Indocyanine green guided sentinel lymph node biopsy may have a high sensitivity for early (T1/T2) colon cancer: A prospective study in Indian patients

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ABSTRACT

Objective: Indocyanine green (ICG) dye guided near infrared fluorescence (NIR) imaging is a promising tool for mapping lymphatics. The aim of this study was to evaluate the role of ICG guided SLN biopsy in Indian colon cancer patients.

Material and Methods: Forty-eight patients of clinically staged T1-T3 node negative colon cancer underwent laparoscopic/open resection. Patients received colonoscopic peritumoral submucosal ICG injections for laparoscopic (n= 32) and subserosal injections for open resections (n= 16) followed by the detection of SLN using NIR camera. SLNs underwent conventional hematoxylin and eosin (H & E) staging with additional serial sectioning and immunohistochemistry for pancytokeratin antibody (ultra-staging). Detection rate and upstaging rate were the primary end points.

Results: Forty-eight patients were recruited. An average of 2.08 ± 1.27 SLNs were identified in 45 patients at a mean time of 8.2 ± 3.68 minutes with a detection rate of 93.75%. Mean age and mean BMI were 59.7 ± 12.54 years and 24.8 ± 4.09 kg/m^2, respectively. Eighteen patients had node positive disease, and SLN was false negative in four of these patients resulting in a sensitivity of 77.77% with a trend towards higher sensitivity for T1-T2 tumours (90% vs. 62.5%, p= 0.068). Upstaging rate was 10%. Negative predictive value (NPV) and accuracy of the procedure were 87.09% and 91.11%, respectively.

Conclusion: ICG guided SLN biopsy can identify metastatic lymph nodes in colon cancer patients that can be missed on H & E staging with relatively higher sensitivity for early (T1/T2) tumours.

Keywords: Sentinel lymph node, colorectal neoplasms, Indocyanine green

INTRODUCTION

Colorectal cancer is the third most common cancer diagnosed in both men and women in the United States and the third leading cause of cancer death in the world (1). The strongest predictive factor for patient survival in patients with colon cancer is lymph node metastasis (2). It has been estimated that 20-30% patients with early-stage node-negative disease will develop distant metastasis despite adequate surgical resection (3). One of the reasons for recurrence in pathologically node-negative patients could be missed micrometastasis and occult tumor cells on routine histopathological examination or inadequate lymph node harvesting leading to understaging (4). Ideally, all harvested lymph nodes should undergo serial sectioning and immunohistochemistry (IHC) routinely to detect these occult metastasis but they are time consuming and expensive (5). Hence, it is not part of routine pathological evaluation.

Sentinel lymph node (SLN) biopsy technique has been described in colon cancer to detect micrometastasis (MM) (6). Near-infrared (NIR) fluorescence imaging for SLN metastasis has been recently used for SLN biopsy with encouraging results (7,8). NIR-fluorescence imaging has high penetration depth and real-time optical guidance which is useful in identifying lymph nodes that are located in unfavorable locations beneath fatty mesocolon (9).

Previous published studies have elucidated the role of SLN biopsy in Caucasian and East Asian patients (6,10). To the best of our knowledge, this is the first study in a south Asian population. This study was conducted to evaluate the role of SLN biopsy using NIR-fluorescence in colon cancer patients with respect to detection rate, upstaging rate, frequency of aberrant lymph node drainage, accuracy, and sensitivity of the SLN biopsy procedure.

MATERIAL and METHODS

Study Design

A single arm prospective cohort study was conducted at a tertiary referral centre. Colon cancer patients who met the eligibility criteria were enrolled from June, 2020 to June, 2022. The study was approved by the Institute Ethics Committee (Ref. No. AIG/IEC-Post BH & R 02/12.2019/ER-01; 10 January, 2020) and was prospectively registered with the clinical trials registry (NCT04351009). The study was HIPAA compliant and adhered to the tenets of Declaration of Helsinki. A written informed consent was obtained from each patient prior to the enrolment.

Biopsy proven colon cancer patients with age at least 18 years old who were scheduled for elective laparoscopic/open colectomy were recruited. Patients had to be willing to provide oral and written informed consent.

