Observational Study

Medicine®

OPEN

Values of ultrasound for diagnosis and management of insulin-induced lipohypertrophy

A prospective cohort study in China

Wei Wang, MD, Rong Huang, MD, Yang Chen, MD, Mei Tu, MD*

Abstract

The aim of this study was to explore the values of ultrasound for diagnosis and management of insulin-induced lipohypertrophy and further analyzing the impact of body mass index and subcutaneous fat thickness on ultrasound manifestations of lipohypertrophy.

In this 3-month, prospective cohort study, a total of 162 patients with diabetes who used insulin therapy more than 1 year with unknown lipohypertrophy status were enrolled into this study. Demographic information, assessment of glycemic control and insulin injection technique were evaluated. Physical and ultrasound examination were separately performed to detect lipohypertrophy by a team of diabetes educator nurses or ultrasonographer in a blinded fashion. Patients with lipohypertrophy received insulin injection technique education based on ultrasound examination and Chinese guideline.

Ultrasound examination detected 41.1% more patients (74.1% vs 52.5%; $P<.001$) with lipohypertrophy and 61.2% more lesions (216 vs 134; $P<.001$) than physical examination. Glycosylated hemoglobin A1c and fasting blood glucose were significantly decreased in patients with lipohypertrophy or subclinical lipohypertrophy (lipohypertrophy without visual and palpation changes) after receiving insulin injection technique education based on ultrasound examination and Chinese guideline than baseline at 3 months ($P<.001$). The proportion of lesions with ultrasound manifestation 2 (distortion of surrounding connective tissue) in obese and STF (>15 mm) groups were no more than 50% and showed a decreased trend with increased subcutaneous fat thickness and body mass index ($P<.001$).

Lipohypertrophy has characteristic ultrasound manifestations which can detect more accurate results than palpation alone and provide detailed information to promote effective education on lipohypertrophy management, thereby improving glycemic control.

Abbreviations: BMI = body mass index, FBG = fasting blood glucose, HbA1c = glycosylated hemoglobin A1c, SFT = subcutaneous fat thickness.

Keywords: diagnosis, glycemic control, lipohypertrophy, ultrasound

1. Introduction

Lipohypertrophy is a common complication of long-term insulin therapy in patients with diabetes characterized by rubber-like or scar-like lesions in the thickened subcutaneous adipose tissue.[1] Cross-sectional studies from different countries such as Spain, Italy, and China reported that the prevalence of lipohypertrophy ranged from 48.7% to 64.4% in patients with long-term insulin therapy.[2–4] Despite the exact prevalence of lipohypertrophy is different in multiple studies, it is indeed common in patients with long-term insulin therapy. Euglycemic clamp test confirmed insulin absorption and action are blunted and considerably more variable when injected into lipohypertrophy, leading to profound deterioration in postprandial glucose control.[5,6] Lipohypertrophy brings great harms to patients with insulin therapy, unexpected hypoglycemic and hyperglycemic events have been reported to be associated with lipohypertrophy,[7,8] while there is no accurate assessment method currently. Traditional physical examination methods lack sensitivity and can only detect lesions with obvious visual and palpation changes. Therefore, a sensitive detection method is urgently needed for clinical diagnosis.

Numerous clinical studies[9–13] demonstrated that lipohypertrophy may have characteristic ultrasound manifestations, while there are lack of congruence in results pertaining to the detection sensitivity of ultrasound and palpation for lipohypertrophy.[9,14] Furthermore, the increased precision of lipohypertrophy assessment whether can improve the glycemic control is still being explored. Ultrasound manifestations of normal subcutaneous tissue structures are often influenced by body mass index (BMI) and STF,[15] whether these factors can influence the ultrasound manifestations of lipohypertrophy are rarely reported. Based on

Editor: Mihnea-Alexandru Găman.

We declared that all authors have read and approved the manuscript and there is no conflict of interest existing in the submission of this manuscript, and manuscript is approved by all authors for publication.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Department of Endocrinology, The First Hospital of Longyan Affiliated to Fujian Medical University, Longyan, Fujian, China.

* Correspondence: Mei Tu, Department of Endocrinology, First Hospital of Longyan Affiliated to Fujian Medical University, Longyan 364000, Fujian, China (e-mail: nfmklysy@163.com).

