Int J Clin Exp Pathol 2017;10(5):5189-5196
www.ijcep.com /ISSN:1936-2625/IJCEP0048698

Original Article

Clinicopathological and genetic features of mutant gastrointestinal stromal tumors with rare lymph node metastasis and literature review

Qiuyu Liu[1], Rongfang He[2], Ziguang Xu[1], Fangfang Fu[3], Shengli Zhou[1], Qianqian Lei[1], Shuang Liang[1], Huanzhou Xue[4]

Departments of [1]Pathology, [3]Image, [4]Liver-Gallbladder-Pancreas Surgery, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, Zhengzhou, Henan Province, China; [2]Department of Pathology, The First Affiliated Hospital of University of South China, Hengyang, Hunan Province, China

Received January 12, 2017; Accepted March 14, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Gastrointestinal stromal tumors (GISTs) are the common neoplasms of the gastrointestinal (GI) tract. Liver and peritoneum are the most metastatic sites, but lymph node metastasis is relatively rare. In this study, 6 cases of GISTs with lymph node metastasis were studied and reviewed the literature. The patients (5 males and 1 female) ranged in age from 48 to 75 years (median, 66 y). Primary tumors developed on intestinal (5 cases) and stomach (1 case), and then 5 cases presented synchronous metastases including liver and/or peritoneal cavity. Intraperitoneal lymph node metastases were detected in all cases. On immunohistochemistry (IHC), tumors cell and metastases were diffusely positive for KIT, DOG1, SDHB, most positive for CD34 and focal for SMA. And exon 11 mutation was detected in all cases. 1 patient died of synchronous intrahepatic biliary adenocarcinoma, and other cases received imatinib mesylate had no signs of tumor recurrence (follow-up about 1-30 months). In conclusion, our results suggest that clinicopathological features of GISTs with rare lymph node metastasis is similar to adult mutated GISTs. High grade GISTs with liver metastasis and/or peritoneal cavity spread, especially intestinal GISTs, should keep on high alert for lymph node metastasis. Patients with lymph node metastatic GISTs usually show exon 11 mutation of *KIT* gene and targeted therapy can get a very good control of tumor progression.

Keywords: Gastrointestinal stromal tumors, metastasis, lymph node, *KIT*, mutation

Introduction

Gastrointestinal stromal tumors (GISTs) are the most mesenchymal tumors in the gastrointestinal (GI) tract, which occur primarily in the older adults of either sex. They could present anywhere along the GI tract but are most commonly located in the stomach (about 60%), jejunum and ileum (about 30%) [1]. For the advanced GISTs, local recurrence and metastasis are frequently observed after adequate resection and adjuvant therapy with tyrosine kinase inhibitor (TKIs). The distribution of metastasis is predictable, with the liver and peritoneum the most common sites. In addition, metastasis to the lung, bone and lymph node is relatively rare [2, 3]. Although a few numbers of reports have been searched lymph node metastasis (LNM) originating from GISTs, the true prevalence remains unknown. In this study, we report 6 mutant GISTs with LNM and examine their pathology, prognosis, and genetic features.

Materials and methods

All of the GISTs cases are retrieved from the medical records and database of Henan Provincial People's Hospital and the First Affiliated Hospital of University of South China in China from 2008 to 2016. We found 6 cases of lymph node involvement among 4100 patients (6/4100, 0.14%). Diagnose were confirmed on the review of the hematoxylin and eosin stained sections by three of the authors before inclusion in the study. In the course of the reviewing, several features were specifically analyzed. These included size, morphology pattern, location, mitotic index and LNM. On the other hand,

