INTERNAL MEDICINE

□ CASE REPORT □

Localized Amyloidosis at the Site of Repeated Insulin Injection in a Diabetic Patient

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Abstract

A 60-year-old woman diabetic patient presented with a subcutaneous mass in right lower abdominal quadrant where recombinant human insulin or insulin analogue had been injected for 16 years. Her diabetes has been insulin resistant with insufficient blood glucose control. The mass was extirpated under the suspicion of neoplasm but it was found to consist of diffuse deposition of eosinophilic amorphous materials mixed with inflammatory change. Congo-red staining demonstrated positive red color and yielded green birefringence by polarized microscopy. Pre-digestion with potassium permanganate was incomplete to quench positive Congo-red stains. Immunostains with insulin antibody were positive for this deposition but not so with amylin or AA or AL amyloid. Thus, the mass was considered to be localized amyloidosis composed of iatrogenic A-Ins type amyloid. Thus, the case suggested that her insulin resistance, i.e. refractoriness of insulin treatment, may be ascribed to poor penetration of injected insulin and human insulin itself or its analogue is amyloidogenic to form a local mass.

Key words: insulin injection, localized amyloidosis, insulin resistance

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Introduction

Amyloidosis is a systemic or local disease in which amyloid substances are deposited extracellularly and impair tissue function. Amyloid substances are derived from a variety of peptides, such as amyloid light chain (AL), immunoglobulins, transthyretin, β_2 microglobulin, amyloid Aβ, calcitonin, and islet amyloid polypeptides (amylin) (1, 2). Although the mechanism by which amyloid fibrils are elaborated has extensively been explored, its precise process is still poorly understood (3).

It has been shown that local amyloid deposition very infrequently takes place at the site of repeated insulin injection in patients with insulin requiring diabetes (4, 5). The amyloid at the injected site was identified as amyloid insulin type (A-Ins) (1, 2). To date, there are only 3 case reports in the English language literature on localized amyloidosis caused by repeated insulin injection in diabetic patients (4, 6). The nature of amyloid in the insulin injection site is considered to be insulin itself (4, 5) or insulin-related substance (6). However, due to the rarity of such cases, its pathogenesis and clinical significance are yet to be determined. We recently encountered a case with a subcutaneous abdominal mass with massive amyloid deposition where insulin had repeatedly been injected for the treatment of diabetes.

Case Report

Clinical history

A 60-year-old woman regularly visited our hospital for treatment of her long-standing diabetes, for which insulin had been administered for blood glucose control since the age of 42. She always used right lower quadrant of the ab-

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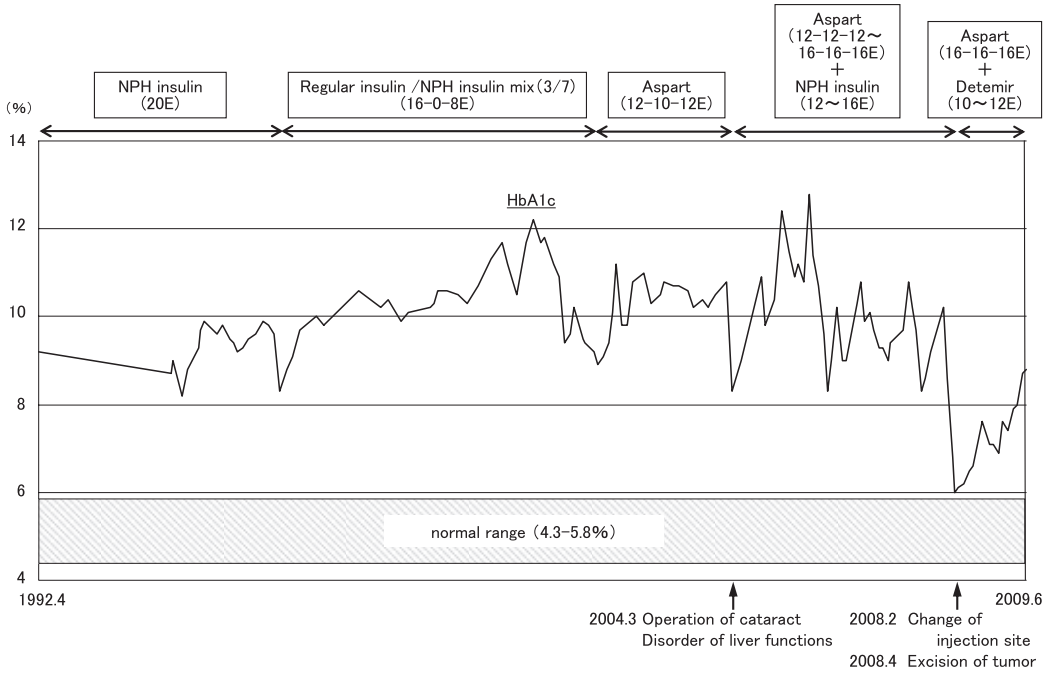

