Decrease of β -cells and Increase of α -cells in a Diabetic Patient with Mitochondrial DNA 3243 (A→G) Mutation

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Abstract

We report here a case of a 53-year-old woman with mitochondrial 3243 (A→G) mutation diabetes. Diabetes mellitus was diagnosed at 39 years of age. At 50 years of age, cardiomyopathy and hypothyroidism were noted. At 53 years of age, the patient was admitted to our hospital for treatment of necrotic ulceration of toes. However, she died of massive heart failure. Pathological examination at autopsy showed a decrease in size, number, and acidophilicity of islets in the pancreas. Immunohistochemical stain revealed a decrease in the β -cell population to $20.5 \pm 9.2\%$ and a relative increase in α -cell population to $48.6 \pm 11.5\%$.

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Key words: diabetes mellitus, islet, insulin, glucagon, mitochondrial DNA 3243 (A→G) mutation

Introduction

Mutation of mitochondrial DNA 3243 (A→G) was first reported by van den Ouweland et al in 1992 (1). Kadowaki et al reported that the incidence of the mutation is 1% of diabetic patients in Japan (2). Gerbitz et al estimated that 1.5% of diabetic patients have the mutation (3). Diabetes with the mitochondrial DNA 3243 (A→G) mutation has several characteristics: progressively decreasing insulin secretion, maternal inheritance, frequent occurrence of deafness, cardiomyopathy, disorder of the cardiac electrical pathway, muscle

atrophy, and rarely, obesity. At onset, many patients can regain sufficient insulin secretion by treatment with sulfonylureas, but most cases require insulin therapy to correct the rapidly diminishing insulin secretion. As there are few reports of pathological analysis of the pancreas (4–7), the association of diminishing insulin secretion with diabetes onset has not been fully clarified. Here, a deceased patient with diabetes with the mitochondrial DNA 3243 (A→G) mutation was autopsied, and the α - and β -cells in the pancreas were examined.

Case Report

A 53-year-old woman with leg edema was admitted to our hospital complaining of ulceration of toes. She had been deaf in the right ear from birth and suffered sensory hearing loss in the left ear from 30 years of age. She had heart failure at 42 and cerebral infarction at 48. Her father had hypertension and acute myocardial infarction, but there was no family history of diabetes mellitus, including her mother and grandmother, or hearing loss. Although urinary sugar was first noted at 34 years of age during pregnancy, she did not consult medical services. With a visual disturbance at 39 years of age, she consulted her family practitioner and was diagnosed with diabetes mellitus. She was medicated with sulfonylurea. However, the patient complained of thirst, and her blood glucose control was poor. Although insulin injection therapy was started at 44 years of age, her HbA1c level was over 8%. She was introduced to our hospital at 49 years of age. Because of her short height, hearing loss, and progressive neuropathy, she was suspected to have diabetes mellitus related to a mitochondrial DNA mutation. The mito-

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Figure 1. Mitochondrial mutation 3243 from adenine to guanine was detected in white blood cells. Total DNA was isolated from blood. A 427 bp fragment encompassing the tRNA Leu (UUR) mutation site located at nucleotide 3243 was amplified by PCR with forward primer, 5'-AAGGTTTCGTTTGTTCACGA (from 3029 to 3048) and reverse primer 5'-AGCGAAGGGTTGTAGTAGCC (from 3437 to 3456). The fragment was digested with Apa-I at 37°C overnight, followed by electrophoresis on a 1% agarose gel. Bands were visualized by ethidium bromide. The mitochondrial DNA of the patient (Lane 4) was partially separated to 214 bp and 213 bp with Apa-I treatment.

chondrial 3243 mutation (A→G) was detected in white blood cell analysis (Fig. 1). Anti-GAD antibody was negative. Suggested by the genetic information, dilated cardiomyo-

pathy and hypothyroidism were found at 50 years of age. Serum levels of TSH, FT3, and FT4 were 5.64 μ U/ml, 2.65 pg/ml, and 1.07 ng/dl, respectively, although anti-thyroglobulin antibody, anti-TPO antibody, and TSH receptor antibody were negative. Dilated cardiomyopathy was treated with 120 mg of ubidecarenone, 40 mg of furosemide, and 2.5 mg of enalapril maleate per day. Hypothyroidism was controlled by 75 μ g of levothyroxine sodium per day. Protein intake was restricted to 40 g per day to protect renal function, and calcium polystyrene sulfonate was used to avoid hyperkalemia. Serum levels of lactic acid and pyruvic acid were increased to 31.8 (normal range: 3.3–14.9) mg/dl and 1.70 (normal range: 0.30–0.94) mg/dl, respectively. The patient had noticed edema in the legs and face related to heart failure since age 51. She was admitted to hospital several times for treatment of diabetic foot ulcers. At age 53 she was admitted complaining of necrotic ulceration of the left first and second toes. Physical examination on admission showed 140.0 cm height, 32.0 kg body weight, 16.3 kg/m² body mass index, 72 beats/min heart rate, 127/85 mmHg blood pressure, and 36.3 °C body temperature. Consciousness was clear and bilateral deafness was obvious. The cervical superficial lymph node was not palpable, the thyroid gland was palpable and elastic hard, a systolic murmur of the heart was audible, respiratory sound was clear, abdominal wall was soft and flat, bilateral dorsal pedis arteries were not palpable, patellar and Achilles tendon reflexes were diminished, tactile sensation and sense of temperature were diminished, and vibration sensations measured by C64 were bilaterally decreased. Diabetic preproliferative retinopathy and macular edema were observed and treated with photocoagulation. There were ulcerations on the left first and second toes. Biochemical analysis revealed elevation of BUN (41.4 mg/dl) and creatinine (1.5 mg/dl), and anemia as 9.7 g/dl of hemoglobin concentration. Cardiomegaly was diagnosed by a 60.6% chest thorax ratio upon chest X-ray. Ultrasound cardiograph