Table 1. Clinical features of GIST with LNM

Case	Age/Gender	Location	Location of LNM	Number of LNM	Site of metastasis	Synchronous disease	Treatment	Fellow-up (Status)
No. 1	72/F	Small intestine	Mesenteric lymph node	1+ve LN	No	Primary intrahepatic biliary adenocarcinoma with 2+ve LNM	SE, CT, RT	5 mo (DOD)
No. 2	48/M	Jejunum	Mesenteric lymph node	1+ve LN	Liver, omentum, mesentery	No	SE, IMA	30 mo (AWD)
No. 3	75/M	Duodenum	Mesenteric lymph node	4+ve LN	Liver, mesentery	No	CBN, SE, IMA	20 mo (AWD)
No. 4	60/M	Small intestine	Mesenteric lymph node	1+ve of 11 LN	Left liver, mesentery, abdominal wall	No	SE, IMA	10 mo (AWD)
No. 5	73/M	Stomach	Lymph node in hepatogastric space	1+ve LN	No	No	SE, IMA	1 mo (AWD)
No.6	49/M	Small intestine	Mesenteric lymph node	1+ve of 2 LN	Mesentery	No	SE, IMA	20 mo (AWD)

NA, data not available; LNM, lymph node metastasis; SE, surgical excision; CNB, coarse needle biopsy; CT, chemotherapy; RT, radiotherapy; IMA, imatinib; DOD, dead of disease; AWD, alive with disease.

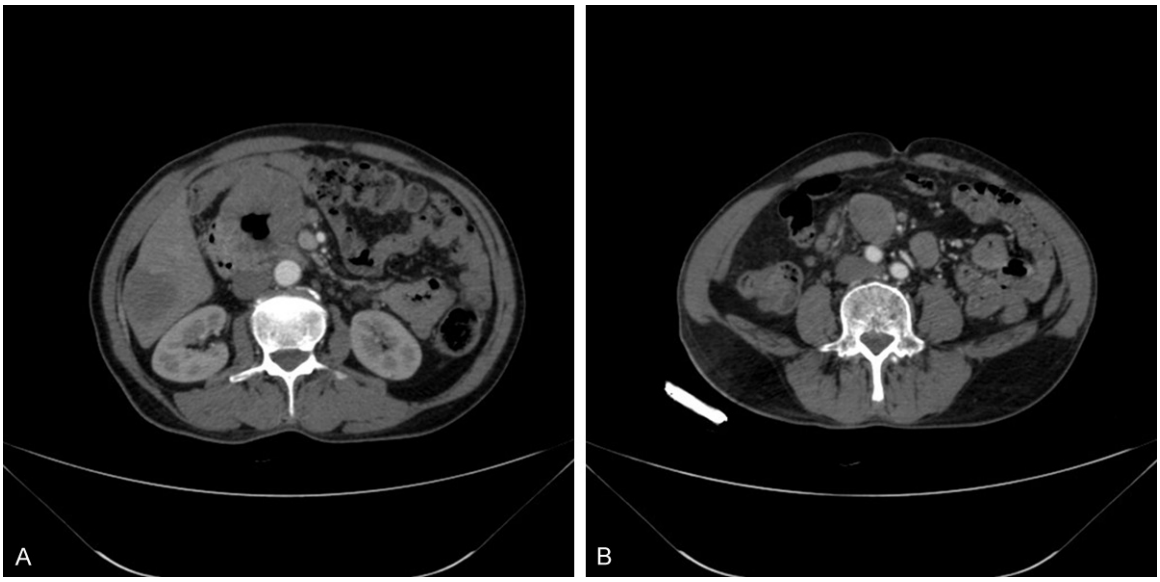

Figure 1. Case 3. 75/M, Computed tomography of primary duodenum GIST and metastases. Soft tissue mass located at the descending portion of the duodenum was displayed as oval and homogeneous density, and the mass necrosis made the tumor connected with intestinal cavity. Lots of round-like and low density shadows were revealed in liver with different size (A). And then, there were multiple enlarged lymph nodes in mesenteric region (B).

we reviewed all the cases and reached consensus on the diagnosis of LNM based on review of clinical history, imaging. Data collected included: sex, age, presenting signs, location, adjuvant therapy, prognosis. Follow-up was obtained in 6 cases.

Immunohistochemistry

Immunostaining was performed on 6 cases of GISTs with LNM using the following antibodies and conditions: KIT (Dako, A4502, dilution 1:300), DOG1 (Leica, clone K9, dilution 1:150), SDHB (Abcam, ab14714, dilution 1:800), CD34 (Dako QBEND10, No. M0823, dilution 1:100), smooth muscle action (SMA, Dako No. M0851, 1:200), Ki67 (Dako QBEND10, No. M7248, dilution 1:100). The Envision Plus detection system (Dako) was used. Appropriate positive and negative controls were used throughout.