How to cite this article: Wang W, Huang R, Chen Y, Tu M. Values of ultrasound for diagnosis and management of insulin-induced lipohypertrophy: a prospective cohort study in China. Medicine 2021;100:29(e26743).

Received: 30 December 2020 / Received in final form: 20 May 2021 / Accepted: 28 June 2021

http://dx.doi.org/10.1097/MD.0000000000026743

the above study, we therefore established a prospective study to explore the values of ultrasound for diagnosis and management of insulin-induced lipohypertrophy, and further analyzing the impact of BMI and subcutaneous fat thickness (SFT) on ultrasound manifestations of lipohypertrophy.

2. Research design and methods

2.1. Study design

This prospective study was conducted with patients from the department of Endocrinology at the Longyan First Hospital who fulfilled the study criteria between July 2020 to August 2020. Study inclusion criteria were:

(1) age ≧18 years, male or female;
(2) diagnosed Type 1 or Type 2 diabetes mellitus;
(3) current treatment with a minimum of one insulin injection daily at least 1 years.

Patients were excluded if they were:

(1) prescribed a glucagon-like peptide-1 agonist;
(2) evidence of dermatitis and cutaneous disease;
(3) Allergic to ultrasound coupling agent.

Demographic information, assessment of glycemic control and insulin injection technique were evaluated by a physician or research nurse. And then, all patients were preformed with physical and ultrasound examination for the presence of lipohypertrophy. This study was approved by the Ethical Committee of Longyan First Hospital (LY-2020–007). Written informed consent from each patient was waived.

2.2. Assessment of lipohypertrophy

All patients were examined for clinical lipohypertrophy on the injection sites through visual inspection and palpation by a single team of nurses certified as diabetes educators. The lipohypertrophy lesion appear to have raised surface or pigmentation and changes in hair distribution at visual inspection or with soft consistency at palpation.

Ultrasound examination was conducted by a portable GELOGIQe machine with an L8–18i-D probe (8–18 MHz;GE Healthcare, Frankfurt, Germany) by a specific ultrasonographer. The settings of the ultrasound were the same for all patients. The ultrasonographer recorded the presence of characteristic ultrasound manifestations such as 1) a nodular shape lesion with a hypoechoic or hyperechoic halo was well circumscribed in the subcutaneous layer (ultrasound manifestation 1); 2)with distortion of surrounding connective tissue (ultrasound manifestation 2); 3) absence of vascularity and capsule (ultrasound manifestation 3); 4) heterogeneous in echotexture compared with surrounding tissue (ultrasound manifestation 4). The ultrasonographer and researchers blinded to the clinical findings. Both ultrasonographer and researchers were involved in the sonographic analysis of lipohypertrophy to reduce inter-operator variability. Lesion matched three of the above characteristic ultrasound manifestations were identified as lipohypertrophy lesion.

The location of lipohypertrophy lesion detected by physical and ultrasound examination were separately documented on a numbered grid system. The presence of lesion within the same area of the grid or within 1 cm from each other were considered to be the same lesion and in agreement.

2.3. Lipohypertrophy management

Patients with lipohypertrophy meeting ultrasound criteria received insulin injection technique education based on ultrasound examination and Chinese guideline for diabetic injection technology[16] (mainly educating patients to avoid injecting at lipohypertrophy lesions and master correct insulin injection technique). Glycosylated hemoglobin A1c (HbA1c), fasting blood glucose (FBG) and total daily insulin dose were used to evaluate the values of ultrasound on lipohypertrophy management.

2.4. Statistical analysis

Data were analyzed by using the SPSS 23.0 software (SPSS Inc. IBM). Descriptive data are expressed as means ± standard deviation (SD). Discrete variables were summarized in frequency tables (N, %). Differences between groups were analyzed using a paired Student *t*-test for normal continuous variables and a non-parametric Wilcoxon test for non-normal data. Contingency tables were evaluated using chi-square. Values of $P<0.05$ were considered statistically significant.