Exclusion criteria included patients with prior colorectal surgery, gross lymph node involvement or tumour infiltration on preoperative imaging or intraoperative staging, history of allergy to iodine containing compounds, indocyanine green or human albumin, history of hyperthyroidism or thyroid adenoma, patients undergoing purely palliative surgery and patients with advanced renal or hepatic insufficiency.

Perioperative Intervention

Exploration of the abdominal cavity was performed to search for any metastatic spread in both open and laparoscopic approaches after induction of anesthesia. Subsequently, patients underwent on-table colonoscopy for laparoscopy cases. All laparoscopic cases received colonoscopic submucosal injections and all open cases received subserosal injections. Twenty-five mg indocyanine green dye (Aurogreen, Aurolab, Madurai, India) diluted in 1 mL of 20% albumin solution and 9 mL of 0.9% normal saline solution was used as described by Ankersmit et al (6). Injections (0.5-1 mL) were given in the submucosa at 2-4 points around the tumor. After colonoscopic injection, excess ICG was washed and suctioned from the lumen. Similarly, subserosal injections were given, the injection sites were pressed with sterile swabs to prevent spillage. Dissection was avoided to prevent damage to the lymphatics. After the injection, a rigid NIR scope (WA53000A, Olympus Medical Systems Corp, Tokyo, Japan) coupled to a laparoscopic NIR camera system (VISERA ELITE II, Olympus Medical Systems Corp., Tokyo, Japan) was used to examine the colon and mesocolon. This system illuminated the target organ with a near-infrared light of 760-780 nm wavelength resulting in emission of ICG fluorescence at wavelengths of 800-850 nm which was detected by the camera system in normal white light mode, fluorescent mode and onlay mode. The first lymph node or group of lymph nodes to show fluorescence were considered the sentinel lymph node. The site of sentinel lymph nodes was determined according to the Japanese Society for Cancer of the Colon and Rectum classificaiton (11). D1 lymph nodes were defiend as lymph nodes along the marginal aretery (paracolic/epicolic), D2 lymph nodes along named tumor bearing arteries (intermediate) and D3 lymph nodes along the origin of main artery (central). All sentinel nodes were marked with laparoscopic titanium clips or 3-0 silk sutures. All sentinel lymph nodes outside the planned resection margins were underwent excision biopsy
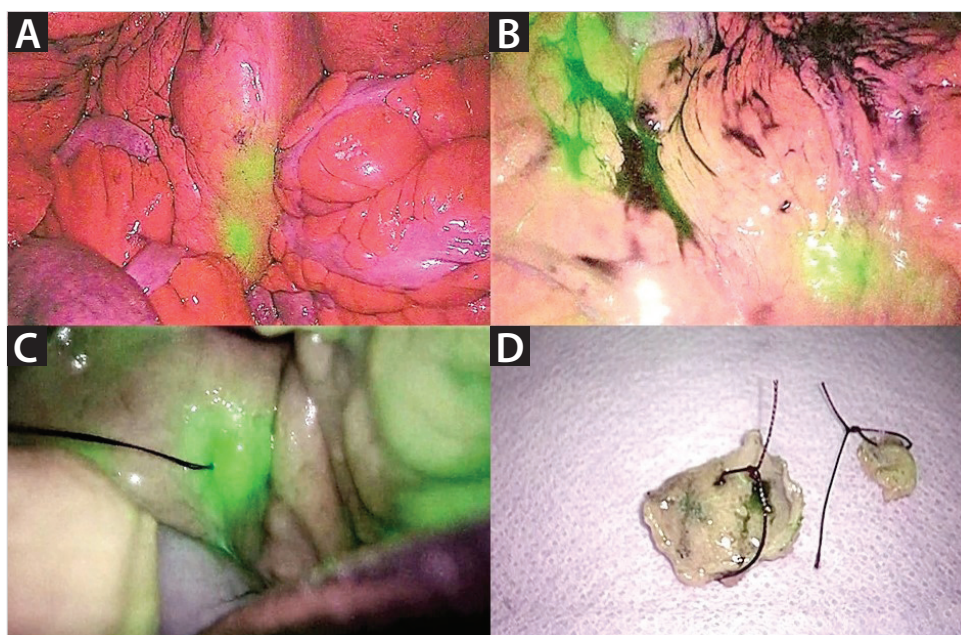

