CASE REPORT

Title: Secondary Hemochromatosis and Hepatocellular Carcinoma Developed from Immunoglobulin G4-Related Disease with Hepatopathy: A Case Report

Authors: Shintaro Kanaka¹*, Youichi Kawano², Shigeki Yokomuro², Fumihiko Ando², Norio Itokawa³, Tsutomu Hatori⁴, Koshi Matsumoto⁵, Yoh Zen⁶, Masao Miyashita², Hiroshi Yoshida¹

1. Department of Surgery, Nippon Medical School, Tokyo, Japan
2. Department of Surgery, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan
3. Department of Internal Medicine, Division of Gastroenterology, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan
4. Department of Clinical Pathology, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan
5. Department of Pathology, Ebina General Hospital, Kanagawa, Japan
6. Department of Diagnostic Pathology, Kobe University Graduate School of Medicine, Kobe, Japan

*Corresponding author: Shintaro Kanaka, Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-Ku, Tokyo 113-8603, Japan.

Phone: +81-3-3822-2131 Fax: +81-3-5685-0989

E-mail: ks32814@nms.ac.jp

Running head: Hemochromatosis and HCC from IgG4-RD
Abstract

Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is a recently characterized entity in which lymphocytes and plasma cells infiltrate various anatomical sites. IgG4-hepatopathy, which is the manifestation of IgG4-RD, is a broader term covering various patterns of liver injury. The clinical course including the malignant potential of IgG4-RD remains unclear. Here we report the first case of secondary hemochromatosis and hepatocellular carcinoma (HCC) developed from IgG4-hepatopathy. A 67-year-old man was admitted to our hospital due to deteriorated glucose tolerance. Blood test results showed hypergammaglobulinemia, especially IgG4. He was readmitted 2 months later with dyspnea due to lung disease and pleural effusion, with elevated transaminase levels. He underwent liver and lung biopsies and was diagnosed with IgG4-RD, and received steroid therapy, which improved his serum IgG4 levels and imaging abnormalities. A follow-up computed tomography (CT) scan conducted 38 months later demonstrated a 50-mm-diameter tumor segments 7 and 8 of the liver. The resected specimen revealed HCC and abundant siderosis in the background liver, leading to a diagnosis of hemochromatosis. IgG4-positive cells were scarce, probably due to corticosteroid therapy. In the present case, IgG4-RD was well controlled with prednisolone (PSL) and immunosuppressive agent, and chronic hepatitis was not so severe, even though the patient subsequently developed HCC. However, extensive siderosis consistent with hemochromatosis was unexpectedly noted. It is suggested that secondary hemochromatosis and HCC developed during
IgG4-RD with hepatopathy. Here we report the present case because it contributes to our understanding of IgG4-RD.

Key words: IgG4-related disease; IgG4-hepatopathy; secondary hemochromatosis; hepatocellular carcinoma

Introduction

Immunoglobulin (Ig) G4-related disease (IgG4-RD) is a recently characterized entity in which a massive inflammatory infiltrate rich in IgG4-positive plasma cells and fibrosis affects various organs.1,2 The pathophysiology and natural clinical course including the relationship with carcinogenesis are still unclear. We herein report a patient who developed secondary hemochromatosis and hepatocellular carcinoma (HCC) during the clinical course of IgG4-RD with hepatopathy.

Case Report

A 67-year-old man was referred to our hospital due to deteriorating glucose intolerance. His medical
history was significant for diabetes that had been treated with oral antidiabetic agents, repaglinide, alogliptin, and pioglitazone for 3 years. Although his serum hemoglobin A1c level had stabilized around 7.0% during that period, it increased to 14.1% in line with a blood glucose level of 486 mg/dL. He was diagnosed with diabetic ketoacidosis and admitted to our hospital. Blood tests revealed high levels of liver enzymes and globulins, particularly IgG. Chest X-ray examination showed blunting of the left costophrenic sulcus, while CT showed left-sided pleural effusion with pleural thickening and atelectasis of the inferior lobe and no abnormality was pointed out in the abdominal CT scan at that time (Fig. 1a). For diagnostic purposes, we aspirated the pleural effusion, which was negative for malignancy but contained many plasma cells. This plasma-cell-rich inflammation raised the possibility of IgG4-related pulmonary disease. Additional serological tests confirmed elevated serum IgG4. Immunostaining of a cell block prepared from the pleural effusion also demonstrated many IgG4-positive plasma cells. Although the diagnosis of lung disease was not established because of a lack of biopsy, he was discharged when his glucose tolerance was improved by insulin therapy.