Genetic mutation

DNA was extracted from formalin-fixed and paraffin-embedded tumor tissue, according to the protocol of the Dneasy Tissue Kit (Cat, 69504, Qiagen, Hilden, Germany). And direct sequencing of polymerase chain reaction (PCR) productions was applied to detect the gene mutations of *KIT* (exon 9, 11, 13, 17) and *PDGFRA* (exon 12, 18) genes. The PCR reaction conditions, recommended by Perkin Elmer, were the standard ones. PCR productions were fractionated on 50 g/L polyacrylamide gelatins and then stained with ethidium bromide. The ABI Prism 310 DNA sequencer (Applied Biosystems) was used to perform the direct sequencing. The Big Dye Terminator cycle sequencing ready reaction Kit (Applied Biosystems Inc. Foster City, Calif.) was used for the sequencing reactions, according to the manufacturer's instructions.

Results

Clinical findings

Clinical features of the patients with LNM are summarized in **Table 1**. 5 patients were males and 1 was female, ranging in age from 48 to 75 years (median, 66 y). The 6 patients presented with pain, abdominal mass and GI bleeding. In addition, Case 1 had a severe jaundice, with synchronous primary intrahepatic biliary adenocarcinoma and GIST of LNM. None of the patients had family history of GIST. The primary GIST tumors of 5 cases were located in the small intestine, and one in the bottom of stomach. Multiple synchronous GISTs were present in 4 patients, almost with liver metastasis and peritoneal cavity spread. 6 patients were confirmed the intraabdominal mass with metastases on the imaging, and showed suspicious

Table 2. Pathological features of primary GISTs with LNM

Case	Size of dominant tumor (cm)	Cell morphology	Necrosis	Mitotic counts (/50HPFs)	Ki-67 index	Gene mutation
No. 1	7	Spindle	Yes	10	20%	*KIT* exon 11 codon 557-558 deletion
No. 2	8.5	Spindle+epithelioid	Yes	13	15%	*KIT* exon 11 codon 551-556 deletion
No. 3	10	Spindle	Yes	20	30%	*KIT* exon 11 codon 557-558 deletion
No. 4	8	Spindle+epithelioid	Yes	12	25%	*KIT* exon 11 codon 557-558 deletion
No. 5	10	Spindle+epithelioid	Yes	15	20%	*KIT* exon 11 codon 559 point mutation (GTT→GAT)
No. 6	9	Spindle	Yes	12	35%	*KIT* exon 11 codon 552-555 deletion

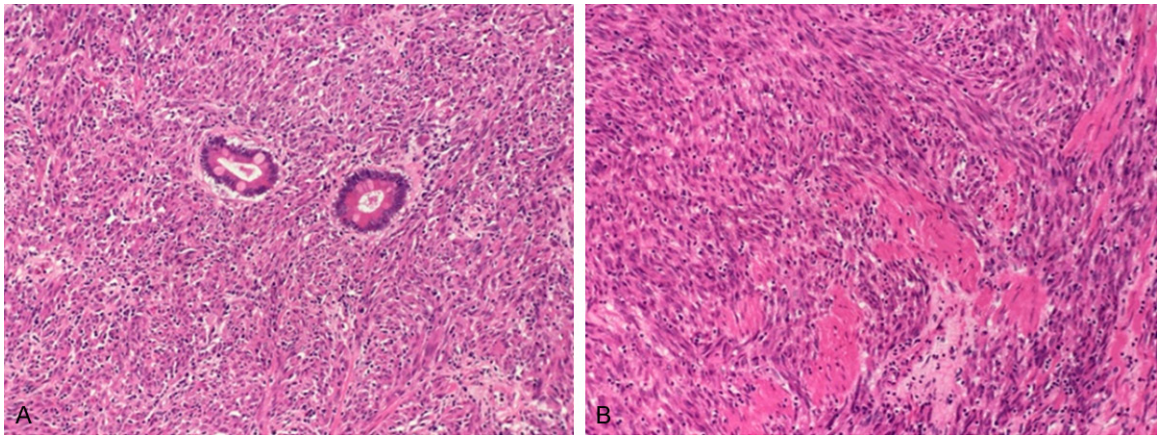

Figure 2. Histopathological appearance of the intestinal GIST. A. The tumor cells infiltrated through the wall of small intestine. It was visible of the residual two intestinal glands. Few of small lymphocyte was found in tumor stroma. (Hematoxylin and eosin stain, ×200). B. The tumor revealed a hypercellular spindle cells, with skeinoid fiber. (Hematoxylin and eosin stain, ×200).