Figure 1. Intermediate sentinel lymph nodes in case of carcinoma sigmoid **(A)**, intermediate sentinel lymph nodes along the middle colic artery in a case of carcinoma hepatic flexure of colon **(B)**, a sentinel lymph node marked with 3-0 silk sutures **(C)**, excised sentinel lymp nodes **(D)**.

but the resection margins were not modified (Figure 1A-D). Standard oncological resections with medial to lateral vessel first approach with high arterial ligation was done in all patients irrespective of laparoscopic or open access.

Pathological Analysis

After resection of the specimen, the tagged lymph node(s) were excised and sent separately as SLN (Figure 1D). Non-sentinel lymph nodes were examined by a central section with H & E staining. All labelled sentinel lymph nodes were processed separately. Lymph nodes less than 5 mm in diameter were embedded entirely in toto whereas larger lymph nodes (>5 mm) were sectioned in slices up to maximally 3 mm and processed to paraffin blocks for hematoxylin and eosin staining. If none of sentinel lymph nodes showed metastasis on initial H & E staining in the presence on negative non-sentinel lymph nodes, the paraffin blocks underwent stepwise sections at intervals of 150 micron-meter. At each level, at least three serial sections were cut at 5 um thickness and one section underwent H & E staining. This was followed by immunohistochemistry with pan-cytokeratin antibody on the other sections if no metastasis was identified on standard H & E staining.

As per the American Joint Committee on Cancer (AJCC) eighth edition, micrometastasis were defined as clumps of tumor cells ≥0.2 mm and <2 mm in diameter or clusters of 20 or more tumor cells. Detection of single cells or clumps of tumor cells <0.2 mm were described as isolated tumor cells (12). Patients with micrometastasis were considered SLN positive. All collected data were entered into a computerized database and processed for statistical analysis. After computing the true positive (TP), true negative (TN), false positive (FP), false negative (FN) sentinel nodes, the sensitivity, accuracy, negative predictive value, upstaging rate and detection rate were calculated.

Outcome

Primary outcome was detection rate which was defined as proportion of successful SLN procedures divided by all executed SLN procedures, upstaging rate and secondary outcomes were sensitivity, accuracy, negative predictive value, and frequency of aberrant lymph node drainage. Number of true positives in patients with positive histopathological findings (TP/TP + FN) was defined as sensitivity. Accuracy was defined as (TN + TP/TN + TP + FP + FN) to calculate the number of times the nodal state was correctly predicted by SLN biopsy. Negative predictive value (TN/TN + FN) was defined as number of times a negative SLN correctly predicted the negative lymph node status of the patient. Upstaging rate was defined as number of patients who turned node positive after advanced histopathology and IHC of patients who were node negative on conventional histopathology.

Statistical Analysis

Statistical analysis-The data for the study was collected using structured pro forma. The results were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) and for continuous variables. The categorical variables were expressed as % frequency distribution. Fisher's exact test was used for categorical variables. A p value <0.05 with two tailed test would be considered as statistically significant. The analysis was carried by using statistical package for social sciences (SPSS 20th version). Proportion test and MedCalc was used for outcomes analysis between T1/T2 and T3/T4 groups.

RESULTS

Total 136 patients of carcinoma colon presented to our institute during the study period i.e June, 2020 to June, 2022. Of these 136 patients, 24 patients had metastatic disease precluding curative resection, 64 patients had locally advanced disease with gross lymph node invasion on preoperative imaging or intraoperative staging and were excluded from the study.

Forty-eight patients were recruited. Thirty-two patients underwent colonoscopic ICG injection during laparoscopic resection and 16 patients underwent subserosal injecitons during open resections. SLN could not be detected in three patients. Two patients had intraperitoneal spillage of the dye leading inability to identify the lymph nodes and one patient had a very fatty mesocolon. Therefore, detection rate was 93.75%. The demographic and pathological characteristics are depicted in Table 1. The location of primary tumour was sigmoid colon (24%), ascending colon (20%), caecum (16%), hepatic flexure (16%), transverse colon (11%), splenic flexure (9%) and descending colon (4%). The most common T stage was T3 (44.44%), followed by T2 (42.22%), T1 (11.11%) and T4a (2.22%). N staging was N0 (67%), N1a (7%), N1b (11%), N2a (11%) and N2b (4%).