Figure 1. **Clinical course of fluctuated levels of glycated hemoglobin and use of insulin in the patient.**

dominal wall for her insulin injection. A month ago, she noticed a mass in the region where insulin was injected and she was referred to the surgical department. The mass was hen-egg sized, ill-defined and elastic soft. For diagnosis, aspiration biopsy cytology was conducted and suspicion of localized amyloidosis was made. The mass was extirpated and subjected to histological examinations.

The patient was 153 cm in height and body weight was 62 kg, and body mass index was 26.5 kg/m^2. Laboratory findings were as follows: anti-GAD antibody was 0.3 U/mL (negative), insulin antibody 6.5% (negative), serum C-peptide 0.4 ng/mL at non-fasting state. Since she was not admitted, fasting blood glucose level and plasma insulin as well as urine C-peptide levels were not examined. Her blood pressure was high with 150-170 mmHg in systolic and 80-90 mmHg in diastolic. For last 5 years, she had continuous albuminuria and slightly elevated concentrations of BUN (24-28 mg/dL) and creatinine (0.73-0.88 mg/dL). Systemic inflammatory markers such as like C-reactive protein (CRP) (0.2 mg/dL) or leukocyte count were within the normal range. Although retinopathy was not pointed out, her eyes suffered from cataract. She did not have a significant history of tuberculosis, rheumatoid arthritis, or other chronic inflammatory diseases. Nor had she any neoplastic disease.

Since April 1992 (43 years old), she had been treated with human insulin preparations (Fig. 1). Insulin products were first human recombinant insulin (Humalin N) for a year, then switched to mixed type of semisynthetic insulin (Penfil N and Penfil R) for 7 years, then insulin aspart (Novorapid30 mix). From February 5, 2009, insulin detemir (Levemir300) was added. For the previous 16 years, glycated hemoglobin levels were 8.3-12.8%, indicating poor blood glucose control. After noticing of a mass in the right side, she started injection on the left side of the lower abdomen and blood glucose control was improved (HbA1c 6.0-8.0%). CT-scan of her abdomen revealed a round mass in the subcutaneous region and inhomogeneous shadow (Fig. 2). After extirpation of the mass, her blood glucose control was further improved with HbA1c 6.0-7.6%.

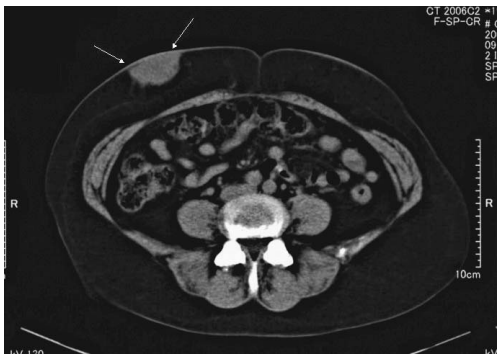

Figure 2. **CT image of a subcutaneous mass in the patient (arrows).**

Informed consent was obtained for this case study from her and all the examinations and description of this case followed the declaration of Helsinki.

