CASE REPORT

Immunohistochemical findings in the pancreatic islets of a patient with transfusional iron overload and diabetes : case report

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Abstract : Excessive iron storage sometimes causes diabetes in patients with hemochromatosis, a disease caused by iron overloading. We performed an immunohistochemical analysis to study an autopsy case of aplastic anemia and diabetic hemochromatosis caused by frequent blood transfusions, and extensive hemosiderin deposition was observed in the liver and pancreas. The pancreatic islets of the patient and a control subject were stained to detect glucagon, insulin, and proinsulin. Significantly lower levels of immunoreactivity with both insulin antibodies and proinsulin antibodies, but not with glucagon antibodies, was observed in the islet cells in the patient's tissue than in the islet cells of the control. Hemosiderin deposition in the islets is known to be exclusively distributed in the β-cells, thus, selective iron-induced damage to the β-cells may have affected insulin synthesis and secretion and led to glucose intolerance in the patient. J. Med. Invest. 57 : 345-349, August, 2010

Keywords **:** *hemochromatosis, iron overload, pancreatic islets, diabetes, immunohistochemical analysis*

INTRODUCTION

A considerable number of studies have demonstrated that iron affects glucose metabolism (1-4). Excessive iron accumulation in patients with hemochromatosis, a disorder caused by iron overloading, often results in the clinical manifestation of type 2 diabetes, which provides clinical evidence that excessive iron storage is strongly associated with the development of type 2 diabetes (3, 5). The mechanism underlying the promoting effect of iron on the development of diabetes is not well known, however, some evidence indicates that free radical formation may play a role in the mechanism by disrupting insulin action and total body glucose disposal. Iron is a powerful pro-oxidant, and oxidative stress increases in the case of glucose intolerance. These observations suggest the possible mechanisms underlying the role of iron in diabetes (1, 2). In addition, histopathological and clinical findings in earlier studies have revealed that selective iron-deposition-induced damage to the β-cells of the pancreatic islets may affect insulin secretion and eventually lead to diabetes (6-8). However, immunohistochemical evidence of this mechanism is quite rudimentary. Here, we report an autopsy case of transfusional iron overload and subsequent diabetes, which was encountered by a review of earlier charts, in order to verify the above observations.

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CASE REPORT

A 30-year-old man who experienced bleeding and had developed anemia from the age of 7 was diagnosed with aplastic anemia in 1993. The primary disease had been treated using antithymocyte globulin (ATG), steroids, immunosuppressants, granulocyte-colony stimulating factor (G-CSF), and anabolic hormones, and he had undergone frequent transfusions for the anemia. His serum ferritin levels were extremely high, -ranging between approximately 3900 ng/ml and 9800 ng/ml-, and he was diagnosed with secondary hemochromatosis. Deferoxamine mesylate, an iron-chelating agent, was used to facilitate iron removal, but the ferritin-reducing effect was only temporary. His casual blood glucose levels constantly ranged between 150 mg/dl and 290 mg/dl and his hyperglycemia was treated with diet alone. The patient died of cerebral hemorrhage and pulmonary hemorrhage, and an autopsy was performed 6 h after death. The liver was enlarged and weighed 1780 g, and part of the liver was discolored, suggesting iron deposition (Fig. 1). Hematoxylin-eosin (HE) immunohistochemical (Fig. 2a) and Berlin blue staining (Fig. 2b) of the liver revealed hemosiderin deposition mainly in the hepatic parenchyma and Kupffer cells, and weak hemosiderin staining was observed in the epithelial cells of the biliary duct. The pancreatic slice exhibited diffuse dark-brown staining, a characteristic feature of hemochromatosis (Fig. 3). Berlin blue staining (Figs. 4, 5) of the pancreas revealed significant hemosiderin deposition, mainly in the acinar tissue, pancreatic islets, interstitium, and pancreatic ducts. Hemosiderin deposition was predominantly observed in the peri-insular acini.

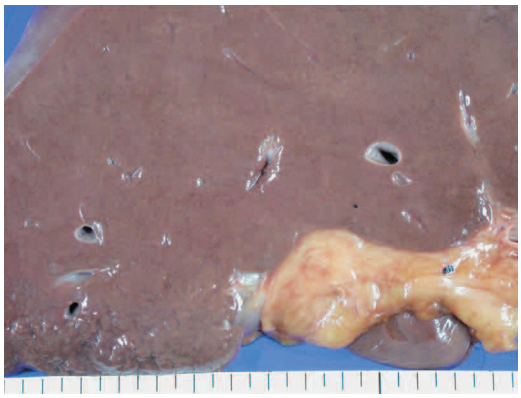

Fig. 1. Macropathology of the liver (slice)