Thirty patients were node negative (pN0) i.e. TN after conventional histopathological examination. Advanced histopathological examination and IHC revealed micrometastasis in three patients who were upstaged and considered as node positive. The upstaging rate was 10% (three in 30 patients) (Figure 2).

Table 1. Demographic and procedural characteristics of the study population

n= 45	Mean (SD)
Age (yrs)	59.7 (12.54)
Sex (M/F)	62.2%/37.8%
BMI (kg/m^2)	24.8 (4.09)
Total no of LN	20.2 (10.06)
Total no SLN	2.08 (1.27)
Time to imaging (mins)	8.2 (3.68)

Figure 2. Overview of the pathological analysis.
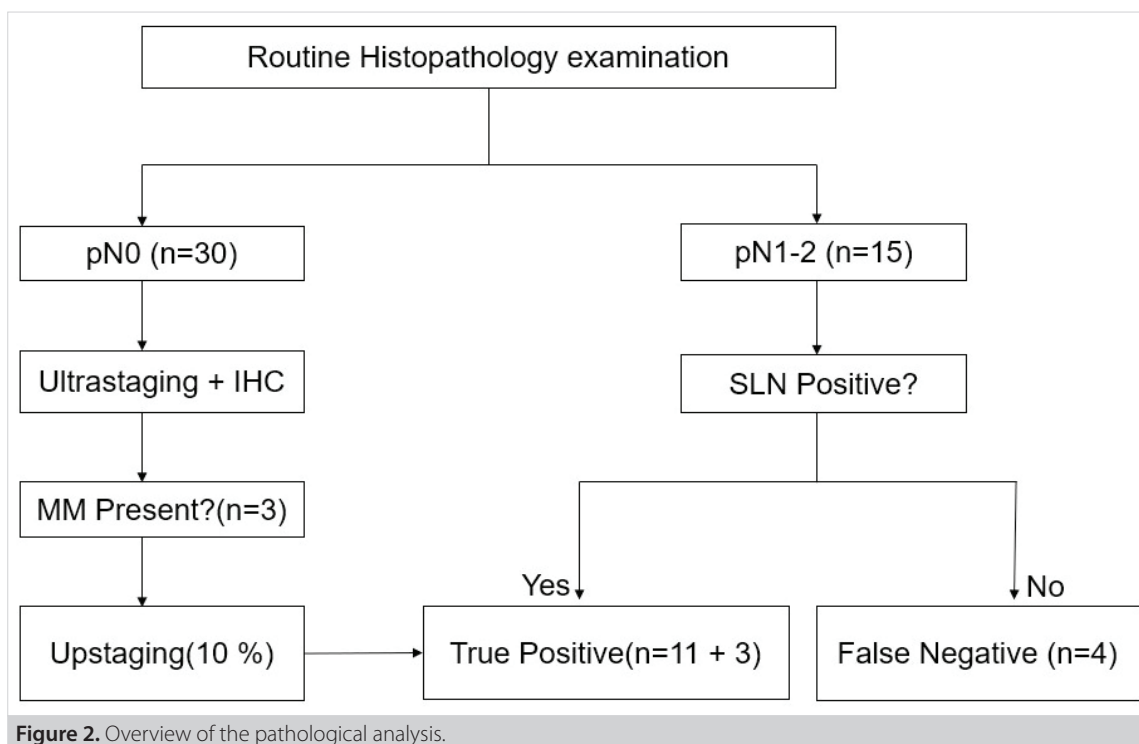
Table 2. Outcomes in early (T1/T2) versus late (T3/T4) tumours			
	T3/T4 (n=21)	**T1/T2 (n=24)**	**P**
Sensitivity	62.5%	90%	0.068
Accuracy	85.71%	95.85%	0.508
Negative predictive value	81.25%	93.33%	0.439
Upstaging rate	7.41%	11.76%	0.586

Total no of patients with positive SLN (TP) was 14 (11 on conventional H & E staining and three patients on ultrastaging and IHC). SLN was negative in four (FN) with positive non-sentinel lymph nodes. In 27 patients (TN), both the non-sentinel lymph nodes and sentinel lymph nodes were negative even after ultrastaging and IHC. Therefore, the sensitivity, negative predictive value, and accuracy of the procedure in our study were 77.77% (14/18), 87.09% (27/31) and 91.11% (26/30), respectively. Specificity and positive predictive value for the procedure was 100% as there were no false positive patients in the study.