Pathological findings

The extirpated mass was 4.5×3.0×2.0 cm in size and the cut surface was glittering yellow-white and homogenous. On Hematoxylin-Eosin sections, most of the space was occupied by eosinophilic homogenous materials (Fig. 3A). There were

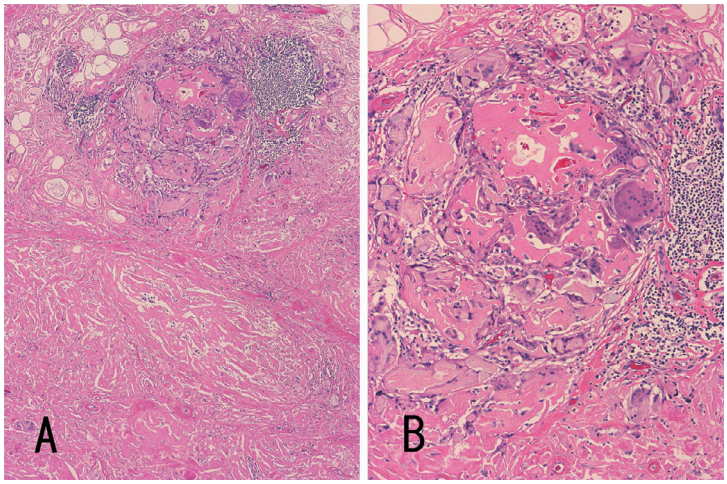

Figure 3. **Light microscopic findings of the abdominal mass showing deposition of eosinophilic amorphous materials (A). There are areas mixed with infiltration of lymphocytes, macrophages and foreign body giant cells, indicating inflammatory reactions against foreign antigens (B). (Original magnification; A, ×120. B, ×360**

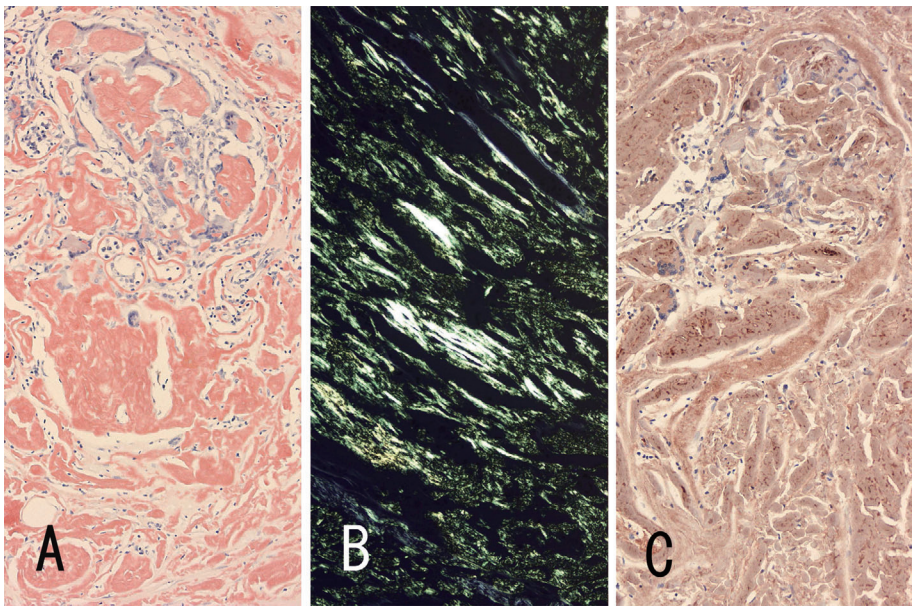

Figure 4. **Confirmation of amyloid as A-Ins type by specific staining. The homogenous depositions are uniformly positive with Congo-red (A), showing green birefringence after potassium permanganate digestion by polarized microscopy (B). Immunostains with insulin show positive reactions (C). (original magnification ×280 for all)**

scattered foci of growth of small blood vessels, infiltration of lymphoid cells, plasma cells and macrophages (Fig. 3B). From place to place, foreign body giant cells surrounded the white transparent amorphous deposits. The eosinophilic homogenous materials were positive with Congo-red stains (Fig. 4A), but incompletely digested with potassium permanganate. They yielded green-birefringence on polarized microscopy even after digestion (Fig. 4B), indicating non-AA type amyloid. Immunohistochemistry demonstrated positive reactions on homogenous materials with insulin antibody (Dako, Carpinteria, CA, USA) (Fig. 4C), but negative for amyloid P (Dako, polyclonal), κ chain (Dako, polyclonal) as well as λ chain (Dako, polyclonal), amylin (Peninsula Lab., San Carlos, CA, USA) and calcitonin (Peninsula Lab.).