The patient was readmitted to our hospital 2 months later with respiratory distress and fatigue. Blood test results from arterial blood revealed in Table 1. Chest CT revealed deterioration of the pulmonary abnormalities with bilateral consolidation and ground-glass opacity, left-sided atelectasis, and enlarged mediastinal lymph nodes (Fig. 1b). Transbronchial lung biopsy demonstrated infiltration of
plasma cells and lymphocytes in the bronchial wall and alveolar septa. Immunostaining revealed >10 IgG4-positive plasma cells per high-power field (HPF; Fig. 1d, e, f). He also underwent a liver biopsy due to liver dysfunction. Microscopically, the liver was moderately fibrotic with periportal and bridging fibrosis. Portal tracts were densely infiltrated by lymphocytes and plasma cells with mild interface hepatitis (Fig. 2a, b). Immunostaining showed many IgG4-positive plasma cells (>10 IgG4-positive cells per HPF) and an IgG4/IgG-positive cell ratio of 50%–60% (Fig. 2c, d). At this point, no iron deposition was present in the liver specimens. Additionally, dysarthria and left-sided hemiplegia occurred during hospitalization. CT showed no remarkable abnormalities but magnetic resonance imaging (MRI) revealed thickening of the meninges, consistent with hypertrophic pachymeningitis, which is one of the characteristics of IgG4-RD in the central nervous system (CNS). Furthermore, a retrospective comparison of the CT images before and after steroid therapy showed a decrease in pancreatic tail thickness, suggesting that the deterioration in glucose intolerance might have been partly due to autoimmune pancreatitis (AIP). Based on these findings, he was diagnosed as IgG4-RD with manifestations in the lungs, pleura, liver, CNS, and pancreas. He was treated with steroid pulse therapy (500 mg methylprednisolone (mPSL) daily by intravenous drip for 3 days) because the main problem was respiratory failure, followed by 20 mg/day of prednisolone (PSL), which improved his symptoms, imaging abnormalities, and laboratory findings (Fig. 1c, 3).
PSL was slowly tapered to 10 mg/day with no relapse over the next 2 years, but he then returned to the hospital with fatigue and nausea. Blood test results were as follows: WBC, 14,660/μL; Hb, 14.7 g/dL; PLT, 11.3×104/μL; IgG, 1388 mg/dL; IgG4, 739 mg/dL; AST, 42 IU/L; and ALT, 50 IU/L. CT and endoscopies showed no abnormality. We suspected that his symptoms might be due to worsening IgG4-RD, and we therefore added an immunosuppressive agent to PSL as diagnostic therapy. After that, his serum IgG and IgG4 levels remained around 100–200 mg/dL after discharge (Fig. 3). A follow-up CT conducted 14 months later demonstrated a 50-mm tumor located in segments 7 and 8 (S7/8) of the liver, which was non-uniformly hyperattenuated in the early phase, washed-out immediately, and showed faint enhancement in the late phase (Fig. 4a, b). MRI using gadolinium ethoxybenzyl-diethylenetriaminepentaacetic acid showed a well-defined solitary tumor that was enhanced in the early phase and washed-out in the late phase (Fig. 4c). Protein induced by vitamin K absence or antagonist-II (PIVKA II) was elevated (1255 mAU/mL; normal range, 4–108 mg/dL) but all other tumor markers including alpha-fetoprotein were within normal limits. These findings suggested HCC, but a benign IgG4-related lymphoplasmacytic inflammatory pseudotumor was also a possibility. We performed a liver biopsy, which confirmed the diagnosis of HCC. The patient underwent liver resection of the right para-median sector one month later. Pathologically, the tumor consisted of atypical hepatocytes arranged in a thin trabecular pattern, in keeping with moderately differentiated HCC (Fig. 4d). The background liver showed multiple bridging fibrosis...
and focal, mild portal inflammation. At the first time of liver biopsy, the liver was moderately fibrotic with periportal and bridging fibrosis, graded as F1 in the New Inuyama classification, but at the time of surgery, the background liver showed multiple bridging fibrosis graded as F2 in the New Inuyama classification. Immunostaining showed a small number of IgG4-positive cells. Hepatocytes also contained brown granules suggesting underlying hemochromatosis (Fig. 4e, f). After surgery, blood tests revealed the ferritin level was 3388.7 ng/mL (normal range, 21.0–282.0 ng/mL). The patient had not had any family history and the first liver biopsy had not revealed iron deposition. Finally, we considered that secondary hemochromatosis and HCC had developed from IgG4-hepatopathy. The patient died of multiple recurrences of HCC just one year later.

