Intractable hypoglycaemia induced by the antibody against exogenous insulin in a Chinese patient with type 1 diabetes

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Introduction:

Circulating insulin antibodies are able to disrupt normal insulin function and cause hypoglycemia, hyperglycemia or both[1]. This kind of antibodies can be produced in patients with or without insulin therapy. If patients who have never been exposed to exogenous insulin generate insulin antibodies in their circulation due to autoimmune disorders and show the symptoms of hypoglycemia or mild insulin resistance, which is named as insulin autoimmune syndrome (IAS) or Hirata's disease[2]. If diabetic patients treated with insulin drugs generate antibodies against exogenous insulin and therefore have symptoms of severe hypoglycaemia or insulin resistance, which is named as exogenous insulin antibody syndrome (EIAS)[3]. IAS is mainly reported in Japanese population while EIAS is very rare regarding its prevalence.

Both in IAS and EIAS, insulin antibodies are proposed as an buffering agent to bind and release insulin dynamically[4]. Since insulin is released from the insulin antibodies under fasting condition, it induces severe fasting hypoglycaemia. On the contrary, postprandial hyperglycemia occurs when insulin is bound by insulin antibodies and can not exert its function to lower blood glucose. However, this mechanism has not been proved by experimental data and remains controversial.

There have been a few rare cases worldwide where patients with type 1 diabetes (T1D) treated with recombinant human insulin have developed a high titre of circulating insulin antibodies. The presence of these antibodies has led to unstable glycaemic control, resulting in a severe form of insulin resistance or recurrent hypoglycemia. In this project, we report a 25-year-old Chinese woman with 13-year history of T1D, who was periodically hospitalized for recurrent abdominal pain and vomiting alternating with spontaneous hypoglycaemia in the last 3 years. She was treated short-acting insulin Lispro via an insulin pump. Although the insulin dosage had been reduced from 10.9 U/day to 3.2 U/day, she gained 32 kg body weight and her haemoglobin A1c (HbA1c) level decreased from 10.9% to 5.9% after the occurrence of hypoglycaemia. This study aimed to investigate why this patient with T1D developed obesity and recurrent hypoglycaemia.

Methods:

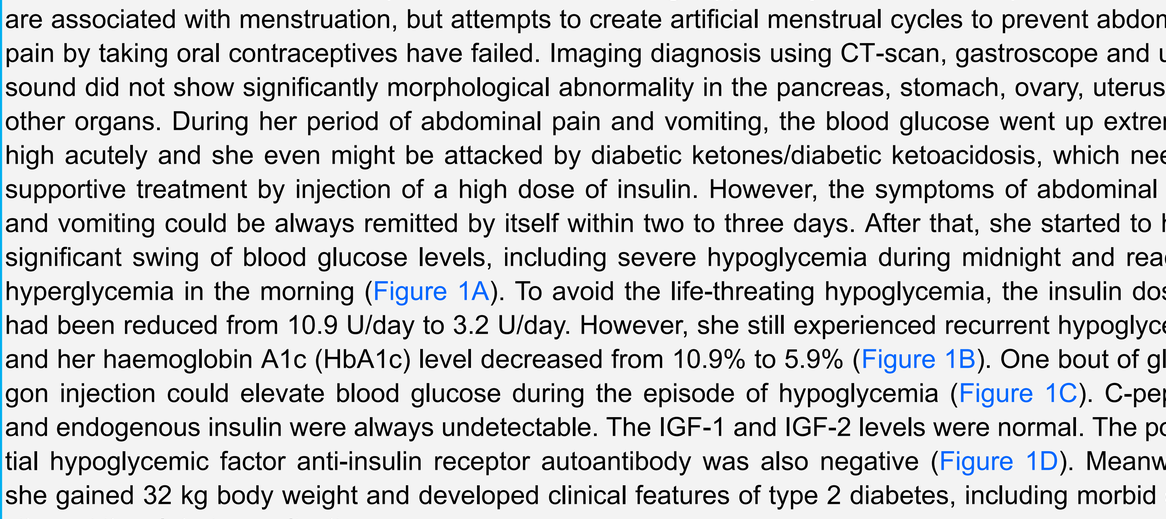
Previous hospitalization records and medical reports of this patient were carefully checked. Global similar cases were searched and compared. A comprehensive biochemical and immunological analysis was performed to measure the levels of circulating factors involved in regulation of glucose homeostasis and insulin actions.