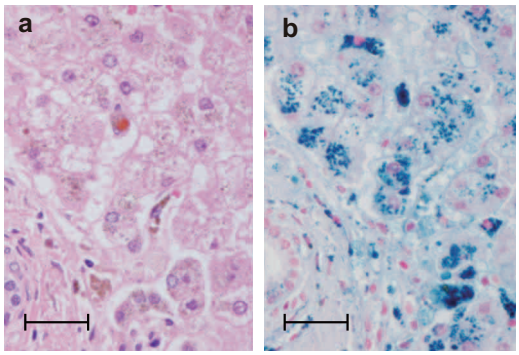

Fig. 2. The hemosiderin in the liver was detected as
a. brown granules by HE staining (×132)
b. blue granules by Berlin blue staining (×132)
The scale bars in Figs. 2 and 4-8 represent 20 μ.

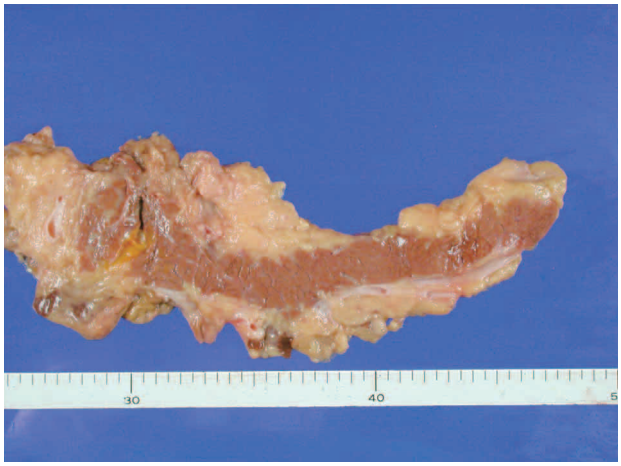

Fig. 3. Macropathology of the pancreas (slice)

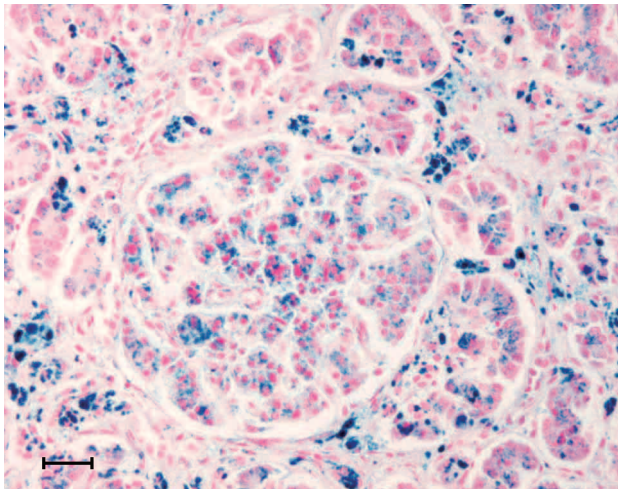

Fig. 4. Significant hemosiderin deposition was detected as blue granules in the acinar tissue and in the islets by Berlin blue staining (×66)

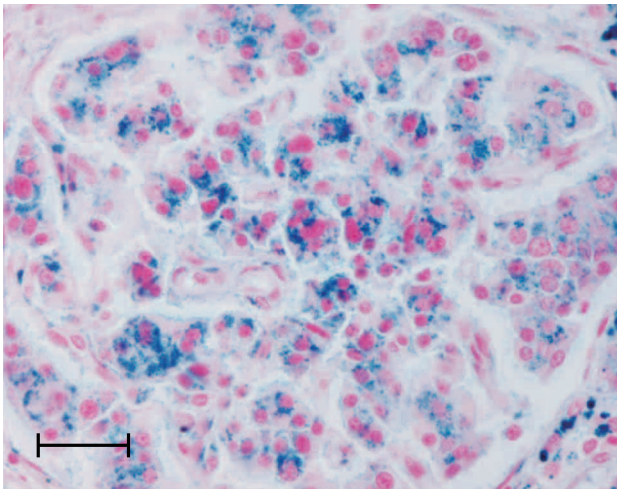

Fig. 5. Hemosiderin deposition in the islet cells (×132)