3. Results

3.1. Clinical characteristics

A total of 162 patients meeting the inclusion and exclusion criteria were selected for the study. The mean age was 59.5 ± 11.8 years, 93 males and 69 females, mean BMI was 25.6 ± 3.6 Kg/m^2, and mean duration of insulin used was 7.8 ± 4.3years. All patients injected with insulin pens. The distribution of needle sizes was: 4 mm needles, 38.0%; 6 mm needles, 39.0%; 8 mm needles, 23.0%. Only 36.7% of patients rotated the injection site. More than 80.0% reused the injection needles.

3.2. Values of ultrasound in detecting and managing lipohypertrophy

Inspection and palpation identified 134 lesions consistent with lipohypertrophy present in 85 (52.5%) patients by a single team of nurses certified as diabetes educators. Ultrasound examination identified 216 lesions meeting ultrasound criteria in 120 (74.1%) patients, all lesions identified by inspection and palpation meeting ultrasound criteria. In addition, 35 (21.6%) patients only meeting ultrasound criteria without lipohypertrophic visual and palpation changes (subclinical lipohypertrophy). Ultrasound examination detected 41.1% more patients (74.1% vs 52.5%; $P<.001$) with lipohypertrophy and 61.2% more lesions (216 vs 134; $P<.001$) than physical examination.

In order to explore the benefit of ultrasound on glycemic control, a total of 120 patients with lipohypertrophy meeting ultrasound criteria received insulin injection technique education based on ultrasound. As expected, after 3 months of insulin injection technique education, patients achieved a lower HbA1c level (7.82% ± 0.44% vs 7.27% ± 0.36%; $P<.001$) and a lower FBG level (8.21 ± 0.4mmol/L vs 7.31 ± 0.35mmol/L; $P<.001$), while there was no significant difference in total daily insulin dose from baseline (54.0 ± 9.6 IU vs 54.5 ± 8.6 IU; $P=.157$). Furthermore, In addition, 35 (29.2%) patients with subclinical lipohypertrophy also achieved a lower HbA1c level (7.67% ± 0.30% vs 7.26% ± 0.31%; $P<.001$) and a lower FBG level (8.19 ± 0.34 mmol/L vs 7.42 ± 0.35mmol/L; $P<.001$), while there was

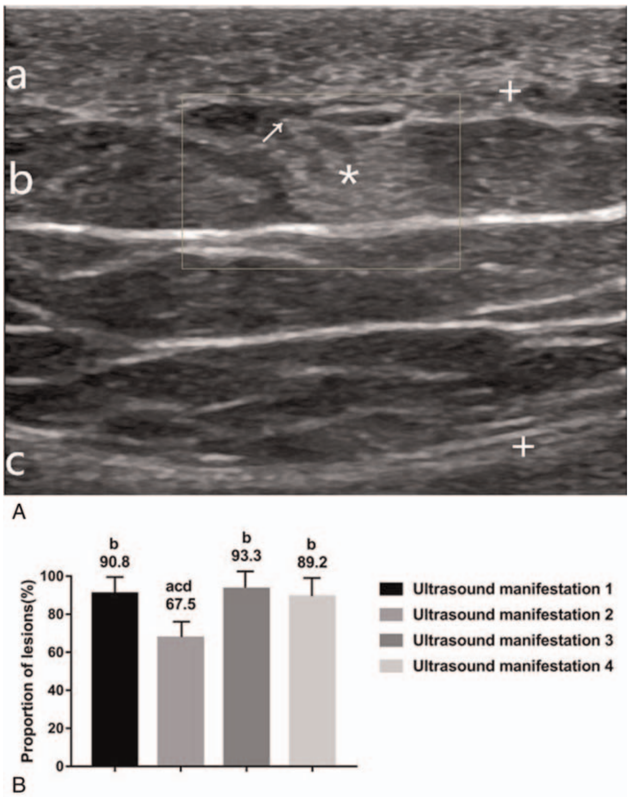

Figure 1. Characteristic ultrasound manifestations of lipohypertrophy (A) and the proportion of lesions with ultrasound manifestations (B). Epidermal and dermal layers (a) are separated from the muscular layer (c) by the subcutaneous layer (b), a nodular shape lesion with a hypoechoic halo or hyperechoic halo (*) was well circumscribed in the subcutaneous layer (ultrasound manifestations); with distortion of surrounding connective tissue (ultrasound manifestation 2); absence of vascularity and capsule (ultrasound manifestation 3); heterogeneous in echotexture compared with surrounding tissue (ultrasound manifestation 4). The distance between the two (+) is the thickness of the subcutaneous fat. $^{a}P<.05$ vs ultrasound manifestation 1; $^{b}P<.05$ vs ultrasound manifestation 2; $^{c}P<.05$ vs ultrasound manifestations; $^{d}P<.05$ vs ultrasound manifestations.

