

Case report

Identification of extrahepatic metastasis of hepatocellular carcinoma using indocyanine green fluorescence imaging

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ABSTRACT

Background/purpose: Intraoperative fluorescence imaging with indocyanine green (ICG) plays a significant role in the identification of hepatocellular carcinoma (HCC) during Hepatectomy. Despite that, few researchers have reported using ICG fluorescence imaging to detect extrahepatic metastases. Herein, we report the successful application of ICG fluorescence imaging in the detection of extrahepatic metastatic lesions from primary liver cancer.

Methods: In two HCC patients, a near-infrared fluorescence imaging system was used to intraoperatively examine the abdominal cavity for implantation metastasis after hepatectomy of the primary tumor, following preoperative intravenous infusion with ICG.

Results: In the first case, the abdominal cavity implantation metastasis exhibited intense fluorescence and clear boundaries of tumors during abdominal tumor resection, and there was no fluorescence in the resection margin. In the second case, a new lesion that was not detected by preoperative imaging examination was found in the omentum by ICG fluorescence imaging. Intraoperative rapid freezing pathology showed that it was an adenocarcinoma. Accordingly, we revised the tumor staging.

Conclusion: ICG fluorescence imaging can not only detect intrahepatic metastases, but also extrahepatic metastases. Furthermore, it can help surgeons correct tumor staging during surgery.

1. Introduction

Hepatocellular carcinoma (HCC) is a common malignancy worldwide that is closely associated with chronic viral hepatitis infection or exposure to toxins such as alcohol or aflatoxin. Hepatic resection remains an effective method to radically treat different liver carcinomas [1]. However, about 70–80% of HCC patients who receive curative resection will suffer a relapse within five years after the operation [2,3,4]. The early recurrence rate is high, possibly due to the small extrahepatic metastases which escape detection by current detection methods used before and during operation. Due to its concealment and to invasive growth in other organs, it is very difficult to reoperate. Nevertheless, effective intraoperative detection may change this situation during reoperation.

Indocyanine green (ICG) is a water-soluble compound that can bind tightly to blood plasma proteins immediately after being injected to the human bloodstream to become confined to the vascular system. Most of

the injected ICG is taken up by hepatic parenchymal cell, followed by excretion into the bile, thus avoiding its metabolization. Recent research has paid increasing attention to the role of ICG in the identification of HCC during hepatectomy. To date, to the best of our knowledge, there have only been two reports describing the use of ICG fluorescence imaging in the detection of extrahepatic metastasis of hepatocellular carcinoma and hepatoblastoma [5,6]. However, whether this imaging technique can detect abdominal cavity implantation metastasis of primary liver cancer postoperatively remains unclear. Moreover, only a few studies have been reported using the ICG fluorescence imaging technique to detect extrahepatic metastases. Herein, we report the successful application of ICG fluorescence imaging in the detection of extrahepatic metastasis from primary liver cancer.

