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Insulin-derived Amyloidosis and Poor Glycemic Control: A Case Series

Terumasa Nagase, MD, PhD,[a] Keiichi Iwaya, MD, PhD,[b] Yoshiki Iwaki, MD, PhD,[a] Fumio Kotake, MD, PhD,[c] Ryuji Uchida, MD, PhD,[d] Tsunao Oh-i, MD, PhD,[e] Hidenori Sekine, MD,[a] Kazuhiro Miwa, MD,[a] Satoshi Murakami, PhD,[a] Tomotada Odaka, MD, PhD,[a] Masahiko Kure, MD, PhD,[a] Yoko Nemoto, MD, PhD,[a] Masayuki Noritake, MD, PhD,[a] Yoshiya Katsura, MD, PhD[a]

[a]Department of Metabolism and Endocrinology, [c]Department of Radiology, [d]Department of Plastic Surgery, and [e]Department of Dermatology, Tokyo Medical University Ibaraki Medical Center, Ami, Ibaraki, Japan; [b]Department of Pathology, National Defense Medical College, Tokorozawa, Saitama, Japan.

ABSTRACT

OBJECTIVES: Insulin-derived amyloidosis is a rare skin-related complication of insulin therapy. The purpose of this study was to show the effects of insulin-derived amyloidosis on blood glucose levels, insulin dose requirements, and insulin absorption.

METHODS: Seven patients were found to have insulin-derived amyloidosis at the Tokyo Medical University Ibaraki Medical Center. The clinical characteristics and insulin therapy of the 7 patients were investigated. Insulin absorption was studied by comparing the serum insulin levels after insulin injections into insulin-derived amyloidosis sites versus injections into normal sites in 4 patients.

RESULTS: When the insulin-derived amyloidosis was discovered, the mean hemoglobin A1c level was 9.3%, and the mean daily insulin dose was 57 units. After changing the injection sites to avoid the insulin-derived amyloidosis, the blood glucose concentrations improved, and the mean daily insulin dose could be reduced to 27 units ($P = .035$; 53% reduction). The insulin absorption at insulin-derived amyloidosis sites was 34% of that at normal sites ($P = .030$).

CONCLUSIONS: Insulin-derived amyloidosis caused poor glycemic control and increased insulin dose requirements because of impairments in insulin absorption.

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KEYWORDS: Amyloidosis; Insulin absorption; Insulin dose; Insulin therapy

Insulin-derived amyloidosis is a rare skin-related complication of insulin therapy. The amyloid fibril protein is derived

published, including ours,[2] describing 11 patients with

amyloid masses that were immunoreactive to insulin antibodies at the insulin injection sites.[1-8] In a previous report, we

and an increase in insulin dose requirements.[2] However, the clinical characteristics of this condition, particularly changes in glycemic control and insulin dose, have not been well described in other reports. The purpose of this study was to show the effects of insulin-derived amyloidosis on blood glucose levels, insulin dose requirements, and insulin absorption.

MATERIALS AND METHODS

Insulin-derived amyloidosis was defined as a subcutaneous mass at the insulin injection site, provided the following conditions were met: The mass proved to be an amyloid

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Requests for reprints should be addressed to Terumasa Nagase, MD, PhD, Tokyo Medical University Ibaraki Medical Center, Department of Metabolism and Endocrinology, 3-20-1 Chuou, Ami, Ibaraki 300-0395, Japan.

E-mail address: tnagase@tokyo-med.ac.jp

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deposit by pathologic study; and the amyloid deposit was positively stained by an antibody against insulin. From 2005 to 2008, 7 patients were found to have insulin-derived amyloidosis at the Tokyo Medical University Ibaraki Medical Center. For pathologic studies, skin incision biopsy of an abdominal mass was performed in 6 patients, and ultrasound-guided percutaneous fine-needle aspiration biopsy of an abdominal mass was performed in 2 patients. Both procedures were performed in 1 patient for verification of the result of fine-needle aspiration biopsy. The obtained samples were stained with Congo red, and immunohistochemical examinations were carried out using monoclonal antibodies against insulin.[2]