no significant difference in total daily insulin dose from baseline (53.1±9.7 IU vs 53.7±8.9 IU; $P=.470$).

3.3. Impacts of BMI on ultrasound manifestations of lipohypertrophy

Characteristic ultrasound manifestations of lipohypertrophy are shown in Figure 1A. The proportion of lesions with ultrasound manifestation (1,2,3, and 4) were 90.8%, 67.5%, 93.3%, and 89.2% separately. Moreover, proportion of lesions with ultrasound manifestation 2 was lower than other three characteristic ultrasound manifestations (Fig. 1B). Ultrasound manifestations of lipohypertrophy in patients with normal weight (36.7%), overweight (33.3%), and obese (30.0%) are shown in Figure 2 (A-C). The proportion of lesions with ultrasound manifestation 2 (Fig. 2D) was significant decreased in obese group than other two groups (38.9% vs 88.6% and 38.9% vs 70.0%; all $P<.001$), while there was no significant difference in the proportion of lesions on ultrasound (1, 3, and 4) among the 3 groups (all $P>.05$).

3.4. Impacts of SFT on ultrasound manifestations of lipohypertrophy

In order to explore the impacts of SFT on ultrasound manifestations of lipohypertrophy, we measured the SFT in lipohypertrophy lesions, the mean SFT in lipohypertrophy lesions was 15.2±6.3 mm, the distribution of SFT in lipohypertrophy lesions >15 mm and 5–15 mm were 49.4% and 50.6%. The ultrasound manifestations of lipohypertrophy in patients with SFT (5–15 mm and >15 mm) are shown in Figure 3A–B. The proportion of lesions with ultrasound manifestation 2 (Fig. 3C) was significant decreased in SFT (5–15 mm) group than SFT (>15 mm) group (49.2% vs 85.2%; $P<.001$), while there was no significant difference in the proportion of lesions on ultrasound (1, 3, and 4) between SFT (>15 mm) group and SFT (5–15 mm) group (all $P>.05$).

4. Discussion

Lipohypertrophy is a common complication of long-term insulin therapy in patient with diabetes which is associated with poor glycemic control and huge extra insulin consumption. This study aimed to explore the values of ultrasound for diagnosis and management of insulin-induced lipohypertrophy, and further analyzing the impact of BMI and SFT on ultrasound manifestations of lipohypertrophy. The results showed that lipohypertrophy has characteristic ultrasound manifestations. Ultrasound detected more patients and more lesions than inspection and palpation. The glycemic control was improved in patients with lipohypertrophy after receiving insulin injection technique education based on ultrasound examination.

Normal abdominal skin consists of epidermal, dermal and subcutaneous layers. Epidermal and dermal layers (smooth linear strong echo, smooth surface, uniform thickness)are separated from the muscular layer by the subcutaneous layer consisting of adipose tissue (isoechoic or hypoechoic) and thicker connective tissue (hyperechoic).[17] The duration of insulin use, frequency/extent of needle or syringe reuse, and site rotation are three independent risk factors for the formation of lipohypertrophy.[18] Subcutaneous tissue injury and fibrotic scar formation caused by repeated injection at the same site are the main reason for formation of hyperechoic halo under ultrasound.[19,20] Incomplete absorption of insulin (hypoechoic component) leads to accumulation of insulin at the injection site, which is the main cause of hypoechoic halo under ultrasound. The superposition and delayed absorption caused by incomplete absorption of insulin may be one of the reasons for frequent hypoglycemia in patients with insulin therapy.[21] Insulin also belongs to a kind of growth factor, which has the functions of promoting growth and inducing adipocyte proliferation and differentiation.[22] Histopathological examination showed that lipohypertrophy can invade the adjacent fibrous and connective tissue, with metabolic activation characteristics such as phagocytosis of lipid droplets and proliferation, without neovascularization.[23] These histopathological features provided a basis for ultrasound manifestations (distortion of surrounding connective tissue and absence of vascularity). It is worth noting that appearance of connective tissue is more likely to be influenced by inter-individual variations of adipose tissue distribution.[24] In the current study, we found that the proportion of lesions with ultrasound manifestation 2 (distortion of surrounding connective tissue) in obese and SFT (>15 mm) groups were no more than 50% and showed a