intraabdominal lymph node at the same time (**Figure 1**).

Pathologic findings

Pathologic features of the patients with LNM are summarized in **Table 2**. Multiple synchronous GISTs were detected in 4 cases. The dominant tumors were still the primary ones, with the size varied from 7 cm to 10 cm (median 8.75 cm). The GISTs were typically described as lobulated and huge mass, and two primary tumors were centrally cystic. On sectioning the tumors varied from yellowish to pale pink, and many contained focal areas of hemorrhage and necrosis.

Histopathology examination revealed hypercellular cell morphology. Spindle cytology dominated in 3 cases, and other 3 cases had mixed epithelioid and spindle cell. The tumor cells showed rather uniform cytology, which arranged in concentric whorls or palisaded-vacuolated morphology and little of intermixed small lymphocytes. Most tumor cells had plump spindle-shaped or (and) ovoid nuclei with dark chromatin and nucleolus, as well as a moderate amount of eosinophilic cytoplasm. Usually focal part of the tumor cell presented the nuclear palisading, but vacuolization was detectable in most cases. The tumor stroma was relatively rarefactive. Mitotic index was about 10-20 per 50 high-power fields, without lymphovascular invasion. The metastases of lymph node were present spindle cell morphology that arranged in a sheet-like or nested growth pattern (**Figures 2-4**).

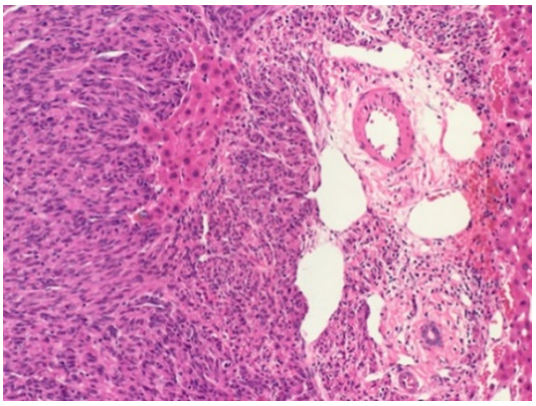

Figure 3. Histopathological appearance of the liver metastasis. The tumor cells infiltrated the hepatic lobule and portal area. (Hematoxylin and eosin stain, ×200).

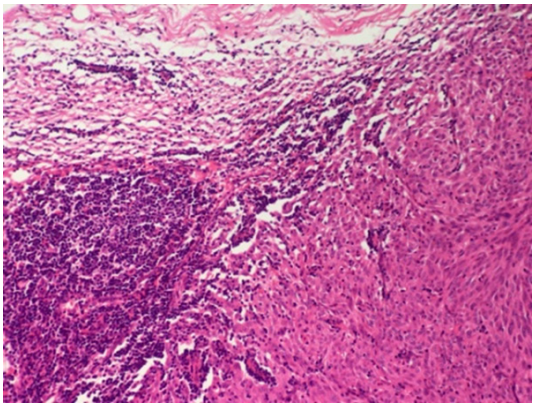

Figure 4. Histopathological appearance of LNM. It was visible that invasive spindle tumor cells presented under capsule of lymph node and there was some residual lymphoid tissue on the left. (Hematoxylin and eosin stain, ×200).

Immunohistochemically, the tumor cells were extensively positive for KIT (6/6) (**Figure 5**), DOG1 (6/6), with >50% of tumor cells positive in each case. CD34 was detected in 5/6 cases, but SMA only rarely (1/6, focally). All 6 cases were SDHB protein positive. In addition, the result of immunohistochemical staining in the primary tumors was in agreement with that in metastases of lymph node and abdominal cavity.