2. Materials and methods

This study was approved by the Institutional Review Board of the

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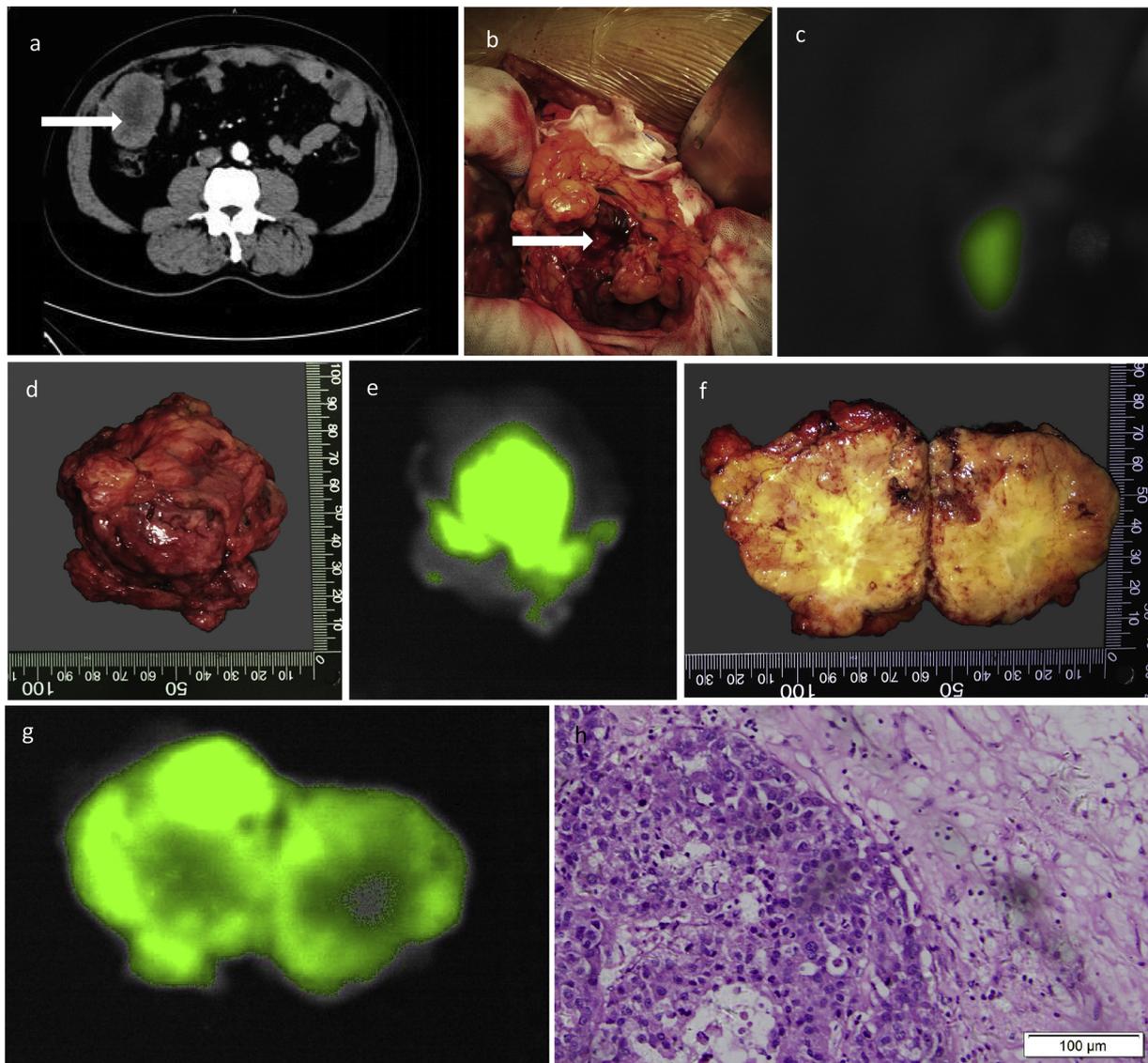


Fig. 1. Case 1: The Computed tomography (CT) scan, together with ICG fluorescence imaging show the gross appearance of the abdominal mass. a: CT scan, the tumor was located in the right middle and lower abdominal region, and appeared to be resectable. b: The gross appearance of the abdominal mass. c: Fluorescence imaging before removal of the abdominal mass enabled the visualization of the tumor lesion, and provided a clear line. d and g: An image of the resected specimens. e and g: ICG fluorescence imaging technique reveals a bright portion with fluorescence signal in the resected specimens of the abdominal mass. h: An image of the lesion section stained with hematoxylin and eosin (H&E) dye, showing the conformable liver cancer metastasis.

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ICG fluorescence imaging was used to detect abdominal cavity implantation metastasis and primary tumor lesions in two patients. The fluorescent dye ICG (Dandong Medical and Pharmaceutical Co, Ltd. Dandong, China) was injected intravenously (i.v.) at a dose of 0.5 mg/kg at 72–96 hours prior to surgery. Informed consent was obtained from the parents of the patients prior to operation. A near-infrared light camera system provided by the Key Laboratory of Molecular Imaging in Beijing, China was used for the ICG fluorescence imaging. This system uses light with an emission wavelength of 785 nm and filters out light of wavelength smaller than 840 nm, which was detected with a near-infrared light (NIL) camera.

Prior to the lesion resection, the ICG-NIL camera system was used to observe the suspicious sites with the head of the camera 30–50 cm away from the suspicious sites. Following the resection of the lesion, the ICG-NIL camera was used to examine whether there was any fluorescence in the resected site or surgical specimens. All specimens after resection were submitted to perform pathological examination.

3. Results

3.1. Case 1: a abdominal cavity implantation metastasis after hepatectomy

A forty-six-year-old male, who had undergone hepatectomy and splenectomy due to liver cancer, was readmitted with a middle and lower abdominal mass. As can be seen in the computed tomography (CT) image shown in Fig. 1a, a huge mass is present in the right middle and lower abdominal region at presentation. After liver surgery, the mass size was decreased from 9.0×7.0 cm (Fig. 1d).