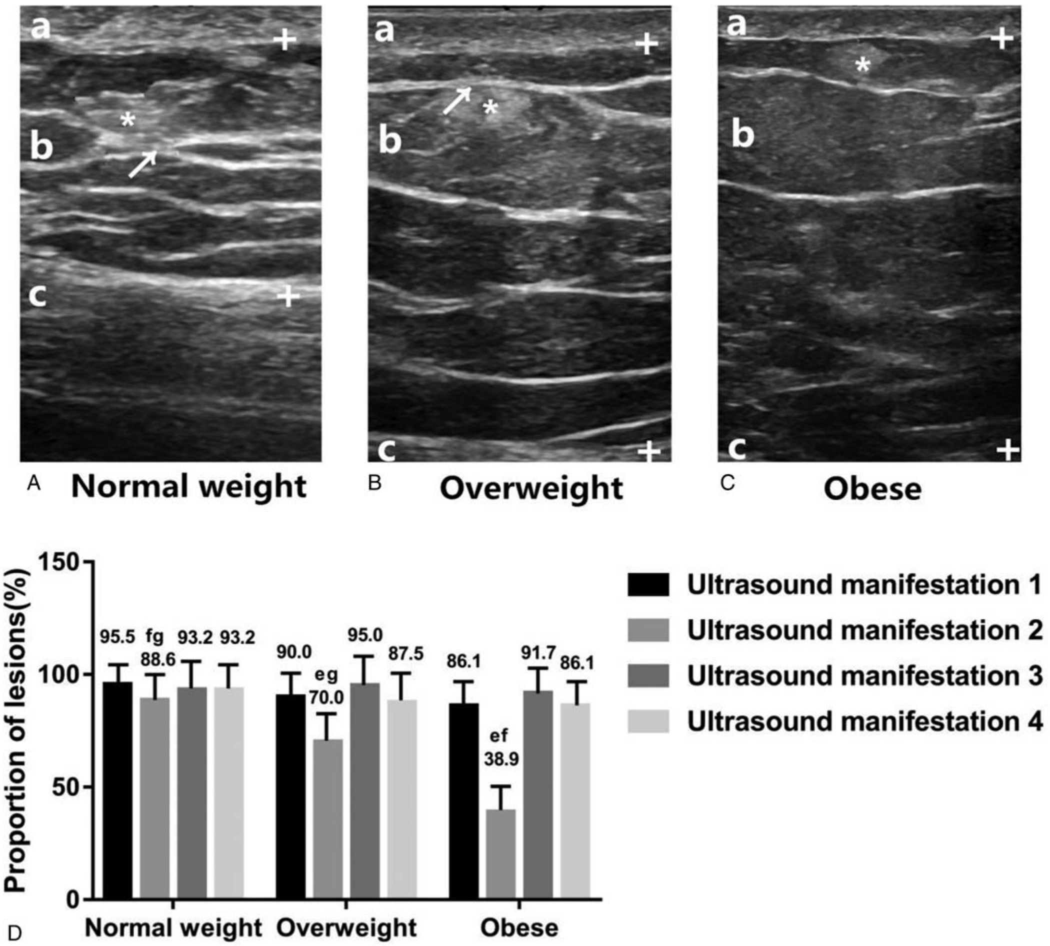

Figure 2. The presence of lipohypertrophy ultrasound manifestations in patients with normal weight (A), overweight (B), and obese (C). The proportion of lesions (D) with ultrasound manifestations (1–4) in normal weight group (n = 44), overweight group (n = 40), and obese group (n = 36). $^{e}P < .05$ vs normal weight group; $^{f}P < .05$ vs overweight group; $^{g}P < .05$ vs obese group.

decreased trend with increased SFT and BMI. These findings in our study may indicated us that the distortion of surrounding connective tissue is not necessary for the diagnosis of lipohypertrophy in patients with obvious obesity.