KIT exon 11 mutation was found in all the 6 cases. Type of the mutation included deletion (4/6) and point mutation (1/6). And then, codon 551-558 deletion of *KIT* exon 11 was detected in 5/6 cases. The metastases still showed same molecular features in common with the primary tumor (**Figure 6**).

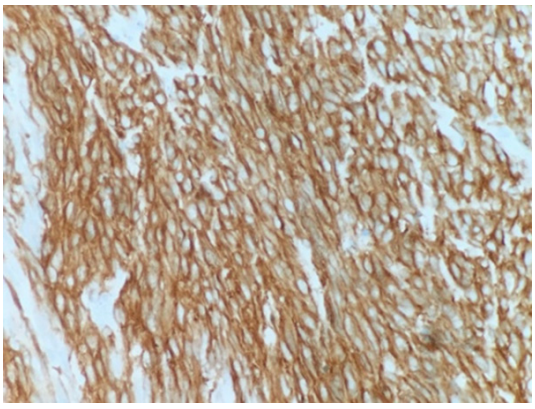

Figure 5. Immunohistochemical results. The tumor cells showed immunoreactivity to KIT at cell membrane. (DAB, ×200).

Follow-up

Six cases developed multiple synchronous tumors, including metastases and different tumor, on the first presentation. In particular, all cases had suspected LNM and 3 had liver metastasis, meanwhile peritoneal cavity spread. So far, one of them, who also had primary intrahepatic biliary adenocarcinoma, died of disease, but other patients were survival for 1-30 months after diagnosis. All the cases accepted surgical operation (including the primary sites and metastases), and one of them also underwent the coarse needle biopsy before operation. Five patients were known having received imatinib mesylate for 1-30 months and none of them had imatinib resistance.

Discussion

GISTs show lots of morphologic, immunohistochemical, and molecular features in common with ICC of GI. And the gold standard of therapy is the complete resection of the local mass. In addition, for the tumors with high or intermediate risk of recurrence, based on the modified National Institutes of Health consensus criteria, the administration of imatinib significantly improve the rate of progression-free survival. Despite this, GISTs still have higher risk of metastatic relapse. The common metastatic sites are the liver (50%-60%) or disseminate throughout the peritoneal cavity (20%-30%) as lots of

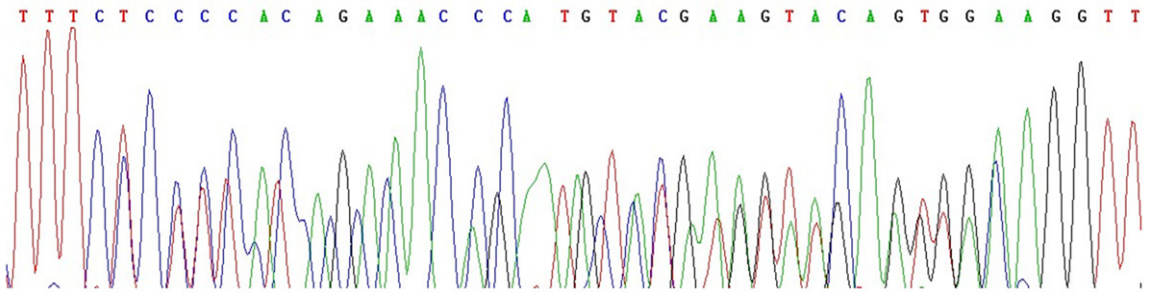

Figure 6. Gene mutation of intestinal GIST. The tumor showed codon 557-558 deletion of *KIT* exon 11.

metastatic nodules, which is the frequent feature for high grade GISTs. Sometimes, the liver metastasis is the only or primary clinical manifestation. On the contrary, it is unusual for GISTs to metastasize outside of the abdomen, but dermal/subcutaneous, bone, brain, lymph node, and lung metastases occur rarely. In a series of reports concerning metastatic GISTs, the incidence of LNM is less than 10%, especially for the mutant GISTs.

