CASE REPORT

Utilizing circulating free DNA in diagnosing early gastric cancer in a patient with situs inversus totalis: A case report and literature review

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Abstract
To offer a pilot view on the application of cfDNA in diagnosing early gastric cancer in patient with situs inversus totalis (SIT), accompanied by a literature review. Within this study, we assessed the feasibility of diagnosing early gastric cancer in a SIT patient by detecting cfDNA concentration and mutations. Meanwhile, a search through PubMed using key words of “situs inversus totalis and gastric cancer” covering English studies from 2008 to 2019 was carried out to provide an associated literature review. Of all the 24 publications we reviewed, 25 patients were diagnosed with SIT and GC. The majority of them (23/25) were from the eastern Asia area. More than 40% (11/25) of the patients were diagnosed at advanced tumor stages. In the case of an early staged GC in our center, when both radiographic and endoscopic exams failed to establish an accurate diagnose, we found that not only the concentration of cfDNA was above normal, the frequent genetic mutations for GC were also detected within preoperative cfDNA. The concomitance of SIT and GC is still a rare incident, only limited cases have been reported and were often diagnosed in late cancer stages. By measuring the concentration and mutations of cfDNA, early GC may be detected in SIT patients.

KEYWORDS
circulating free DNA (cfDNA), early diagnose, gastric cancer (GC), situs inversus totalis (SIT)

1 | BACKGROUND

Situs inversus totalis (SIT) is a rare situation in clinic and occurs approximately in every 10 000 to 50 000 people. So far, the genetic cause of SIT has only been partly revealed. Some pathological conditions such as bronchiectasis, chronic sinusitis and male infertility, were proved to be related to SIT. On the other hand, ever since the first case of a gastric cancer in a patient with SIT was described, reports of the rare association were still limited. Moreover, the main topics of these reports were still concerning about surgical techniques, while few studies focused on the early detections of GC in SIT patients which was also of great importance in prolonging the overall survival. So, in the study, we report the case of a SIT patient associated with GC, whose early diagnose was established with the help of circulating free DNA (cfDNA), a novel and rising biomarker for various of cancers.

1.1 | Case presentation

A 53-year-old male Chinese with a clean medical history visited our outpatient department for regular physical examinations. The laboratory tests including routine blood test, biochemistry tumor markers...
determination showed no significant abnormality. Upper gastrointestinal contrast and computed tomography (CT) reported a total situs inversus viscera and spotted a thickening area in the proximal stomach (Figure 1A) which called for differentiation to rule out GC. A lamellar erosion was observed during gastroscopic exam and was furtherly proved to be chronic superficial gastritis with intestinal metaplasia by biopsy. However, the concentration of circulating free DNA (cfDNA) was higher (12.32 ng/mL) than normal. Meanwhile, genome sequencing of cfDNA obtained before surgery spotted GC related gene mutations, suggesting a possibility of malignancy. Therefore, a second endoscopic exam was arranged and a diagnosis of high-grade intraepithelial neoplasia of subcardial gland epithelium was offered.

Eventually, laparoscopic radical proximal subtotal gastrectomy with D2 regional lymphadenectomy and jejunum interposition anastomosis were carried out. One square centimeter superficial erosive ulcer was detected during surgery and was completely removed. The postoperative pathology turned out to be gastric adenocarcinoma (Figure 1B). Microscopic exam observed invasion of the muscularis mucosae (T1) and no metastasis of all the 10 lymph nodes harvested (N0).

The postoperative recovery was uneventful. The patient restored exhaustion and defecation 4 days after surgery, and started liquid intake in the fifth postoperative day and then semifluid diet in the seventh postoperative day, and was discharged 2 weeks after the operation. The serum cfDNA was extracted and analyzed 1 week after the surgery, this time, the concentration dropped to 0.66 ng/mL, and numbers of detectable mutations decreased.

We followed the patients for 6 months. CT scan and blood tests suggested a smooth recovery and no signs of tumor residual or recurrence.

1.2 | Sample collection and preparation

Blood samples were obtained from the ulnar vein in the first morning after hospitalization and on the seventh postoperative day. Once drawn, all samples were processed within 3 hours through a standard procedure: first, the blood was contained in the 10 milliliter EDTA tube (BD, Plymouth, UK) and was spun at 3000 rpm for 10 minutes to separate the plasma from the blood cells; second, the plasma from the first step went through another spin at 13000 rpm for 10 minutes to remove the cellular debris. Peripheral blood mononuclear cells (PBMCs) were collected from the blood cells from the first step. Both the plasma and PBMCs were stored at the temperature of −80°C for future use after clarification. We reported the detailed methods of PBMCs and cfDNA acquiring in a previous study.

