**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

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**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.
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)
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.

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
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.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Toshio Kitazawa for his excellent technical assistance.

REFERENCES