Hemoglobin A1c values were measured by high-performance liquid chromatography and converted to the values certified by the National Glycohemoglobin Standardization Program.[9] For insulin absorption studies, 10 units of insulin lispro or insulin aspart were injected into an abdominal insulin-derived amyloidosis site or an abdominal normal site before breakfast on different days in random order. The studies were performed in 6 patients from whom informed consent was obtained; later, 2 patients were excluded because the endogenous insulin secretions were not negligible. Blood samples were taken before the insulin injection and at 30, 60, and 120 minutes after injection (additional time points were obtained at 90 minutes in patients 3 and 5, at 180 and 240 minutes in patient 1 and 5). Plasma glucose levels were measured on an automated chemical analyzer. Serum insulin and C-peptide values were measured by immunochemiluminometric assays. Area under the curve of serum insulin values from 0 to 120 minutes ($AUC_{0\text{-}120min}$) was calculated using the trapezoidal method, and the peak insulin value (C_{max}) was established. The mean $AUC_{0\text{-}120min}$ and C_{max} after insulin injections into amyloidosis sites versus normal sites were compared by the paired *t* test. Statistical analyses were performed using SPSS version 11.0 (SPSS Inc, Chicago, Ill). All study protocols were approved by the ethics committee of Tokyo Medical University, and all subjects provided written informed consent to participate in this study.

CLINICAL SIGNIFICANCE

- When insulin-derived amyloidosis was discovered at the insulin injection site, patients had poor glycemic control with a mean hemoglobin A1c value of 9.3%.
- After changing the injection sites to avoid the insulin-derived amyloidosis, the blood glucose concentrations improved and the mean daily insulin dose could be reduced by 53%.
- The insulin absorption at insulin-derived amyloidosis sites was 34% of that at normal sites.

RESULTS

The typical physical appearance of insulin-derived amyloidosis consisted of nodules at the insulin injection sites (**Figure 1**). A hard mass, usually 2 to 5 cm in diameter, was palpated at each nodular site. All the masses were located at the sites where the patients repeatedly injected insulin.

The 7 cases are summarized in the **Table**, although the clinical features of patient 1 have been described.[2] Six patients were admitted to the Tokyo Medical University Ibaraki Medical Center because of poor glycemic control (patients 1 and 3), diabetic retinopathy (patient 2), hypoglycemic coma (patient 4), cerebral infarction (patient 5), and diabetic gangrene (patient 7). Patient 5 had a history of an admission for hypoglycemia. In the patients, insulin-derived amyloidosis was discovered during admission. In patient 6, insulin-derived amyloidosis was identified during outpatient management. Of the 7 patients, 6 had type 1 diabetes and 1 had type 2 diabetes. The mean duration of diabetes was 20 years, and the mean duration of insulin therapy was 18 years. All patients had been using insulin analogues for multiple daily insulin injections.

When the insulin-derived amyloidosis was discovered, the patients had poor glycemic control, as reflected by a mean hemoglobin A1c value of 9.3% (range, 8.5%-10.2%). Furthermore, they were using higher doses of insulin; the mean daily insulin dose was 57 units, corresponding to 1.00 U/kg body weight (**Table**). After discovery of the masses, the patients were instructed to change the injection sites to avoid the masses, and then blood glucose concentrations improved. Insulin requirements decreased to a mean daily dose of 27 units ($P = .035$), corresponding to 0.48 U/kg body weight ($P = .040$) (**Table**). Thus, insulin requirements were reduced by 53% by changing the site of insulin injection.

Insulin absorption studies revealed that serum insulin values after insulin injections into insulin-derived amyloidosis sites (**Figure 2**, open circles) were markedly lower than those after injections into normal sites (**Figure 2**, closed circles). The mean $AUC_{0\text{-}120min}$ was 456 pmol/L·h (amyloidosis sites) versus 1258 pmol/L·h (normal sites) ($P = .030$). The mean ratio of $AUC_{0\text{-}120min}$ (amyloidosis site to normal site) was 0.34. The mean C_{max} was 383 pmol/L (amyloidosis sites) versus 1071 pmol/L (normal sites) ($P = .066$). The mean ratio of C_{max} (amyloidosis site to normal site) was 0.34. C-peptide values before and during the studies among all subjects were 0.13 nmol/L or less. Blood glucose values after insulin injections into insulin-derived amyloidosis sites (**Figure 3**, open circles) were relatively higher than those after injections into normal sites (**Figure 3**, closed circles).