There was no significant difference in sensitivity another outcome measures between the submucosal (n= 30) and subserosal groups (n= 15). There was a non-significant trend towards higher sensitivity in early (T1/T2) tumour when compared to late (T3/T4) tumours as depicted in Table 2.

One patient with a hepatic flexure carcinoma had an aberrant lymph node drainage at the splenic flexure paracolic node which was harvested separately but non-metastatic on pathological examination, ultrastaging and IHC. Hence, the aberrant lymph node drainage rate was 2.22%.

DISCUSSION

In this study we evaluated role of sentinel lymph node biopsy by indocyanine green in patients with carcinoma colon who underwent oncological resections with curative intent.

In our study the detection rate was 93.75%. SLN could not be detected in three patients due to intraperitoneal spillage of dye and fatty mesocolon. Previously, Anderson et al. had described intraperitoneal spillage of dye during sub-serosal injections but did not attribute it to cause false negative lymph nodes (13). Currie et al. have also described intraperitoneal spillage of dye during colonoscopic submucosal injections (14). In our study, the intraperitoneal spillage can be attributed to the learning curve involved in mastering the colonoscopic sub-mucosal injection technique. Multiple authors have described a learning

curve of 5-30 cases for ICG injection (15-17).

Previous meta-analysis has not shown any difference between submucosal and sub-serosal techniques of ICG injection (18,19). On the contrary, Ankersmit et al. in their single centre study found the detection rate higher with submucosal injection and opined that uptake of dye by tumour draining lymphatics is more efficient after submucosal injection (6). Carrara et al. in their study of 95 patients with non-metastatic colorectal cancer described a detection rate 96.8% with peritumoral laparoscopic injections (20). There was no significant difference in primary and secondary outcomes between the submucosal and subserosal techniques during our study. Our study was not designed or adequately powered to evaluate differences between the two techniques. Additionally, the subserosal injections were given in open surgeries where tactile feedback helped in correct positioning and limiting spillage compared to a previous study where laparoscopic access was used.

The mean time to detection was 8.2 ± 3.68 minutes and the average number of SLN identified was 2.08 ± 1.27. A larger number of sentinel lymph nodes identified during the procedure is undesirable but analysing lymph nodes with serial sections and IHC is expensive and time-consuming. Watanabe et al. studied 31 patients of carcinoma splenic flexure of colon with 2.5 mg ICG peritumoral submucosal injections and observed lymph flow after 30 minutes resulting in a very high SLN yield of 10.4 ± 4.73 which is undesirable (10). Therefore, it has been opined that lymphatic flow should be followed in real time after ICG injection to minimize the number of lymph nodes identified (6).

The upstaging rate in our study was 10% (three in 30 patients). Our upstaging rate is comparable to a recently published meta-analysis which had shown a pooled upstaging rate of 15% among five high quality studies (range 6% to 23%) (6). On the other hand a recently published study from Italy of 95 patients had an upstaging rate of only 1.08% (1 in 95 patients) (20). Desguetz et al. in their meta-analysis of 1794 patients (1201 colon, 332 rectum) in 33 studies found a micrometastasis rate of 9% (18,21).

The sensitivity, negative predictive value, and accuracy of the procedure in our study was 77.77%, 87.09% and 91.11%, respectively. Overall sensitivity in our study was relatively lower in our study at 77.77% due to the high number of T3/T4 tumours (46.66%) in the study population. Emile et al. conducted a meta-analysis of 12 studies with 248 patients where the median sensitivity and accuracy rates were 73.7% and 75.7% respectively with a pooled sensitivity and specificity of 71% and 84.6% (18). The percentage of patients with early-stage CRC varied among the studies from 30 to 100% (median= 41%). In six studies, patients with early-stage tumors comprised less than 50% of the sample size and the median sensitivity, specificity, and accuracy rates were 76%, 87.2%, and 68.8%, respectively which was relatively lower than studies with >50% early-stage tumors.