We then performed an exploratory laparotomy and resection of the abdominal mass using the fluorescence ICG imaging technique. The procedure of surgery was as follows: First, after entering the abdomen layer by layer according to the predetermined surgical incision, the location of the tumor was preliminarily determined to be in the right lower abdomen by palpation and naked eyes, but the boundary between the tumor and normal tissue could not be accurately established (Fig. 1b). Second, intense fluorescence was observed in the viable tumor tissue. Additionally, fluorescence imaging quickly showed the

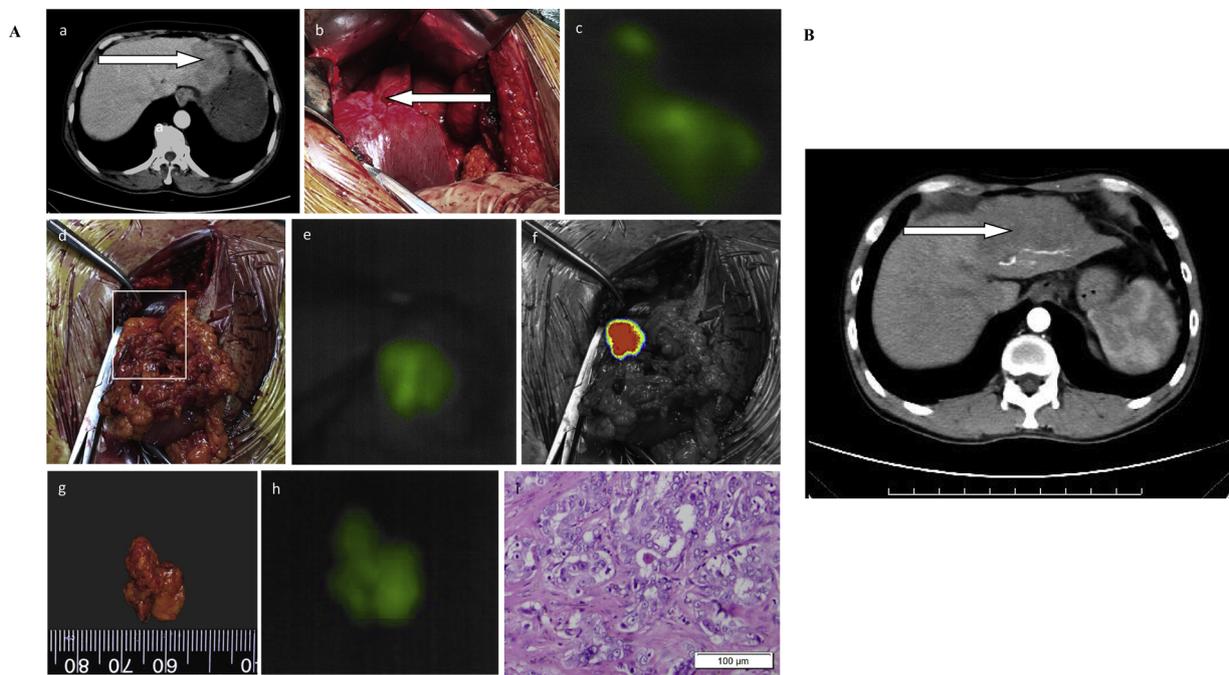


Fig. 2. Case 2, A : The CT scan, together with ICG fluorescence imaging show the gross appearance of the liver tumor lesions. a: CT scan, the tumor was located in the left lobe of the liver with no lesions found on the omentum and could be resected via the left lobectomy. b: Gross appearance of the tumor on the left liver lobe. c: Fluorescence imaging before removal of left liver tumor enabled the visualization of the tumor lesion, which provided a clear tumor margin by observing the fluorescence signal. d, e: An image of a new lesion detected by ICG fluorescence imaging, where no tumor was previously found by visual inspection. f: The overlay images of a new single nodule. g: An image of the resected specimens. h: ICG fluorescence imaging technique reveals a bright total fluorescence signal in the resected specimens of extrahepatic metastasis. i: An image of the extrahepatic metastasis lesion section stained with H&E dye, adenocarcinoma. B: The CT scan at three months after surgery: The tumor on the left liver lobe was markedly enlarged.

location and clear boundaries of tumors during the operation (Fig. 1c). As a result, the operation time was greatly shortened. Third, according to the tumor boundary established by fluorescence imaging, the resection line, which was about 2 cm away from the tumor boundary, was preliminarily set to ensure that the tumor is completely resected. Fourth, the tumor was removed along the preset line using real time navigation ICG fluorescence imaging. Fifth, after surgery, using ICG fluorescence imaging to detect the cutting edge and the resection margin revealed no fluorescence. Thus, we concluded that the tumor had been completely resected. The subsequent pathological examination showed HCC, conformable liver cancer metastasis, and acquired R0 cut edge (Fig. 1h). The patient has been in complete remission for 1 year after operation, and no tumor recurrence has been observed.