Lipohyertrophy has reported to be associated with adverse blood glucose events, worse glycemic control, more insulin consumption and cost implications, insulin injection technique education is the important means to manage lipohypertrophy. The Insulin Injection Technique Survey showed that the proportion of patients mastered correct insulin injection technique in China are sub-optimal, most of them do not realize they have lipohypertrophy and or are unaware of the hazards of injecting into lipohypertrophy.[25] Thus, early and accurate detection of lipohypertrophy and educating patients to avoiding injection at lipohypertrophy are the key steps of insulin injection technique education. Previous studies showed a lack of congruence in results pertaining to the detection sensitivity of ultrasound and palpation for lipohypertrophy lesions. Kasperska-Czyzyk T[14] reported that palpation detected 64% more lipohypertrophy lesions than ultrasound. Conversely, with increased sensitivity of the ultrasound probe, several recent studies[9,11,26] demonstrated that ultrasound can detect 50% more lesions and 5 cm^2 more areas of lipohypertrophy lesions than palpation, the results in our study further confirmed that ultrasound can identify patients with lipohypertrophy more frequently than palpation and inspection, and demonstrated the great advantage of ultrasound in detecting subclinical lipohypertrophy, which is also significantly independently associated with the non-optimal glycemic control (OR = 9.97, 95% CI: 3.46–28.75).[27] Moreover, ultrasound detection can also provide more details about the nature and severity of lipohypertrophy (size, distribution and elasticity) and accurately measure the SFT which can help educators better conduct better conduct insulin injection education. In our study, Patients with lipohypertrophy meeting ultrasound criteria received insulin injection technique education based on ultrasound examination (mainly educating patients to avoid injecting at lipohypertrophy lesions and master correct insulin injection technique), patients achieved lower HbA1c and FBG levels without increasing insulin dose after 3 months. In addition, patients with subclinical lipohypertrophy also achieved better glycemic control which is often overlooked due to the inaccurate identification of physical examination. These results in our study indicated us that ultrasound can help patients with lipohypertrophy to improve glycemic control by accurately detecting lipohypertrophy lesion.

To our knowledge, this is the first study in China to apply ultrasound to detect and manage lipohypertrophy and analyze