Sensitivity for T1/T2 tumours was 90% which is comparable to other published literature. Other authors have also opined that SLNB procedure has better sensitivity for early stage procedures than advanced carcinoma (93.1% vs. 58.8%) (22,23).

In vivo approach for SLN mapping can help to identify aberrant lymph node drainage. Some authors have used it to modify the mesocolic resection margins (10,13,24,25). We identified aberrant lymph node drainage in one (2.22%) patient in our study.

Additionally, in vivo SLN approach in early tumour may enable us to identify patients who might benefit from a local segmental excision if the SLN are negative for metastasis thus decreasing the morbidity associated with extensive resection (26). In the future, well planned large sample size randomized controlled trials should be done to address this issue.

The limitations of our study were the small sample size with relatively higher number of advanced lesions.

CONCLUSION

ICG guided sentinel lymph node biopsy in colon cancer is a promising tool to enable clinicians identify patients with lymph nodal metastasis in colon cancer. It has a high sensitivity for early (T1/T2) patients in the Indian population which confirms the findings of previous publications. Early (T1, T2) colon cancer patients might benefit from upstaging and subsequent adjuvant in this setting. Additionally, limited segmental resections might be considered for early tumours which are sentinel node negative. Further trials are needed to confirm this hypothesis.

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İndosiyanin yeşili rehberliğinde sentinel lenf nodu biyopsisi erken (T1/T2) kolon kanseri için yüksek duyarlılığa sahip olabilir: Hintli hastalarda prospektif bir çalışma

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ÖZET

Giriş ve Amaç: İndosiyanin yeşili (ICG) boya rehberliğinde yakın kızılötesi floresan (NIR) görüntüleme, lenfatikleri haritalamak için umut verici bir araçtır. Bu çalışmanın amacı, Hintli kolon kanseri hastalarında ICG rehberliğinde SLN biyopsisinin rolünü değerlendirmektir.

Gereç ve Yöntem: Klinik olarak evrelenmiş T1-T3 düğümü negatif kolon kanseri olan 48 hastaya laparoskopik/açık rezeksiyon uygulandı. Hastalara laparoskopik (n= 32) için kolonoskopik peritümöral submukozal ICG enjeksiyonları ve açık rezeksiyonlar için subserozal enjeksiyonlar (n= 16) yapıldı, ardından NIR kamera kullanılarak SLN saptandı. SLN'lere ek seri kesitleme ve pansitokeratin antikoru için immünohistokimya (ultra evreleme) ile geleneksel hematoksilen ve eozin (H & E) evrelemesi uygulandı. Tespit oranı ve ileri evreleme oranı birincil son noktalardı.

Bulgular: Kırk sekiz hasta çalışmaya alındı. Kırk beş hastada, ortalama 8,2 ± 3,68 dakikada ortalama 2,08 ± 1,27 SLN saptandı ve tespit oranı %93,75 idi. Ortalama yaş ve ortalama VKİ sırasıyla 59,7 ± 12,54 yıl ve 24,8 ± 4,09 kg/m^2 idi. On sekiz hastada nod pozitif hastalık vardı ve bu hastaların dördünde SLN yanlış negatifti ve T1-T2 tümörleri için daha yüksek duyarlılığa doğru bir eğilimle birlikte %77,77'lik bir duyarlılıkla sonuçlandı (%90'a karşı %62,5, p= 0,068). Evreleme oranı %10 idi. Negatif prediktif değer (NPV) ve işlemin doğruluğu sırasıyla %87,09 ve %91,11 idi.

Sonuç: ICG rehberliğinde SLN biyopsisi, kolon kanseri hastalarında erken (T1/T2) tümörler için nispeten daha yüksek hassasiyetle H & E evrelemesinde gözden kaçabilen metastatik lenf nodlarını belirleyebilir.

Anahtar Kelimeler: Sentinel lenf nodu, kolorektal neoplazmalar, İndosiyanin yeşili