Based on the study and reports of GISTs, most common special subtype with LNM is mainly the succinate dehydrogenase (SDH) deficient GISTs. The SDH-deficient GISTs especially include pediatric GISTs and those associated with Carney triad (CT) or Carney-Stratakis syndromes (CSS) [4]. However, the special SDH-deficient GISTs lack *KIT* or *PDGFRA* mutations (is wild type in relation to those mutations), features commonly seen in adult and mutant GISTs, such as clinical manifestations and pathological features are only rarely encountered in SDH-deficient GISTs. Interestingly, in our series of GISTs with LNM, most of the clinical pathological characteristics were same as the adult and mutant GISTs and different with the SDH-deficient GISTs, including the following five aspects.

Firstly, it is well known that SDH-deficient GISTs exclusively among gastric GISTs, and usually occur in children and young adults, with predilection to female [4, 5]. They are often multiple nodules and have a chronic course similar to that of pediatric and CT GISTs [4]. On the contrary, most of mutant GISTs with LNM in our study developed in the small intestine with single mass. There was a significant overall male predominance: 1 female and 5 males, and they were all adult patients. Especially multiple synchronous GISTs were present in patients, almost with liver metastasis and peritoneal cavity spread at the same time. Meanwhile one additional patient had primary intrahepatic biliary adenocarcinoma and GIST with LNM separately.

Secondly, there are significant differences of the pathological morphology between the mutant GISTs and SDH-deficient GISTs with LNM. SDH-deficient GISTs typically show epithelioid hypercellular morphology and plexiform muscularis propria involvement [4]. But the palisaded-vacuolated or sclerosing morphology of the tumor cells are very rare, which are commonly seen in adult GISTs [4]. In addition, lymphovascular invasion around the tumor nodules of SDH-deficient GISTs is common, but rare in mutant or adult GISTs. In our group, the primary and metastatic tumors showed spindle cell (3 cases) or mixed epithelioid and spindle cell (3 cases) without single epithelioid morphology. The primary tumors demonstrated expansive growth, including the metastases, and rather rare lymphovascular invasion. There could be involvement of the whole layer of GI, especially the muscularis and serosa layer. Also tumor size and mitotic activity were large and high. The tumor cells in our series showed similar histology to the classic mutant GISTs and usually presented extensive hemorrhage and necrosis.

Thirdly, immunohistochemically the only and greatest difference between SDH-deficient GISTs and our group was SDHB protein staining, which is also one of their identification points. Both the two groups were extensively positive for KIT and DOG1 protein, often with more than 50% of tumor cells positive. So the Immunophenotyping of our group was extremely similar to the classic mutant GISTs.

Fourthly, most GISTs (about 80%-85%) are driven by the gain of function mutations in either *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*) [6]. However, about 10%-15% GISTs lack such mutations, which are called wild type GISTs (WT-GISTs). These WT-GISTs include three clinicopathologic subgroups: pediatric GISTs, those associated with neurofibromatosis type I, and sporadic WT-GISTs [4]. And this is also another identification points between SDH-deficient GISTs and our group. In addition, the frequency of *KIT* mutation was significantly increased in the GISTs with higher mitosis (≥5/50HPFs) and larger size (≥5 cm) of tumors. *KIT* mutational status has prognostic significance for GISTs' outcome. 5 year relapse-free survival (RFS) rate was dramatically higher in patients with *KIT* exon 11 deletion than in those with other type of *KIT* exon 11 mutations. And then, the deletion of *KIT* exon 11, especially codon 557-558 deletion, was a valuable predictor of prognosis for GISTs [6]. In our group, all the 6 cases found *KIT* exon 11 mutation. And codon 551-558 deletion of *KIT* exon 11 was detected in 5/6 cases.