The frozen primary tumor and matched adjacent gastric tissues were obtained. The tumor tissue was confirmed to be consisted with at least 50% tumor cell content by postoperative pathology. DNA was extracted from the tissues using DNeasy Blood & Tissue Kit (QIAGEN), and was stored at −80°C for future sequencing.

1.3 | Whole exome sequencing and targeted deep sequencing

DNA acquired from tissue samples was further analyzed by WES while the plasma cfDNA was sequenced using a panel of 556 genes for digestive cancers. Germline DNA extracted from PBMCs was used as normal control. The sequencing was performed on the high

![FIGURE 1](image-url)
| Year  | Authors                          | Patient number | Surgical strategies                        | Tumor stages*                | Regions     |
|-------|----------------------------------|----------------|-------------------------------------------|-----------------------------|-------------|
| 2008  | El Bachir Benjelloun, et al.      | 1              | Subtotal gastrectomy with D2 lymphadenectomy | Advanced (pT3N³)            | Morocco     |
| 2010  | TOMOHIRO HARUKI, et al.           | 1              | Bowel bypass                              | Advanced (stage IV)         | Japan       |
| 2011  | Hong Beom Kim, et al.             | 1              | RADG with D1 + β lymph node dissection    | Stage IIb, pT3N3a.          | Korea       |
| 2011  | Kyung Won Seo, et al.             | 1              | LADG with D1 + β lymph node dissection    | Stage IA, pT1, pN0, sH0, sP0, sM0, | Korea       |
| 2012  | Pan Ke, et al.                    | 1              | proximal gastrectomy with D2 lymph node dissection | Advanced (pN4)              | China       |
| 2013  | Hirohito Fujikawa, et al.         | 1              | LADG with D1 lymph node dissection        | Stage IA, pT1[sm], pN0, sH0, sP0, sM0 | Japan       |
| 2013  | Sa-Hong Min, et al.               | 2              | LADG with D1a lymph node dissection       | Stage 1B, pT2N0             | Korea       |
| 2014  | Taro Isobe, et al.                | 1              | Total gastrectomy with D2 lymph node dissection | Stage IIIC, pT4aN3bH0P0CY0M0 | Japan       |
| 2014  | Yasuo Sumi, et al.                | 1              | LADG with standard lymph node dissection  | Stage IB, pT1b1, pN1, sH0, sM0 | Japan       |
| 2015  | Min-Feng Ye, et al.               | 1              | LADG and D2 lymph node dissection         | Stage IIB, pT4aN0M0         | China       |
| 2015  | Mamoru Morimoto, et al.           | 1              | LATG with standard lymph node dissection  | Stage 1A, pT1aN0M0          | Japan       |
| 2015  | Zhu H, et al.                     | 1              | Distal gastrectomy with D2 lymphadenectomy | Stage IIia, pT4aN1M0,       | China       |
| 2016  | Yu Kigasawa, et al.               | 1              | LADG and D1* lymph node dissection        | Stage IA, pT1b (sm2), pN0, pM0, | Japan       |
| 2017  | Rana Alhossaini, et al.           | 1              | Robotic distal gastrectomy                | Stage IA, pT1aN0M0          | Korea       |
| 2017  | Kengo Shibata, et al.             | 1              | LTG and modified D2 lymph node dissection | Stage IB, pT3N1             | Japan       |
| 2017  | Yinghao Cao, et al.               | 1              | Total gastrectomy with D2 lymph node dissection | Advanced (pT3N1M0)          | China       |
| 2017  | Byoung Jo Suh                    | 1              | Subtotal gastrectomy with D2 lymph node dissection | Early (pT1N0M0)            | Korea       |
| 2018  | Hai-Bin Dai, et al.               | 1              | Lower two-third stomach resection         | Advanced (pT4aN2M0)        | China       |
| 2018  | Youichi Miyakoa, et al.           | 1              | Endoscopic submucosal dissection          | Early (Na)                 | Japan       |
| 2018  | Ebubekir Gündes, et al.           | 1              | Distal subtotal gastrectomy and D1 lymph node dissection | Early (pT1bN0M0)          | Turkey      |
| 2018  | Yuki Aitsu, et al.                | 1              | RADG with D1* lymph node dissection       | Stage IA, pT1b (SM), int, INF-β, ly0, v0, pPM0, pDM0, pN0 | Japan       |
| 2018  | Tsutomu Namikawa, et al.          | 1              | Total gastrectomy with regional lymph node dissection | Early (pT1N0M0)            | Japan       |
| 2019  | Toshiyasu Ojima, et al.           | 1              | Robotic distal gastrectomy and D2 lymph node dissection | Stage IB, pT1b (SM1), ly0, v0, pPM0, pDM0, pN1 (1/24) | Japan       |
| 2019  | Wangsheng Xue, et al.             | 1              | Distal gastrectomy and D2 lymph node dissection | Advanced (Adrenal metastasis) | China       |