**Discussion**

This case demonstrates the possibility of secondary hemochromatosis and HCC developed during IgG4-RD with hepatopathy. IgG4-RD is a new entity which a massive inflammatory infiltrate rich in IgG4-positive plasma cells and fibrosis affects various organs and the pathophysiology still unclear. IgG4-RD affects various organs, most commonly the pancreas, followed by the salivary glands, kidneys, lacrimal glands, and aorta.3 Sclerosing cholangitis, cholecystitis, lymphoplasmacytic inflammatory pseudotumor, and microscopic liver injury are part of the IgG4-RD spectrum of hepatobiliary tract disorders.4,5 IgG4-related liver injury has been described as IgG4-AIH, which
resembles classic AIH apart from elevated serum and tissue IgG4 levels, or as IgG4-hepatopathy, which is a broader entity covering various microscopic changes (e.g., portal inflammation, lobular injury, cholestasis) related to systemic IgG4-RD. In 2016, Nakanuma et al. proposed new diagnostic criteria for IgG4-related AIH. They suggested that patients could be diagnosed with definite or probable AIH based on the IAIHG scoring system. IgG4-related AIH could be definitively diagnosed if patients met all four of the following conditions: (1) serum IgG4 concentration ≥135 mg/dL; (2) ≥10 IgG4-positive cells per HPF in liver tissue; (3) chronic hepatitis with zonal and bridging necrosis or broad collapse; and (4) metachronous or synchronous association with other organ manifestations of IgG4-RD. In the present case, the patient had an IAIHG score of 17, indicating definite AIH. However, his specimen revealed no zonal necrosis and no interface hepatitis, and lobular injury were less conspicuous than in typical AIH, while he fulfilled three of the four parameters. Furthermore, recent clinicopathological analysis identified IgG4-AIH as a subtype of AIH rather than hepatic manifestation of systemic IgG4-RD. We, therefore, considered that the diagnostic term IgG4 hepatopathy was more appropriate in the present case.

Steroids are known to be an effective treatment for the symptoms of IgG4-RD. In the present case, the patient’s symptoms deteriorated when the mPSL was slowly tapered to 10 mg daily. There are currently no guidelines for treating PSL-resistant IgG4-RD, and combination of steroids and immunosuppressive agents to PSL-resistant IgG4 is still controversial. Some studies have reported
on the efficacy of rituximab as a chimeric IgG1 monoclonal antibody against CD20.\textsuperscript{11} Although rituximab is currently not approved for IgG4-RD in Japan, it might be useful in patients whose symptoms are difficult to control with corticosteroids or who have a long history of diabetes. In the present case, IgG4-RD was well controlled after the addition of the immunosuppressive agent, even though the patient subsequently developed HCC. As evidence, the resected liver tissue lacked dense inflammatory infiltrate. However, extensive siderosis consistent with hemochromatosis was unexpectedly noted.

Hemochromatosis is divided into two types, hereditary (HH) and secondary, by its causes. Secondary hemochromatosis could occur in ineffective erythropoiesis, such as myelodysplastic syndrome (MDS) or aplastic anemia, in patients who have received a large number of erythrocyte transfusions, in chronic hepatitis from non-alcoholic steatohepatitis (NASH), or hepatitis C and cirrhosis.\textsuperscript{12} HCC occurs in approximately 30\% of HH cases.\textsuperscript{13} However, there have only been five reported cases of HCC developing in patients with secondary hemochromatosis.\textsuperscript{14,15} Recently, in cases of hepatitis C and NASH, it has been found that even if the degree of liver damage is mild, iron absorption and iron deposition on hepatocytes become excessive such as in secondary hemochromatosis.\textsuperscript{16-18} Furthermore, there are some reports that such hepatic iron overload might induce HCC.\textsuperscript{19} If there is a relationship between IgG4-hepatopathy and iron overloads, such as hepatitis C or NASH, it is possible that HCC has developed from IgG4-hepatopathy.
The relationship between IgG4-RD and carcinogenesis is controversial. Some reports have suggested that pancreatic carcinoma could develop from AIP, and various carcinomas occurred in patients with IgG4-RD during long-term follow-up.\textsuperscript{20} IgG4 has a role in cancer inflammation.\textsuperscript{21} There is a hypothesis that cancer could become a trigger for IgG4-RD such as one of the paraneoplastic syndrome.\textsuperscript{22,23} Although few reports are available, it is also possible that there is a relationship between IgG4-hepatopathy and HCC.

**Conclusion**

We have presented a rare case of secondary hemochromatosis and HCC that developed during the clinical course of IgG4-related disease. Further studies are needed to clarify the clinical course of IgG4-related disease.

