Letter to the Editor
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Polyethylene Glycol Precipitation to Avoid Misdiagnosis of Insulin Autoimmune Syndrome: A Case Report and Testing Pathway in Medical Laboratories

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Dear Editor,

Insulin autoimmune syndrome (IAS), also known as Hirata's disease, manifests as a hypoglycemic syndrome due to abnormally elevated insulin autoantibodies (IAA) without a history of exogenous insulin exposure [1, 2]. Patients with autoimmune or hematological disorders are prone to developing IAS [3, 4]. The use of drugs containing sulfur or sulfhydryl groups, such as methimazole, glutathione, imipenem, penicillin G, and hydrazinebendazole, can trigger IAS [5]. However, IAS is currently poorly understood by professionals at medical laboratories due to its rare incidence [6]. We report tests regarding a case of IAS in which the application of polyethylene glycol (PEG) precipitation prevented a misdiagnosis and will provide medical laboratories with a testing pathway for such patients (Fig. 1). This study was approved by the Ethics Committee of the West China Hospital, Sichuan University, Chengdu, China[No. 903 (2021)].

A 31-year-old Chinese male previously registered at another hospital with a history of recurrent hypoglycemia, loss of consciousness, and blood glucose of 1.2 mmol/L, recovering after glucose administration was admitted to our hospital on January 21, 2021. He had no history of diabetes mellitus and denied taking exogenous insulin or any hypoglycemic drugs, thus excluding drug-induced hypoglycemia. The patient had a history of hyperthyroidism and presented with hyperphagia, easy hunger, weight loss, excessive sweating, fear of heat, palpitations, hand tremors, and awakening at night. Methimazole (10 mg) was administered orally, twice daily.

The results of the thyroid function test conducted at the previous hospital indicated hyperthyroidism, the suppressed release of thyroid-stimulating hormone (TSH), and grossly elevated levels of free triiodothyronine (FT3) and free thyroxine (FT4). Other parameters included low blood glucose level of 2.4 mmol/L, C-peptide level of 5.9 nmol/L, serum insulin level of 2.72 nmol/L, cortisol level of 168.5 nmol/L, and a negative IAA detected using immunoblotting. Magnetic resonance imaging of the pancreas was unremarkable, excluding insulinoma.

On admission to our hospital, two weeks later, further evaluations were performed. The 75 *g* oral glucose tolerance test showed fasting glucose of 2.19 mmol/L and 2-hour postprandial glucose of 19.11 mmol/L (Table 1). Insulin levels at various time points of the insulin/C-peptide release test were out of the test range (>1,000 μU/mL), and C-peptide levels were 5.94 nmol/L at baseline and 7.34 nmol/L at 120 min. The insulin/C-peptide ratio was >1 at each time point. Thyroid function tests indicated hyperthyroidism with TSH <0.005 mU/L, FT3 33.4 pmol/L, and FT4 >100 pmol/L. IAA was 90.9 cut-off index (reference range:

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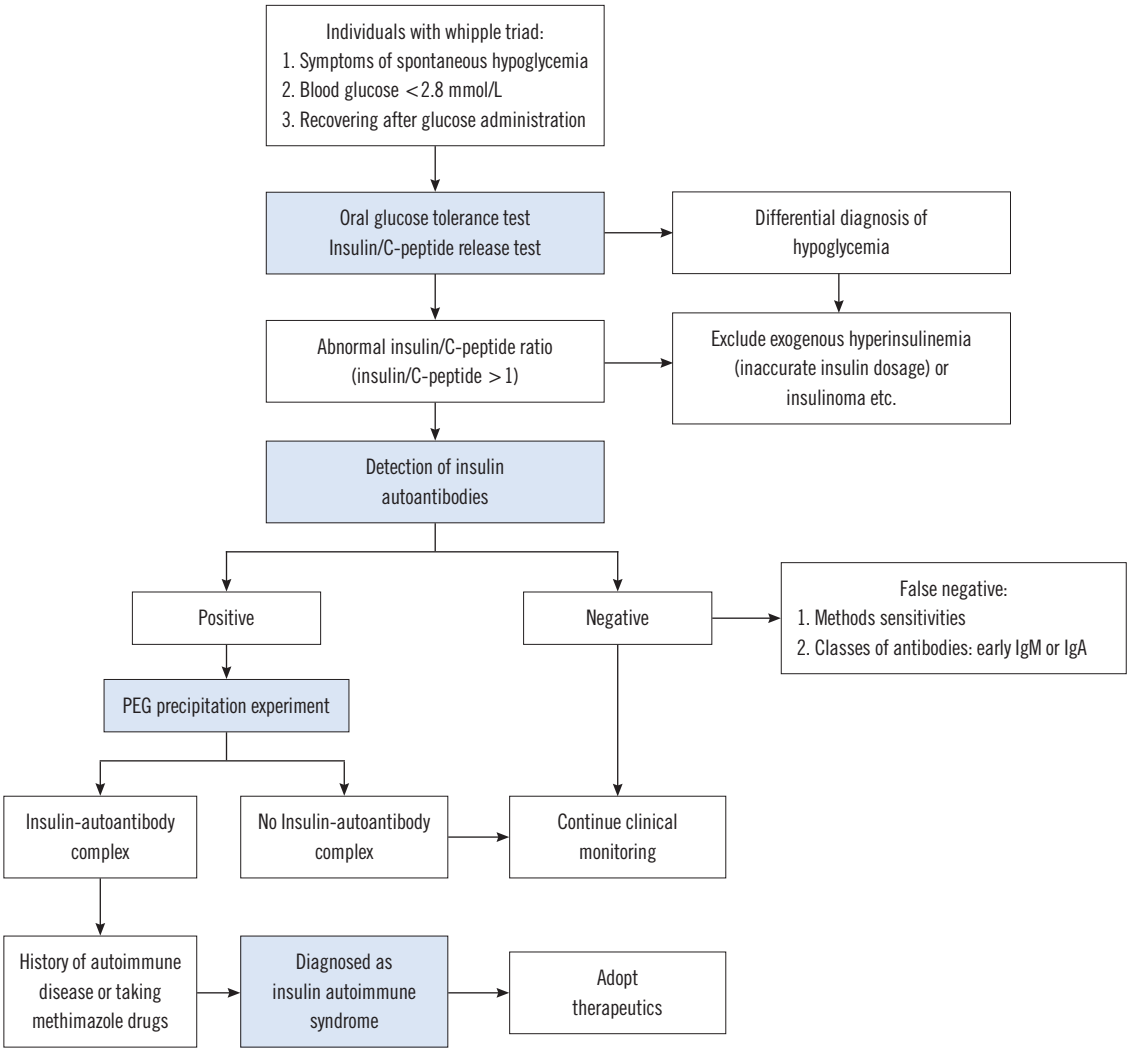