DISCUSSION

This study showed that insulin-derived amyloidosis caused worsening blood glucose control and increased insulin dose requirements in insulin-treated diabetic patients. All 7 patients in this study had poor glycemic control at the time the amyloidosis was identified, and their blood glucose levels

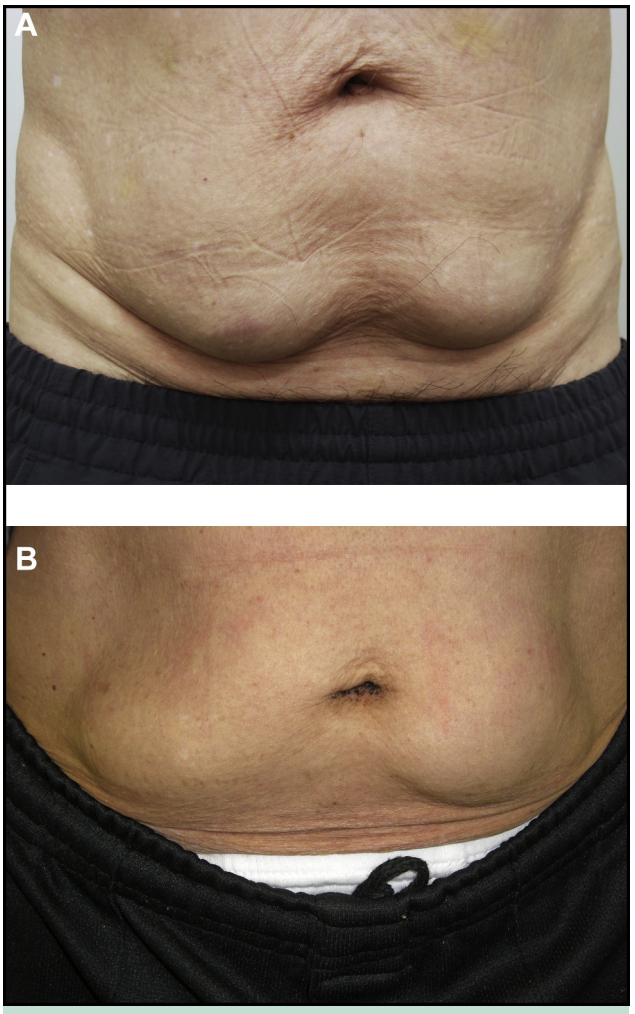

Figure 1 Typical physical appearance of insulin-derived amyloidosis in 2 patients: patient 4 (**A**) and patient 6 (**B**).

improved when insulin was injected at sites other than insulin-derived amyloidosis. Further, their insulin dose requirements were reduced significantly by changing the injection sites. In addition, we presumed that the insulin-derived amyloidosis indirectly resulted in an episode of severe hypoglycemia leading to hospitalization in 2 of the patients probably because they injected an increased dose of insulin into a normal site.[2,8]

This study also revealed that insulin absorption was profoundly impaired at insulin-derived amyloidosis sites. The $AUC_{0\text{-}120min}$ and C_{max} after insulin injections into amyloidosis sites were 34% of those after insulin injections into normal sites (**Figure 2**). In the study, the large portion of insulin injected into insulin-derived amyloidosis sites did not appear in the serum. We speculate that there was increased local degradation of insulin at the insulin-derived amyloidosis sites, as may occur in lipohypertrophic tissue.[10] Alternatively, insulin might become trapped in the amyloid fibrils of insulin-derived amyloidosis after injection.[11]

The mechanism of insulin-derived amyloidosis formation is unknown. Whereas local amyloidosis may occur during continuous subcutaneous insulin infusion in humans and rats,[12] it is not known how the amyloid deposit is produced by usual insulin injections. One factor may be the repeated insulin injections at the same site, which was seen in all of our patients and many previously reported cases,[1,3,6,8] because such injections may induce nucleus formation of amyloid fibril.[11]

Study Limitations

This study has a limitation because the exact prevalence of insulin-derived amyloidosis is not known. To date, only 11 cases have been reported in the English literature, but we have experienced the 7 cases in only 1 institute. Therefore,

Table Clinical Characteristics and Insulin Therapy of the 7 Patients with Insulin-derived Amyloidosis