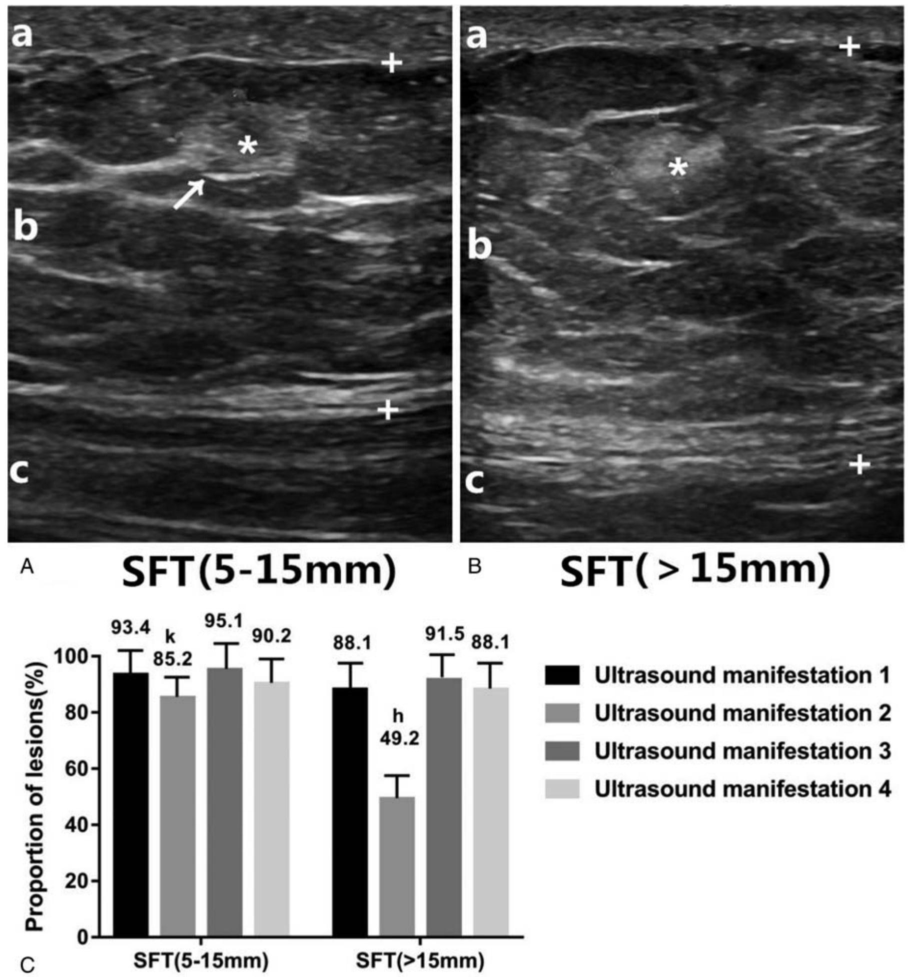

Figure 3. The presence of lipohypertrophy ultrasound manifestations in patients with SFT (5–15 mm) (A) and SFT (>15 mm) (B). The proportion of lesions (C) with ultrasound manifestation (1–4) in SFT (5–15 mm) group (n=61) and SFT (>15 mm) group (n=59). SFT = subcutaneous fat thickness. $^{k}P<.05$ vs SFT (5–15 mm) group; $^{h}P<.05$ vs SFT (>15 mm)group. SFT = subcutaneous fat thickness.

the impact of BMI and SFT on ultrasound manifestations of lipohypertrophy. The results in our study had confirmed the feasibility and accuracy of ultrasound detection in detecting and managing lipohypertrophy. There still have some limitations in our study. First, this study was limited by manpower and financial resources, only 162 patients were enrolled into this study and carried out in a single care center. Second, the results in our study had not confirmed the cost-effectiveness of ultrasound in comparison to palpation, more research with robust study design is need to verify.

In conclusion, the overall evidence in our study implicates that ultrasound detection can detect more accurate results than palpation alone and provide detailed information, which can promote effective education on injection management to improve self-management and diabetes outcomes. The use of ultrasound detection in routine lipohypertrophy assessment is foreseeable.

Acknowledgments

The authors thank the department of ultrasonography of our hospital providing the ultrasound equipment and technical support.

Author contributions

Data curation: Wei Wang.
Investigation: Wei Wang, Rong Huang, Yang Chen, Mei Tu.
Software: Wei Wang.
Writing – original draft: Wei Wang.
Writing – review & editing: Mei Tu.

References

[1] Thow JC, Johnson AB, Marsden S, et al. Morphology of palpably abnormal injection sites and effects on absorption of isophane (NPH) insulin. Diabetic Med 1990;7:795–9.
[2] Blanco M, Hernández MT, Strauss KW, et al. Prevalence and risk factors of lipohypertrophy in insulin-injecting patients with diabetes. Diabetes Metab 2013;39:445–53.
[3] Grassi G, Scuntero P, Trepiccioni R, et al. Optimizing insulin injection technique and its effect on blood glucose control [J]. J Clin Transl Endocrinol 2014;1:145–50.
[4] Ji L, Sun Z, Li Q, et al. Lipohypertrophy in China: prevalence, risk factors, insulin consumption, and clinical impact. Diabetes Technol Ther 2017;19:61–7.
[5] Famulla S, Hövelmann U, Fischer A, et al. Insulin injection into lipohypertrophic tissue: blunted and more variable insulin absorption and action and impaired postprandial glucose control. Diabetes Care 2016;39:1486–92.