Fig. 1. Testing pathway of insulin autoimmune syndrome in medical laboratories.

Table 1. Results of the oral glucose tolerance test and insulin/C-peptide release test

Time	Glucose (mmol/L)	Insulin (nmol/L)*			C-peptide (nmol/L)		Insulin/C-peptide	
	Original results	Original results	Serum dilution	PEG precipitation	Original results	PEG precipitation	Serum dilution	PEG precipitation
0 min	2.19	>6.97	26.99	2.60	5.94	1.24	4.54	2.10
60 min	15.68	>6.97	26.17	3.36	7.14	3.44	3.66	0.98
120 min	19.11	>6.97	31.83	3.85	7.34	4.00	4.34	0.96
180 min	12.74	>6.97	34.81	4.28	7.88	4.18	4.42	1.02
240 min	6.09	>6.97	38.29	4.06	6.92	3.16	5.53	1.28
300 min	1.59	>6.97	33.24	3.14	5.84	1.53	5.69	2.05

*Insulin: nmol/L=(μU/mL×6.965)/1,000.
Abbreviation: PEG, polyethylene glycol.

<1 as detected by chemiluminescence in our laboratory.

Given the inconsistent insulin and C-peptide levels and IAA findings between our hospital and the previous hospital, we used several algorithms to rule out potential interference. The quality of insulin in the laboratory was controlled, and the patient samples were re-tested to remove any random errors.

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Samples were also obtained from patients with low insulin levels. Serum dilution experiments revealed high insulin levels at various time points in the insulin/C-peptide release test (Table 1). A PEG precipitation experiment was conducted [7, 8]. Patient serum (1 mL) was mixed with an equal volume of 20% PEG 2000 liquid. Serum from patients with normal insulin levels was used as control. After incubation at 37°C for 1 hour, samples were centrifuged at 1,500×*g* for 15 min, and the insulin levels in the supernatant were measured. Insulin levels in the patient sample were significantly lower after PEG precipitation, while the precipitation had no effect on the control samples, implying the formation of insulin-IAA complexes (Table 1). We also analyzed the current patient's genotype and found it to be associated with the HLA DRB1*0406 [9].

Discrepant results of tests conducted at our hospital and the previous hospital may be attributed to differences in method sensitivities or reagents, such as antibodies. We could not completely exclude the possibility of other types of insulin-binding antibodies, such as early IgM or IgA, as the existing kits used to detect IAA primarily target IgG antibodies. Therefore, even if IAA test results are negative, IAS cannot be completely excluded. However, PEG precipitation is a simple, quick, and inexpensive method that can detect all immunoglobulin classes and subclasses [10]. Collectively, our results highlight the importance of PEG precipitation for avoiding the misdiagnosis of IAS.

In summary, a diagnosis of IAS was confirmed by combining laboratory findings with the patient's history of hyperthyroidism and methimazole use. This case report provides medical laboratories with suggestions for inconsistent insulin and C-peptide levels. PEG precipitation can be paramount in assisting the diagnosis of IAS. Omitting or delaying the use of PEG precipitation can be clinically misleading in patients with different autoantibody classes and may lead to unnecessary hospitalization or procedures.

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AUTHOR CONTRIBUTIONS

Zeng YP collected the data and wrote the manuscript. Li GX interpreted the data and provided ideas for detection. Song HL designed the study and revised the manuscript accordingly.

CONFLICTS OF INTEREST

None.

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