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (y)	62	32	82	71	80	68	58
Sex	M	M	F	M	F	M	M
Type of Diabetes	Type 1	Type 1	Type 1	Type 1	Type 1	Type 1	Type 2
Duration of Diabetes (y)	20	25	17	30	13	18	17
HbA1c (%)	9.0	9.7	8.8	8.5	10.2	9.5	9.6
Duration of Insulin (y)	20	25	12	25	13	18	15
Insulin Formulations*	L+G	A+G	L+G	L+G	A+N	L+G	L+G
Daily Insulin Dose							
Before (U/d)	116	76	30	47	44	33	52
After (U/d)	24	35	17	26	26	26	34
Daily Insulin Dose per Weight							
Before (U/kg/d)	2.11	1.00	0.76	0.75	0.92	0.75	0.71
After (U/kg/d)	0.42	0.49	0.43	0.41	0.58	0.58	0.46

HbA1c = hemoglobin A1c.

*Insulin formulations are as follows: L, insulin lispro; G, insulin glargine; A, insulin aspart; and N, neutral protamine Hagedorn insulin. All patients continued to use the same insulin formulations after changing the insulin injection sites, except for patient 5 in whom neutral protamine Hagedorn insulin was replaced by insulin detemir.

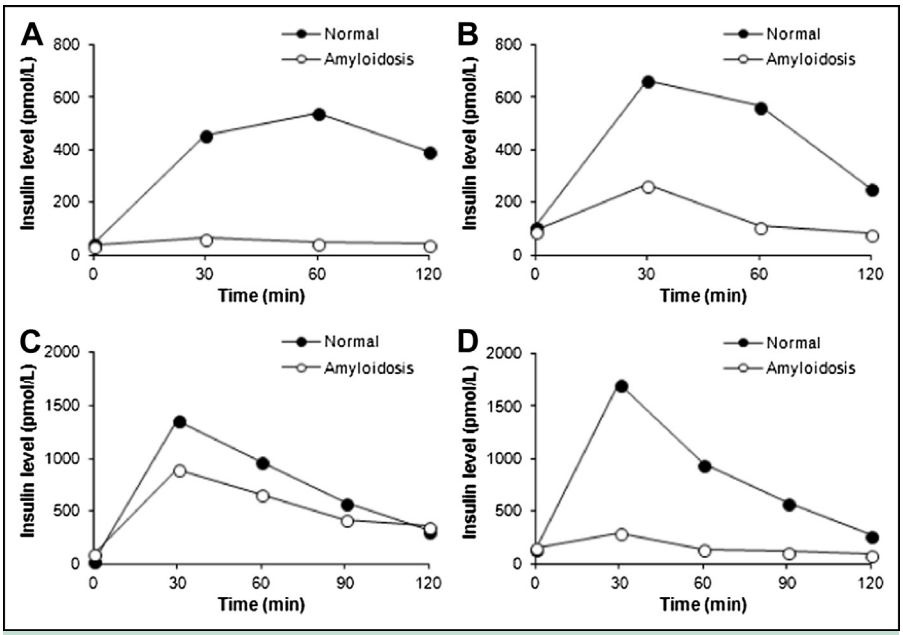

Figure 2 Serum insulin levels after 10 units of an insulin injection into an abdominal insulin-derived amyloidosis site (*open circles*) or a normal site (*closed circles*). (**A**) Insulin lispro injected in patient 1. (**B**) Insulin aspart injected in patient 2. (**C**) Insulin lispro injected in patient 3. (**D**) Insulin aspart injected in patient 5.

insulin-derived amyloidosis may be a more common complication of insulin therapy than previously thought and a more common manifestation of amyloid-related disease. In addition, a recent study indicated that insulin amyloid fibrils have 2 different forms with corresponding differences in cellular toxicity,[13] which may have important clinical implications.