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Figure legends

Figure 1. (a) Thoracic CT at the first admission revealed left-sided pleural effusion with pleural thickening and atelectasis of the inferior lobe. (b) Thoracic CT at the second admission revealed bilateral pneumonia and ground-glass opacity, and worsening left-sided atelectasis. (c) Pleural CT improved rapidly after mPSL therapy. (d, e, f) Lung biopsy findings. (d) Plasma cells and lymphocyte infiltration in the bronchial wall and alveoli (hematoxylin and eosin (H&E) staining, ×100). (e, f) Immunostaining showed >10 IgG4-positive plasma cells per HPF. The IgG4/IgG ratio was approximately 40%. (e) IgG stain (×100). (f) IgG4 stain (×100).
**Figure 2. Liver biopsy findings.** (a) Bridging fibrosis and interface hepatitis (H&E stain, ×40). (b) Incomplete hepatocyte rosette formation (H&E stain, ×100). (c, d) More than 10 IgG4-positive cells were observed per high power field and almost 50%–60% of IgG-positive cells were IgG4-positive plasma cells. (c) IgG stain (×100). (d) IgG4 stain (×100).

**Figure 3. Changes in serum IgG, IgG4, and ALT throughout the clinical course.**

**Figure 4.** (a, b) CT demonstrated a 50-mm-diameter tumor located at S7/8. The tumor was non-uniformly hyperattenuated in the early phase, washed-out immediately, and showed faint enhancement in the late phase. (c) MRI showed a well-defined tumor that was enhanced in the early phase. (d, e, f) Pathological findings. (d) Pathologically, the patient was diagnosed with well- and/or moderately-differentiated HCC. Multinuclear atypical epithelial cells with abundant eosinophilic cytoplasm were arranged in a cord-like pattern with partial necrosis (H&E stain, ×200). (e) The remaining hepatic tissue showed multiple bridging fibrosis and focal, mild portal inflammation (H&E stain, ×40). (f) Immunostaining showed a small number of IgG4-positive cells. Hepatocytes also contained brown granules. IgG4 stain (×40).
Fig 1

(a) CT scan of the chest showing bilateral pulmonary infiltrates.
(b) CT scan of the chest showing consolidation in the right lung.
(c) CT scan of the chest showing normal lung parenchyma.
(d) Histopathological examination showing interstitial fibrosis.
(e) Immunohistochemical staining for CD3+ T-cells.
(f) Immunohistochemical staining for CD8+ T-cells.
Fig 2
Fig 3

PSL (half pulse 500 mg/day 3 days, maintenance therapy (20 - 10 mg/day))

AZA (50 - 100 mg/day)

CPA (100 mg/day)

(mg/dL)  
0  1000  2000  3000  4000  5000  6000  7000  8000

I U/L  
0  20  40  60  80  100  120  140  160

(month)

First visit 2 4 6 9 12 18 27 31 37

--- IgG  --- IgG4  --- ALT

CPA: cyclophosphamide, AZA: azathioprine, PSL: prednisolone
Fig 4
Table 1. arterial blood test on second visit

| Blood gas          | Biochemistry          |
|--------------------|-----------------------|
| pH                 | CRP 4.8 mg/dL         |
| pO2 61.5 mmHg      | IgG 7329 mg/dL        |
| pCO2 31.3 mmHg     | IgG4 3510 mg/dL       |
| HCO3 25.2 mmol/L   | AST 105 U/L           |
| BE 2.8 mmol/L      | ALT 106 U/L           |
|                    | γGT 322 U/L           |
|                    | ALP 790 IU/L          |
|                    | AMY 27 IU/L           |
|                    | ANA 1:80              |
|                    | AMA 1:20              |
|                    | Virus marker (-)      |

**Hematology**

| WBC 13000 /μL | CRP 4.8 mg/dL |
|---------------|---------------|
| RBC 448 ×10^6/μL | IgG 7329 mg/dL |
| Hb 11.9 g/μL | IgG4 3510 mg/dL |
| Plt 20.9 ×10^4/μL | AST 105 U/L |

pH: potential of hydrogen
pO2: partial pressure of oxygen
pCO2: partial pressure of carbon dioxide
BE: base excess
WBC: white blood cell
RBC: red blood cell
Hb: hemoglobin
Plt: platelet
CRP: C-reactive protein
AST: aspartate aminotransferase
ALT: alanine aminotransferase
γGT: gamma-glutamyl transpeptidase
ALP: alkaline phosphatase
AMY: amylase
ANA: antinuclear antibody
AMA: antimitochondrial antibodies