[6] Hövelmann U, Famulla S, Hermanski L, et al. Insulin injection into regions with lipohypertrophy (LHT) worsens postprandial (PP) blood glucose (BG) versus injections into normal adipose tissue (NAT). Diabetes 2015;64(suppl 1):A254–5.
[7] Boon IS, Saeed MA. Cautionary tale: subdural haematoma following frequent hypoglycaemia from insulin-induced lipohypertrophy. BMJ Case Rep 2015;2015:bcr2015212855.
[8] Barola A, Tiwari P, Bhansali A. Insulin-mediated lipohypertrophy: an uncommon cause of diabetic ketoacidosis. BMJ Case Rep 2017;2017: bcr220387-2017.
[9] Volkova N, Davidenko I, Rudakova J, et al. Insulin-induced lipohypertrophy diagnostics in diabetic patients: subcutaneous fat ultrasonography; proceedings of the 17th European Congress of Endocrinology. BioScientifica, F 2015.
[10] Bertuzzi F, Meneghini E, Bruschi E, et al. Ultrasound characterization of insulin induced lipohypertrophy in type 1 diabetes mellitus. J Endocrinol Invest 2017;40:1107–13.
[11] Kapeluto JE, Paty BW, Chang SD, et al. Ultrasound detection of insulin-induced lipohypertrophy in Type 1 and Type 2 diabetes. Diabet Med 2018;35:1383–90.
[12] Klimontov V, Lazarev M, Makhotin A, et al. Insulin-induced lipohypertrophy: clinical and ultrasound characteristics. Diabetes Mellitus 2018;21:143–54.
[13] Lazarev M, Klinontov V, Makhotin A, et al. Quantitative ultrasound characteristics of insulin-induced lipohypertrophy in subjects with diabetes. Diabetologia 2018;61:S125.
[14] Kasperska-Czyzyk T, Stefanski P, Elwertowski M, et al. Ultrasonographic assessment of subcutaneous lipohypertrophy at insulin injection sites [J]. Diabetes research and clinical practice 2006;50:78.
[15] Gibney MA, Arce CH, Byron KJ, et al. Skin and subcutaneous adipose layer thickness in adults with diabetes at sites used for insulin injections: implications for needle length recommendations. Curr Med Res Opin 2010;26:1519–30.
[16] Ji L, Guo X, Huang J. Chinese guideline for diabetic injection technology. Chin J Diabetes Mellitus 2017;9:79–105.
[17] Alfageme RF. Ultrasound skin imaging. Actas dermo-sifiliograficas 2014;105:891–9.
[18] Vardar B, Kizilci S. Incidence of lipohypertrophy in diabetic patients and a study of influencing factors. Diabetes Res Clin Pract 2007;77:231–6.
[19] Johansson UB, Amsberg S, Hannerz L, et al. Impaired absorption of insulin aspart from lipohypertrophic injection sites. Diabetes Care 2005;28:2025–7.
[20] Wallymahmed ME, Littler P, Clegg C, et al. Nodules of fibrocollagenous scar tissue induced by subcutaneous insulin injections: a cause of poor diabetic control. Postgrad Med J 2004;80:732–3.
[21] Gentile S, Strollo F, Della T, et al. Skin complications of insulin injections: a case presentation and a possible explanation of hypoglycaemia. Diabetes Res Clin Pract 2018;138:284–7.
[22] Hauner H, Stockamp B, Haastert B. Prevalence of lipohypertrophy in insulin-treated diabetic patients and predisposing factors. Exp Clin Endocrinol Diabetes 1996;104:106–9.
[23] Fujikura J, Fujimoto M, Yasue S, et al. Insulin-induced lipohypertrophy: report of a case with histopathology. Endocr J 2005;52:623–8.
[24] Nedelec B, Forget NJ, Hurtubise T, et al. Skin characteristics: normative data for elasticity, erythema, melanin, and thickness at 16 different anatomical locations. Skin Res Technol 2016;22:263–75.
[25] Song Z, Guo X, Ji L, et al. Insulin injection technique in China compared with the rest of the World[J]. Diabetes Ther 2018;9:2357–68.
[26] Gentile S, Guarino G, Giancaterini A, et al. A suitable palpation technique allows to identify skin lipohypertrophic lesions in insulin-treated people with diabetes. Springer Plus 2016;5:563.
[27] Luo D, Shi Y, Zhu M, et al. Subclinical lipohypertrophy–Easily ignored complications of insulin therapy [J]. J Diabetes Complicat 2020;35:107806.