Discussion

More than 20 amyloid proteins and 24 precursor proteins are now identified to attribute to formation of amyloid fibrils through common pathways (1, 2). In patients with diabetes, except for pancreatic islets, localized amyloid deposition is unusual and known to occur only at the site of insulin injection. A survey of the English language literature dis-

Table 1. Summary of Previous Cases with Localized Amyloidosis at the Site of Insulin Injection

Author	Gender	Age	Diabetes	Injection site	Duration of insulin	Tumor size (Max)	Insulin reagents	HbA1c	Amyloid
Dische Fe, et al[4]	male	22	type 1	left thigh	8 year	4cm	Porcine	12.4%	A-Ins
Swift B[5]	male	34	type 1	right thigh	17 year	7cm	Porcine	8.3%	Not stated
Albert SG, et al[6]	male	59	type 1	left abdomen	12 year	—	NPH insulin Lispro	10.9%	Not stated
Shikama Y, et al	female	59	type 1	right abdomen	16 year	4.5cm	NPH insulin Regular insulin Aspart Detemir	6.0～12.2%	A-Ins

closed only 3 cases of localized insulin-induced amyloidosis (Table 1) (4, 6). Localized amyloid at the site of insulin injection is now termed as iatrogenic endocrine amyloidosis composed of A-Ins type amyloid of which the precursor is insulin peptide (1, 2). In previous cases, amyloid formation was suspected to be correlated with non-human insulin products, in particular those of porcine origin (4, 5). However, in the present case, the use was limited to only human recombinant insulin or human insulin semisynthetic analogue, and therefore the species of insulin does not seem to be a major reason for the amyloidogenesis in insulin injected sites. In our case, predigestion with potassium permanganate seemed to be incomplete to diminish Congo-red color, so that the deposition may be misinterpreted as AA type amyloid. Confirmation of green birefringence as well as immunohistochemistry is important for the identification of A-Ins type amyloid.

Histological findings demonstrated clear evidence of chronic active inflammatory changes as well as foreign body reactions with multinucleated giant cells around and in the amyloid depositions. There are macrophages that phagocytose amyloid fibrils. Such inflammatory reactions and refractoriness of insulin peptides to digestion by macrophages may possibly contribute to the amyloid fibril formation (7). It appears that the inflammatory processes may be limited to local, not for systemic because serum markers for inflammation like CRP or leukocyte counts were within normal. During this process, accumulated insulin itself and other compounds related to insulin preparations resistant to digestion may be modified to insoluble fibrillar proteins. Alternatively, innate incapability to digest insulin may also play a role in amyloidogenesis. Zaghi et al (8) proposed that impaired activity of the insulin degrading enzyme associated with insulin resistance exerts production of amyloids in the amyloid plaques of the brain of Alzheimer patients. In fact, insulin degrading enzyme is shown to be present in a variety of tissues and cells including macrophages and lymphocytes (9, 10). The present case was also in a condition of insulin resistance with poor blood glucose control. Inflammatory cells around the amyloid in subcutaneous tissues could not digest insulin by insulin degrading enzyme of lymphocytes and macrophages. The emergence of many foreign body type giant cells may support this contention in our case. Consequently, accumulated insulin peptides in soft tissues undergo aggregation with reactive fibrotic processes, and finally result in amyloid fibrils (3).

In previous cases of insulin-induced amyloidosis, it took from 8 to 17 years of insulin injection history for the discovery of a mass of 3-7 cm in size in either the thigh or abdominal wall (4, 6). One of the previous cases was insulin resistant with elevated HbA1c levels as in our case (6). Since blood glucose control became markedly improved shortly after resection of the tumor or after the change of the injection site, the presence of the amyloid mass itself perhaps due to poor penetration of insulin may have contributed to the insulin resistance or in other words, refractoriness of insulin treatment.

The present case thus emphasizes the necessity for the patient care staff to regularly check-up the insulin injection site to prevent infection, inflammation or mass formation. In addition, patient education for the alternate use of insulin injection site is crucial for the avoidance of localized amyloidogensis, which may happen to underlie insulin resistance in poorly controlled diabetic patients. It is hoped that such attention in turn prevents the development of vascular complications and provides a better quality of life in insulin-requiring diabetic patients.

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