (with EVG). They also showed that use of Elastic stain enhanced the detection of VI particularly in Dukes A and B tumors (33% of Dukes A and B tumors were found to have VI in elastic tissue (EVG) stained sections as compared to 21.5% in H&E stained sections). In a study of 92 cases of CRC the observed incidence of VI increased by 44% when Elastic stained sections were examined. A recent study has emphasized upon the use of Elastic stain to minimize interobserver variability in detection of VI in CRC among GI and Non GI pathologists. In the present study VI was seen in 27 cases which were originally negative on H&E.

Prognostic importance of intramural VI is under debate and recent guidelines of Royal College on CRC have declared it mandatory to assess and record all levels of vascular invasion, including submucosal, intramural and extramural. Several articles have emphasized upon the prognostic relevance of extramural VI only. Intramural vessels are smaller, thinner, and have less smooth muscle than their extramural counterparts. They are also found in the middle portion of the tumour and not at edge of it, so detection of their invasion by tumor can be easily missed when using only an H&E stain. The elastic stain was particularly helpful in these situations. We, however, combined extramural and intramural VI for the statistical analysis, because we were most interested in an overall comparison between these methods in terms of identifying any form of VI.

Conclusion

There is very little, if any, trend of specialist reporting in Pakistan and very few institutions are doing it to some extent. Cases of CRC are randomly reported by all Pathologists. Use of Elastic stains can significantly aid in detection of VI in CRC by a general histopathologist sitting in a remote setting. These stains are cost effective and less time consuming and therefore should be incorporated in guidelines for reporting cases of CRC so that this important prognostic indicator can be reported with accuracy.

Conflict of Interest
This study has no conflict of interest to declare by any author.

References