Table 1. Laboratory Data on Admission

Na: 133 mEq/l	K: 5.4 mEq/l	Cl: 95 mEq/l
BUN: 41.4 mg/dl	Cre: 1.5 mg/dl	UA: 8.1 mg/dl
TP: 6.9 g/dl	Alb: 4.0 g/dl	T.bil: 0.5 mg/dl
AST: 24 IU/l	ALT: 16 IU/l	LDH: 579 IU/l
CRP: 1.40 mg/dl	ESR: 69 mm/h	FPG: 71 mg/dl
HbA1c: 7.3%		
WBC: 5,700/ μ l	RBC: 309 $\times$ 10 ⁴ / μ l	Hb: 9.7 g/dl
Ht: 29.5%	Plt: 27.7 $\times$ 10 ⁴ / μ l	
TSH: 5.64 mU/ml	FT3: 2.65 pg/ml	FT4: 1.07 ng/dl
Anti-GAD Ab: negative		Anti-thyroglobulin antibody: negative
Anti-TPO antibody: negative		TSH receptor antibody: negative
Chest X-ray: CTR 60.6%		ECG: ST depression in I, II, ν F, ν A- ν E
UCG: EF 0.30, severe MR and TR, Dd 39 mm, Ds 31 mm		
Urinary Exam.: pH 6.0, protein (+), sugar (-), OB (-), keton body (-)		
U-protein: 360 mg/day	U-Alb: 153.0 mg/day	Ccr: 15.0 ml/min,
U-C-peptide: 4.0 μ g/day		
Glucagon-induced incremental C-peptide at 6 min: 0.3 ng/ml (1.5–1.2)

HE stain $\times 50$

Figure 2. Hematoxylin-eosin staining of pancreas. The islet is acidophilic. Magnification is $\times 50$.

demonstrated heart failure with 0.30 of ejection fraction and severe mitral and tricuspid regurgitation. ST depression in I, II, aV_F, and V₄–V₆ induction was demonstrated by electrocardiography. Although urinary protein excretion was only 360 mg/day, the creatinine clearance level was decreased to 15.0 ml/min. Insulin secretion had diminished because urinary C-peptide was decreased to 4.0 μ g/day and glucagon-induced incremental C-peptide at 6 minutes was 0.3 ng/ml (Table 1). The patient died in hospital at age 53 of severe heart failure despite intensive therapy, in April 1999. Pathophysiological examination of the pancreas at autopsy revealed decreased size, number, and acidophilicity of islets (Fig. 2). Alpha- and β -cells were identified by immunohistochemical staining with insulin and glucagon (Fig. 3), which showed a decrease in β -cell population ($20.5 \pm 9.2\%$) and an increase in α -cell population ($48.6 \pm 11.5\%$) (Fig. 4). Fibrosis and ragged fiber were observed in myocardial muscle and also in muscles of iliacus, psoas, and tongue. The kidney ex-

Figure 4. The percentage of insulin-positive and glucagon-positive cells was $20.5 \pm 9.2\%$ and $48.6 \pm 9.2\%$, respectively, in twenty-one islets. There is a decrease in β -cell population and an increase in α -cell population.

hibited diabetic nephropathy.

Discussion

The patient had the mitochondrial mutation 3243 (A $\rightarrow$ G), detected by peripheral white blood cell analysis, with diabetes mellitus, sensory deafness, cardiomyopathy, and hypothyroidism. Diabetes mellitus with a mitochondrial DNA mutation is known to result in a rapid and severe decrease in insulin secretion, which was the main cause of the onset of diabetes in this case. Only a few reports have investigated the pathological features of the pancreas in diabetic patients with a mitochondrial DNA mutation (A $\rightarrow$ G) (4–7). The four cases to date all reported a large decrease in the pancreatic β -cell population, as seen in this patient. Although an increase in the population of α -cells was observed in this case

immunohistochemical stain (anti-glucagon) $\times 100$

(anti-insulin) $\times 100$

Figure 3. Immunohistochemical staining of representative islets. Magnification is $\times 100$. The same islet stained for glucagon (left) and insulin (right).

as well as in the case reported by Togashi et al (7), Kobayashi et al (5) found a decrease in the number of α -cells. Miyazawa et al (4) and Kuzuya (6) did not mention any pathological alterations of the α -cells. However, the dramatic decrease in the β -cell population together with the increase in α -cell population in this case may suggest a model of pancreatic pathological changes in typical type 2 diabetes. The greater reduction in β -cell population together with the increase in α -cells should decrease insulin secretion and disturb glucose metabolism more profoundly. Most immunocytochemical studies report a smaller reduction in β -cell population (30–50%) in typical type 2 diabetes and only a slight increase in the α -cell population (8–10). Kobayashi et al suggests that mitochondrial DNA mutation 3243 may reduce mitochondrial enzymes by cytochrome c oxidase activity and increased succinate dehydrogenase, which accelerates damage to β -cells through toxic hydroxyl radical generation by the electron transport system (5). Although we did not observe superoxide generation or apoptosis of islet cells, the dramatic reduction in the population of β -cells and the increase in the population of α -cells in the islets of this patient suggests the pathogenesis of type 2 diabetes.

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