Case Report:

A 25-year-old Chinese woman was diagnosed with T1D in 2007 and treated with insulin pump due to extremely high blood glucose levels. She tested positive for high titers of anti-GAD65 autoantibody while the C-peptide was almost undetectable. Since December of 2016, she has been hospitalized almost once a month due to abdominal pain and severe vomiting. Apparently, the abdominal pain and vomiting are associated with menstruation, but attempts to create artificial menstrual cycles to prevent abdominal pain by taking oral contraceptives have failed. Imaging diagnosis using CT-scan, gastroscope and ultrasound did not show significantly morphological abnormality in the pancreas, stomach, ovary, uterus and other organs. During her period of abdominal pain and vomiting, the blood glucose went up extremely high acutely and she even might be attacked by diabetic ketones/diabetic ketoacidosis, which needed supportive treatment by injection of a high dose of insulin. However, the symptoms of abdominal pain and vomiting could be always remitted by itself within two to three days. After that, she started to have significant swing of blood glucose levels, including severe hypoglycemia during midnight and reactive hyperglycemia in the morning (Figure 1A). To avoid the life-threating hypoglycemia, the insulin dosage had been reduced from 10.9 U/day to 3.2 U/day. However, she still experienced recurrent hypoglycemia and her haemoglobin A1c (HbA1c) level decreased from 10.9% to 5.9% (Figure 1B). One bout of glucagon injection could elevate blood glucose during the episode of hypoglycemia (Figure 1C). C-peptide and endogenous insulin were always undetectable. The IGF-1 and IGF-2 levels were normal. The potential hypoglycemic factor anti-insulin receptor autoantibody was also negative (Figure 1D). Meanwhile, she gained 32 kg body weight and developed clinical features of type 2 diabetes, including morbid obesity and its related complications.

Investigation:

1. Uncontrollable hypoglycemia independent of glucagon function, endogenous insulin or anti-insulin receptor autoantibody in this patient



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Time after glucagon injection	Glucose mmol/l	C-peptide ng/ml	Endogenous Insulin uU/ml
0'	2.44	<0.01	0.42
15'	5.65	<0.01	0.54
30'	8.95	<0.01	0.40
60'	7.51	<0.01	0.32

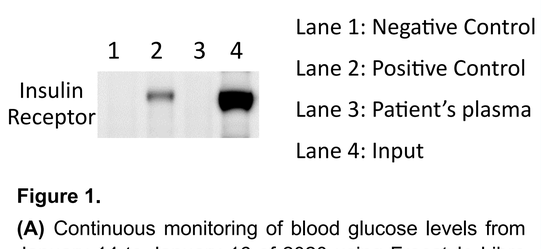


Figure 1.

(A) Continuous monitoring of blood glucose levels from January 14 to January 16 of 2020 using Freestyle Libre system. The daily average blood glucose value was 6.7-7.4 mmol/L. The time percentage of normal blood glucose was 49%-62%. The time percentage of low blood glucose was 24%-28%. The time percentage of high blood glucose was 10%-23%.

(B) HbA1c levels of this patient. It was obvious that the patient's HbA1c was dramatically decreased from June to December of 2017, during which the patient started to have severe hypoglycemia.

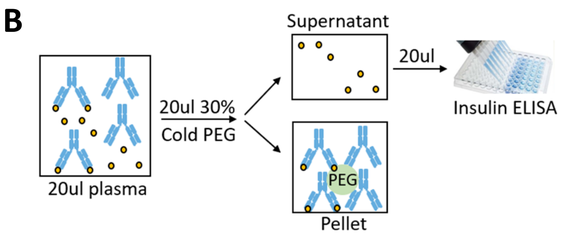
(C) During one episode of hypoglycemia, glucagon tolerance test was performed in this patient. One bout of glucagon injection could significantly increase the blood glucose levels, suggestive of normal glycogen storage in this patient. Both C-peptide and endogenous insulin were almost undetectable, indicating the hypoglycemia was independent of production or secretion of endogenous insulin. Endogenous insulin was measured by Cobas E601 system (Roche, USA).