Abbreviations: LADG, laparoscopy-assisted distal gastrectomy; LATG, laparoscopy-assisted total gastrectomy; LTG, laparoscopic total gastrectomy; Na, not available; RADG: robot-assisted radical distal gastrectomy; TLDG, totally laparoscopic distal gastrectomy.

*tumor stages were cited from corresponding publications with no further alterations made.
throughput platform of Illumina. The average depth for WES was 237.7x (203.5x-272.1x) and 13 000x (11 000x-18 000x) for TDS.

We found mutated genes in the preoperative cfDNA such as ARKND36, MERTK and TCS1. WES revealed a more comprehensive mutation distribution in the tumor tissue. Various of GC related genes were detected mutated including ARKND36, MERTK and TCS1. However, in the postoperative cfDNA, only mutation of CDKN1A was confirmed (Figure 1C).

1.4 | Gastric cancer stage review from literatures published in recent years

We reviewed literatures concerning about gastric cancer with SIT from the year 2008 to 2019. Due to the low morbidity of SIT, only limited publications (24) were found and most of them were presented in the form of case reports. We analyzed the detailed tumor stages in those articles and summarized them in Table 1.

2 | DISCUSSION

So far, the key of GC management lies on early detection and intervention. Due to the asymptomatic feature, GC is often discovered at advanced stages, resulting in poor prognosis. Moreover, anatomical abnormalities in SIT patients cast extra difficulties and interferences for clinical diagnoses. As shown in our review, 44% (11/25) of the reported cases were in the advanced stage, highlighting the need to enable early detection for GC in SIT patients. More importantly, we found that more than 27% (3/11) cases in Japan were presented in late stage. Considering the achievements in GC management in Japan, the situation for SIT patients with GC is not optimistic.

Therefore, measurements that can contribute to early diagnose of GC in SIT patients is urgently demanded. Great efforts have been made to fulfill the goal, however, the results are not satisfactory. Until now, the modalities contributing to early detection of GC are restrained to serum markers, endoscopes and imaging exams.

The serum tumor biomarker such as CA-125 and CA-174 achieved low specificity and positivity, while image exams like CT and GI were only able to detect lesions of certain size. The gastroscope are considered to be the golden measurement, yet, still limited by the difficulty of sampling and the experience of the physicians.

In our case, results of serum markers were unremarkable. CT scan and GI spotted the diseased area, yet, were unable to uncover the nature. Although, the endoscope located the lesion and offered a general description, the first biopsy, on the other hand, failed to establish the correct diagnose due to the unsuccessful sampling.

Circulating free DNA (cfDNA) has been drawing attentions in recent years for its application in oncology studies including early diagnose, treatment evaluation and clinical surveillance. By assessing the quantity and genetic mutations harbored in cfDNA, researchers are able to obtain non-invasive, repeatable and tumor-specific information so as to establish accurate and in-time diagnosis, select effective therapy and optimal follow-up scheme.

Our case complied with the features of cfDNA. As we reported, the concentration of cfDNA elevated before surgery and decreased to normal after tumor removal. The same phenomenon was observed when analyzing cfDNA genome-wise. Abundant cancer related mutations were detected within cfDNA before operation and disappeared when then tumor was resected. It’s noteworthy that, the mutated genes harbored within preoperative cfDNA were also confirmed to be present in tumor tissues by WES.

Another interesting discovery in our study was that, GC in SIT patients manifested accordant genetic alterations with that in normal population. Many of the detected mutated genes, BMP6, EEA1, KLK4, TMRSS5, to name a few, were all proved to be involved in GC carcinogenesis and development. Also, we found some alterations more exclusive to Chinese population such as IGFBP3 and TP53BP1.44,45

There are still some limitations to our study. The patient number and race were both very finite, therefore, the results should be cautiously interpreted before assessed in a larger cohort.

To conclude, our review revealed an imperative need for prompt diagnose for GC in SIT patients. cfDNA may function as a complementary modality to contribute to early detection.

ETHICS STATEMENT

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (the Institutional Review Board of Jiangsu cancer Hospital, Nanjing Medical University) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from every patient.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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