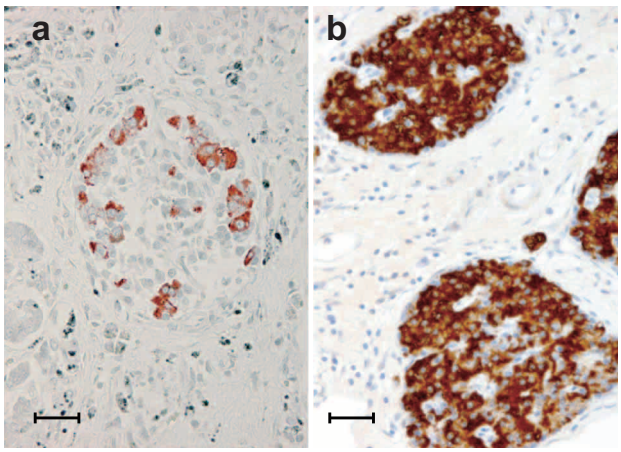

Fig. 7. Immunohistochemical staining for insulin
a. Islet cells of the patient (×66)
b. Islet cells of the control (×66)

The tissue sections of pancreas were also double-stained for glucagon, insulin, proinsulin, and hemosiderin. 3-Amino-9-ethylcarbazole (AEC) was used as the chromogen instead of 3, 3'-diaminobenzidine tetrahydrochloride (DAB), because the brown color reaction obtained with DAB is similar to that of hemosiderin. Immunohistochemical staining of the patient's pancreas (Fig. 6a) with rabbit polyclonal anti-human glucagon antibody (Dako, USA) yielded a staining pattern for glucagon that was similar to or the same as that seen in the control islets of the non iron-overloaded subjects (Fig. 6b), suggesting that the ability of the α-cells to secrete glucagon was preserved. In contrast, staining with guinea pig polyclonal anti-insulin antibody (Dako) revealed that immunoreactive β-cells were quite sparse in the patient's tissues (Fig. 7a), but were abundant and stained intensely in the control tissues (Fig. 7b). Similar results were obtained on staining in the patient's tissue (Fig. 8a) and the control tissue (Fig. 8b) with a mouse monoclonal anti-proinsulin antibody (Abcam, UK). These findings suggest that severe iron deposition in β-cells may interfere with their function and that the β-cells may lose their ability to produce insulin as a result.

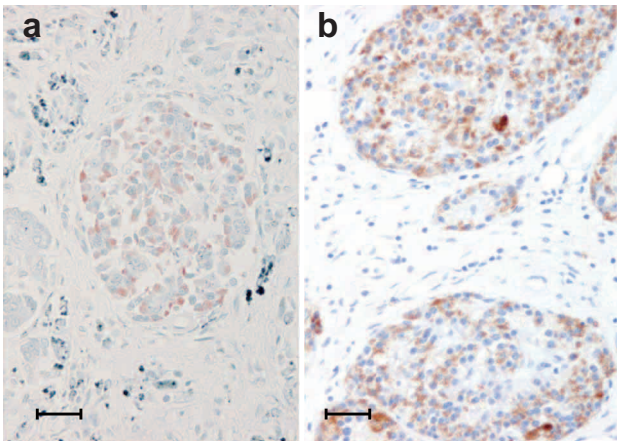

Fig. 6. Immunohistochemical staining for glucagon
a. Islet cells of the patient (×66)
b. Islet cells of the control (×66)

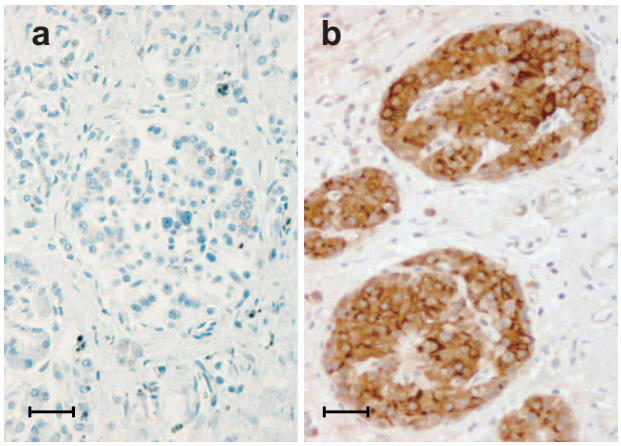

Fig. 8. Immunohistochemical staining for proinsulin
a. Islet cells of the patient (×66)
b. Islet cells of the control (×66)

DISCUSSION

Our patient developed transfusional secondary hemochromatosis and hyperglycemia, and immunohistochemical analysis revealed severe dysfunction of the β-cells of the pancreatic islets. Rahier et

al. (7) reported 7 diabetic patients with primary or secondary iron overload and observed a marked decrease in the number of immunoreactive β-cells in the 4 insulin-requiring diabetic patients among them, which could explain their clinical symptom. A relatively normal number of β-cells was found in other 2 patients without insulin usage, suggesting that the degree of glucose intolerance was related to functional β-cell deficiency. The number of immunoreactive β-cells in our own case was extremely low and the cells stained weakly, suggesting dysfunction of the β-cells due to [1] a decrease in their number, although we did not confirm this possibility by morphometry, [2] failure to synthesize and secrete adequate amounts of immunoreactive insulin, or [3] both of these. In comparison with the control, α-cells in our patient showed normal to slightly less than normal staining. All these findings confirm the findings of the earlier studies (7, 8), including clinical findings of excessive glucagon secretion and deficient insulin response to arginine administration (6), and support the scientific data indicating selective iron deposition in the β-cells in patients with hemochromatosis.