3.2. Case 2: primary liver tumor with an omentum metastasis

A sixty-seven-year-old male was diagnosed with the primary liver tumor. In the CT scan, a big liver mass was observed in the left lobe, but no lesions were found on the omentum (Fig. 2A-a), thus this case was diagnosed as stage B HCC based on Barcelona Clinic Liver Cancer (BCLC) staging system.

Before resecting the left liver cancer lesions, we examined the liver using the ICG fluorescence imaging. Intense fluorescence was observed in the tumor tissue on the left liver lobe, which provided a clear tumor margin by observing the fluorescence signal (Fig. 2A-c). Then, the abdominal cavity was detected by ICG fluorescent imaging. A new lesion, which had not been detected by preoperative imaging examination was found on the omentum by ICG fluorescence imaging (Fig. 2A-e,f). Intraoperative rapid freezing pathology showed that it was an adenocarcinoma (Fig. 2A-i). Accordingly, we revised the tumor stage to C. Additionally, we changed the operation to left hepatic lesion and omentum biopsy. After the operation, on the basis of the pathological examination results, we decided to treat the tumors by Transhepatic

Arterial Chemotherapy and Embolization (TACE), chemotherapy or biologic targeted therapy.

However, after discussing the treatment and prognosis after the surgery with the patient's family, they rejected TACE, chemotherapy and biological targeted therapy for economic reasons and decided to use an adjuvant treatment with An Kang Xin Jiao Nang (inking), which is auxiliary drug for the treatment of lung cancer, stomach cancer, liver cancer and other tumors (Made by Anhui Gaoshan Pharmaceutical Co. Ltd., Anhui, China). After 3 months, the patient was reexamined by CT, and the tumor was significantly enlarged (Fig. 2B), and the alpha fetoprotein was > 1000 ng/ml. After 6 months, we learned that the patient had died by phone.

4. Discussion

ICG is considered a safe reagent and has been approved for use in clinical practice to determine hepatic function, liver and gastric blood flow, cardiac output, etc. In the field of liver surgery, the role of ICG fluorescence imaging in the detection of HCC or metastases has been widely demonstrated [7,8]. To the best of our knowledge, the detection of metastases is mostly limited to intrahepatic metastases, and only a few studies have been reported using the ICG fluorescence imaging technique to detect extrahepatic metastases, such as Atsushi Nanashima et al. [9] found tumor thrombus, right adrenal infiltrating lesion and lymph node metastasis of hepatocellular carcinoma by ICG fluorescence imaging. However, most of these metastases are adjacent to primary cancer lesions or direct tumor invasion. So, its value in clinical application for the detection of extrahepatic metastases is still unclear. This study mainly aimed to demonstrate the potential application of ICG fluorescence imaging in the detection of extrahepatic metastases during operation.

To the best of our knowledge, the effectiveness of ICG fluorescence imaging in the detection of HCC is determined by whether the ICG can

be cleared by liver cells [10]. Relying on the imaging technique, impaired bile excretion can be visualized in or surrounding the tumor tissues. In our cases, intense fluorescence was observed in both the viable tumor tissue in abdominal cavity and the postoperative specimens during the operation, such as in case 1. However, no fluorescence was detected in the resection margin, which was applicable to both the primary tumor and recurrent tumor, providing additional evidence and information on the absorption and metabolism of ICG. In addition, it can not only shorten the resection time of extrahepatic metastatic foci, but also guide the complete resection of the tumor, such as in case 1. At the same time, it can detect small extrahepatic metastatic lesions that were not detected by preoperative imaging examination. Moreover, it can also guide the tumor staging process, such as in case 2. These findings showed that ICG fluorescence imaging is not only conducive to the detection of intrahepatic metastases, but also can be potentially applied in the detection of small extrahepatic metastases missed by preoperative imaging. Among the advantages is that, on the one hand, ICG fluorescent imaging can be employed to explore the extrahepatic metastasis, and, on the other hand, to intraoperatively diagnose a lesion and staging found accidentally. Whether there is a metastatic lesion in the resected specimen can be confirmed instantly after the extirpation.

However, this study has some limitations. The main limitation is that this is a case report study, with very few samples and no control study, thus it is difficult to accurately evaluate the long-term survival effect of surgical intervention associated with this imaging technique. Second, it exhibits low sensitivity in detecting deeper (10 vs 2 mm) and smaller (11 vs 18 mm) nodules [11]. Third, the fluorescence emitted by the ICG only shows tissue penetration of 10 mm or so [12]. The last limitation is that the large size of the CCD camera restricts its use in surgery of small infants or in laparoscopic/thoracoscopic surgery.

In conclusion, the ICG navigation system has great potential for detecting extrahepatic metastasis from primary hepatocellular carcinoma and guided tumor staging.

Competing interests

The authors declare that they have no competing interests.

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