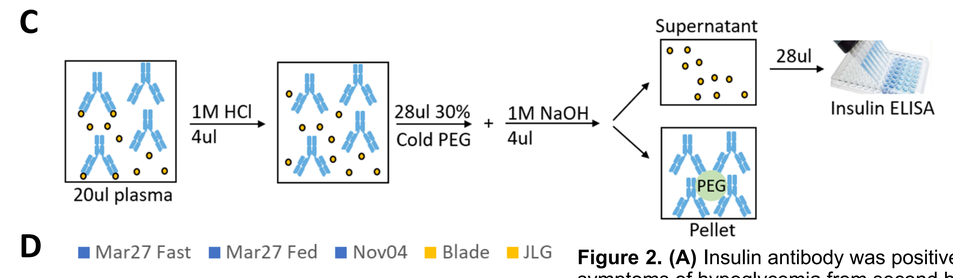
(D) Immunoprecipitation was performed to test whether the patient's plasma had anti-insulin receptor autoantibody (IRAb). The gel picture showed IRAb was negative.

2. Insulin antibody and exogenous insulin bound by insulin antibody were detected in patient's plasma during the episode of hypoglycemia

A

Date	Insulin antibody	Reference range
2017-Mar-05	3.27	0-5
2017-Nov-21	11.21	0-5
2019-Jue-17	25.42	0-5
2020-Jul-09	18.64	0-5





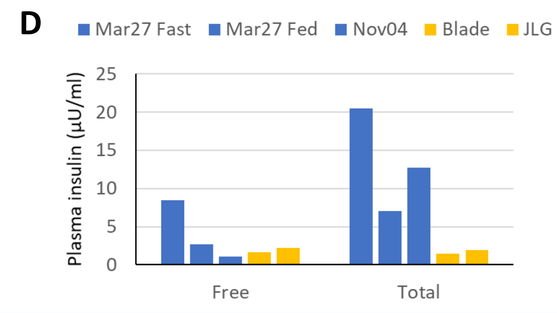


Figure 2. (A) Insulin antibody was positive after the occurrence of symptoms of hypoglycemia from second half of the year 2017. **(B)** Working model for free insulin measurement using PEG precipitation and insulin ELISA kit, which can detect both endogenous and exogenous insulin. **(C)** Working model for total insulin measurement. Briefly, the bound insulin was released from insulin antibody by HCl treatment and all the antibody was removed by PEG precipitation. Finally, the supernatant was neutralized by NaOH and all the free insulin was measured by insulin ELISA kit. **(D)** Free and total insulin in patient's plasma samples collected at three different time points (blue bars) measured by methods as described in B and C. The yellow bars represent samples from two healthy individuals, who have no insulin antibody in their plasms.