In the earlier report, immunohistochemical and electron microscopic analysis revealed that the iron deposits were restricted to β-cells and were associated with progressive loss of the endocrine granules in the β-cell (7), but the mechanism how this exclusive iron deposition takes place is unclear (7, 8). One possible explanation is that the number of transferring receptors (TfRs) in the β-cell is higher than that in the α-cell. It has been suggested that iron uptake by islet cells in vivo is regulated and mediated by TfR, and the predominance of TfR expression in the β-cells of iron-overloaded rats may result in selective iron deposition in β-cells and predispose them to damage that leads to diabetes (9). Kulaksiz et al. (10) recently reported noteworthy findings with the bioactive peptide hepcidin, which regulates iron uptake in the intestine. Immunohistochemical and immunoelectron microscopic analyses revealed that hepcidin was exclusively localized to β-cells and that it was confined to the insulin-storing secretory granules of the β-cells. This finding suggests that in addition to their known function in blood glucose regulation, pancreatic β-cells may be involved in iron metabolism.

In addition to β-cell dysfunction, genetic diabetes and cirrhosis may interfere in diabetic hemochromatosis (6). Iron deposition in muscle can cause muscle damage, thereby decreasing glucose uptake by the muscle, and iron accumulation in the liver may interfere with hepatic insulin extraction, thereby causing insulin resistance (5). In the cases of older children with thalassemia treated with long-term hypertransfusion therapy, insulin resistance and increased insulin secretion develop before diabetes (11). Thus, the development of diabetes in patients with hemochromatosis may be due to a combination of insulin deficiency and insulin resistance, and this deficiency may be caused by [1] exhaustion of β-cells, [2] iron deposition in the islet cells, or [3] a combination of both (11).

In conclusion, we performed immunohistochemical staining of the pancreas of a patient with transfusional iron overload to clarify the relationship between iron overload and pancreatic β-cell impairment that may result in hyperglycemia. Owing to the recent advances in early diagnosis and treatment of hemochromatosis, very few patients present with hemochromatosis that is serious enough to cause diabetes. Therefore, we consider that the present case is quite informative and that our report has certain significance in understanding the cause and mechanism of diabetes in patients with hemochromatosis.

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REFERENCES

1. Frenandez-Real JM, Lopez-Bermejo A, Ricart W : Cross-talk between iron metabolism and diabetes. Diabetes 51 : 2348-2354, 2002
2. Ford ES, Cogswell ME : Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 22 : 1978-1983, 1999
3. McClain DA, Abraham D, Rogers J, Brady R, Gault P, Ajioka R, Kushner JP : High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditary haemochromatosis. Diabetologia 49 : 1661-1669, 2006
4. Tuomainen T-P, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T, Kaplan GA, Salonen JT : Body iron stores are associated with serum insulin and blood glucose concentrations. Diabetes Care 20 : 426-428, 1997

5. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB : Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 291 : 711-717, 2004
6. Nelson RL, Baldus WP, Rubenstein AH, Go VLW, Service FJ : Pancreatic alpha-cell function in diabetic haemochromatotic subjects. J Clin Endocrinol Metab 49 : 412-416, 1979
7. Rahier J, Loozen S, Goebbels RM, Abrahem M : The haemochromatotic human pancreas : a quantitative immunohistochemical and ultrastructural study. Diabetologia 30 : 5-12, 1987
8. Lu J-P, Hayashi K : Selective iron deposition in pancreatic islet B cells of transfusional iron-overloaded autopsy cases. Pathol Int 44 : 194-199, 1994
9. Lu J-P, Hayashi K, Okada S, Awai M : Transferrin receptors and selective iron deposition in pancreatic B cells of iron-overloaded rats. Acta Pathol Jpn 41 : 647-652, 1991
10. Kulaksiz H, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y : Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. J Endocrinol 197 : 241-249, 2008
11. Merkel PA, Simonson DC, Amiel SA, Plewe G, Sherwin RS, Pearson HA, Tamborlane WV : N Engl J Med 318 : 809-814, 1988