Finally, there is another difference between our group and SDH-deficient GISTs on the prognostic indicator and treatment. Patients with SDH-deficient GISTs usually should get complete tumor resection. Because they often have a chronic course, whether or not accompanied by LNM, so the lifelong follow-up is necessary with attention to the potential of secondary tumors and metastases. However, for the metastatic mutant GISTs in our group, most have the synchronous liver metastasis and/or peritoneal cavity spread, which often indicate late clinical stage and poor prognosis [7, 8], and M Tokunaga et al thought that this type of GISTs should be regarded as an advanced disease [7]. The choice of clinical treatment usually based on the progress of the disease, such as the complete primary and metastatic tumors resection as possible, or taking the targeted therapy first of all. The adjuvant therapy of *KIT* tyrosine kinase inhibitor after operation is required based on the *KIT/PDGFRA* mutation [9]. Due to the rarity of the LNM, lymph node dissection is not routinely performed. Even so, preoperative radiographic evaluation and Intraoperative exploration is necessary, and then the LNM should be excised [10].

In conclusion, high grade mutated GISTs with liver metastasis and/or peritoneal cavity spread, especially intestinal GISTs, should keep on high alert for LNM. Patients with lymph node metastatic GISTs usually show exon 11 mutation of *KIT* gene and had a worse biological behavior. In addition, preoperative radiologic screening, such as CT or MRI imaging, is very important.

Disclosure of conflict of interest

None.

Address correspondence to: Huanzhou Xue, Department of Liver-Gallbladder-Pancreas Surgery, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, 7 Weiwu Road, Zhengzhou 450003, Henan Province, China. Tel: +86-371-6558-0511; E-mail: xhz32291316@163.com

References

[1] Liu Q, Kan Y, Sun T, He H, Zhang M, Xu Z, Fu F, Kong L, Xue H. Gastrointestinal stromal tumor with multiple bone, liver, lung and abdominopelvic cavity metastases: significant clinicopathologic characteristics of an unusual case. Int J Clin Exp Pathol 2016; 9: 3999-4003.

[2] Vassos N, Agaimy A, Hohenberger W, Croner RS. Extraabdominal lymph node metastasis in gastrointestinal stromal tumors (GIST). J Gastrointest Surg 2011; 15: 1232-6.

[3] Shafizad A, Mohammadianpanah M, Nasrolahi H, Mokhtari M, Mousavi SA. Lymph Node Metastasis in Gastrointestinal Stromal Tumor (GIST): to Report a Case. Iran J Cancer Prev 2014; 7: 171-4.

[4] Miettinen M, Wang ZF, Sarlomorikala M, Osuch C, Rutkowski P, Lasota J. Succinate dehydrogenase-deficient GISTs: a clinicopathologic, immunohistochemical, and molecular genetic study of 66 gastric GISTs with predilection to young age. Am J Surg Pathol 2011; 35: 1712-21.

[5] Noomene R, Mchirgui L, Chammakhi-Jemli C, Cherif A. [Young female multiple gastric GIST presenting with lymph node metastasis: sporadic or incomplete Carney's triad? Presse Méd 2014; 43: 478-9.

[6] Lin Y, Lei Z, Zhao W, Wang Y, Bo L, Yao H, Yu H. Clinicopathological significance of c-KIT mutation in gastrointestinal stromal tumors: a systematic review and meta-analysis. Sci Rep 2015; 5: 330-44.

[7] Tokunaga M, Ohyama S, Hiki N, Fukunaga T, Yamamoto N, Yamaguchi T. Incidence and prognostic value of lymph node metastasis on

c-Kit-positive gastrointestinal stromal tumors of the stomach. Hepatogastroenterology 2011; 58: 1224-8.

[8] Waki N, Yoshida R, Yamashita K, Taguchi K. Colonic gastrointestinal stromal tumor (GIST) with lymph node metastasis concomitant with adenoma, adenocarcinoma, and neuroendocrine carcinoma-a case report. Nippon Daicho Komonbyo Gakkai Zasshi 2015; 68: 34-9.

[9] Kinoshita J, Kaji M, Sakurai K, Kawahara Y, Amaya K, Terada I, Yamamoto S, Maeda K, Shimizu K. A case of gastrointestinal stromal tumor of the stomach with lymph node metastasis treated with laparoscopic and endoscopic cooperative surgery. Nihon Gekakei Rengo Gakkaishi 2014; 39: 204-10.

[10] Gong N, Wong CS, Chu YC. Is lymph node metastasis a common feature of gastrointestinal stromal tumor? PET/CT correlation. Clin Nucl Med 2011; 36: 678-82.