3. Insulin withdraw study

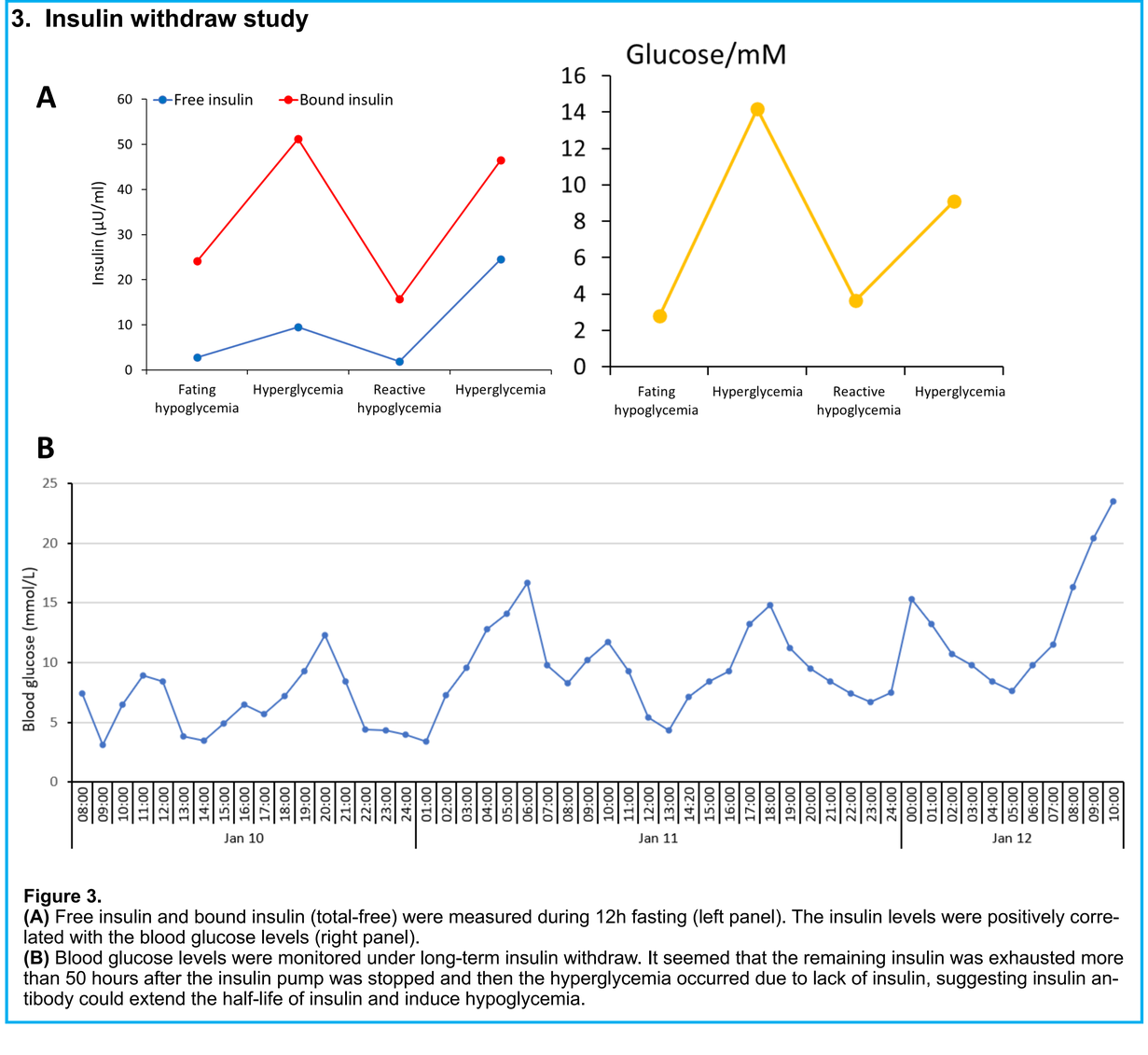


Figure 3.
(A) Free insulin and bound insulin (total-free) were measured during 12h fasting (left panel). The insulin levels were positively correlated with the blood glucose levels (right panel).
(B) Blood glucose levels were monitored under long-term insulin withdraw. It seemed that the remaining insulin was exhausted more than 50 hours after the insulin pump was stopped and then the hyperglycemia occurred due to lack of insulin, suggesting insulin antibody could extend the half-life of insulin and induce hypoglycemia.

4. Proposed working mechanisms whereby how insulin antibodies extend the half-life of insulin

Figure 4. Part of the bound insulin binds to insulin receptor (IR) and generates a cross-linking structure of insulin antibody-insulin-IR, which prevents insulin degradation and facilitates insulin release from the IR after functioning. In details, the neonatal Fc receptor (FcRn) could bind with insulin antibody Fc domain and prevent free insulin degradation by lysosome. Thus, antibody-dependent enhancement of the pharmacological effect of exogenous insulin leads to intermittent hypoglycemia, finally causing morbid obesity

Conclusion:

1. Antibody against exogenous insulin is able to lead to hypoglycemia in patients with T1D, although it is very rare.
2. Measurements of endogenous insulin, exogenous insulin, free insulin and bound insulin are helpful for diagnosis of autoimmune hypoglycemia.
3. Insulin antibody extends the half-life of insulin via unclear mechanisms, which needs to be investigated in the future.

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