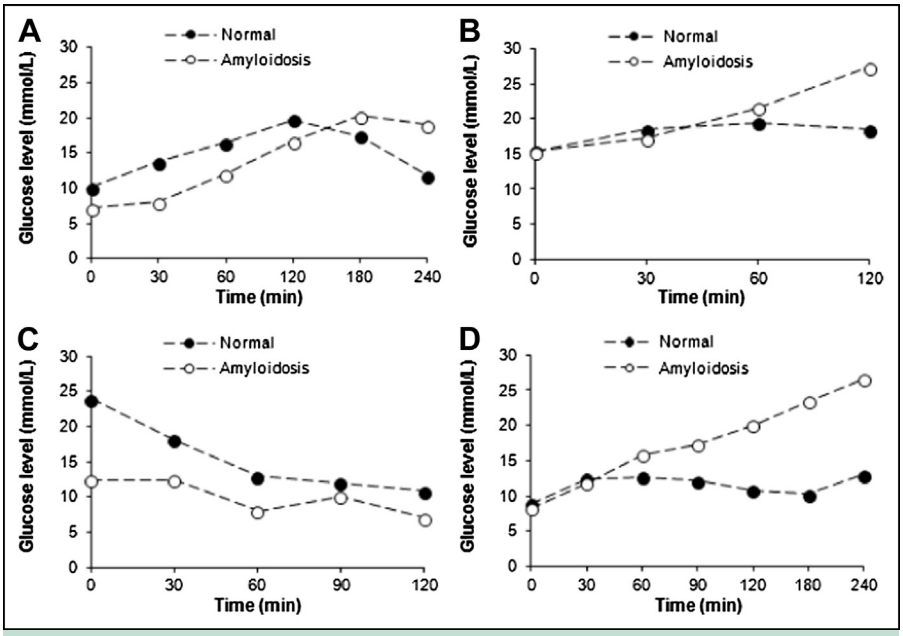

Figure 3 Plasma glucose levels after 10 units of an insulin injection into an abdominal insulin-derived amyloidosis site (*open circles*) or a normal site (*closed circles*). (**A**) Insulin lispro injected in patient 1. (**B**) Insulin aspart injected in patient 2. (**C**) Insulin lispro injected in patient 3. (**D**) Insulin aspart injected in patient 5.

CONCLUSIONS

Insulin-derived amyloidosis can cause poor glycemic control and increased insulin dose requirements in insulin-treated patients because of impairments in insulin absorption. Physicians caring for insulin-treated diabetic patients should be mindful of insulin-derived amyloidosis and specifically assess for it on physical examination, especially in patients with increasing insulin requirements or erratic blood glucose levels.

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References

1. Dische FE, Wernstedt C, Westermark GT, et al. Insulin as an amyloid-fibril protein at sites of repeated insulin injections in a diabetic patient. *Diabetologia*. 1988;31:158-161.
2. Nagase T, Katsura Y, Iwaki Y, et al. The insulin ball. *Lancet*. 2009;373:184.
3. Swift B. Examination of insulin injection sites: an unexpected finding of localized amyloidosis. *Diabet Med*. 2002;19:881-882.
4. Yumlu S, Barany R, Eriksson M, Röcken C. Localized insulin-derived amyloidosis in patients with diabetes mellitus: a case report. *Hum Pathol*. 2009;40:1655-1660.
5. Lonsdale-Eccles AA, Gonda P, Gilbertson JA, Haworth AE. Localized cutaneous amyloid at an insulin injection site. *Clin Exp Dermatol*. 2009;34:1027-1028.
6. Shikama Y, Kitazawa J, Yagihashi N, et al. Localized amyloidosis at the site of repeated insulin injection in a diabetic patient. *Intern Med*. 2010;49:397-401.
7. Sie MPS, van der Wiel HE, Smedts FMM, de Boer AC. Human recombinant insulin and amyloidosis: an unexpected association. *Neth J Med*. 2010;68:138-140.
8. Endo JO, Röcken C, Lamb S, et al. Nodular amyloidosis in a diabetic patient with frequent hypoglycemia: sequelae of repeatedly injecting insulin without site rotation. *J Am Acad Dermatol*. 2010;63:e113-e114.
9. Kashiwagi A, Kasuga M, Araki E, et al. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *Diabetol Int*. 2012;3:8-10.
10. Johansson UB, Amsberg S, Hannerz L, et al. Impaired absorption of insulin aspart from lipohypertrophic injection sites. *Diabetes Care*. 2005;28:2025-2027.
11. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med*. 2003;349:583-596.
12. Störkel S, Schneider HM, Müntefering H, Kashiwagi S. Iatrogenic, insulin-dependent, local amyloidosis. *Lab Invest*. 1983;48:108-111.
13. Zako T, Sakono M, Hashimoto N, et al. Bovine insulin filaments induced by reducing disulfide bonds show a different morphology, secondary structure, and cell toxicity from intact insulin amyloid fibrils. *Biophys J*. 2009;96:3331-3340.