

# Insulin autoimmune syndrome without hypoglycemia: A different perspective of method interference

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## ABSTRACT

Insulin autoimmune syndrome (IAS) is a serious autoimmune disorder that may cause spontaneous hypoglycemia. IAS is characterized by hyperinsulinemia, normal C-peptide levels and positive anti-insulin antibody. The diagnosis is confirmed by demonstrating the presence of macroinsulin complex by polyethylene glycol (PEG) precipitation or gel filtration chromatography. Although some macrohormones like macroprolactin and some macroenzymes such as macroamylase are seen commonly, macroinsulinemia is a rare condition. In this report, we presented an IAS case from laboratory perspective by using three different immunoassays with different performances in eliminating macroinsulin interference. Besides presenting a case with IAS without hypoglycemia we evaluated the contribution of different immunoassays to the diagnosis of this syndrome. Immunoassays have different features, considering the analysis of macroinsulin or bioavailable insulin. In this case, the superiority or handicap of these immunoassays will be discussed in terms of analysis of total or free insulin.

**Keywords:** Free insulin; insulin antibody; insulin autoimmune syndrome; macroinsulin.

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Insulin is a peptide hormone secreted by beta cells of pancreas and has a crucial role for carbohydrate metabolism in human body. Hyperinsulinemia causes hypoglycemia. Some autoimmune disorders of insulin and its receptor, such as insulin autoimmune syndrome (IAS), may result in hypoglycemia [1]. Exogenous insulin treatment is a common cause of generation of insulin antibodies. However, insulin autoantibodies can be seen in some people who have never administered exogenous insulin and these may have clinical remarks, especially for postprandial hypoglycemia [2, 3].

Insulinoma and IAS are introduced as the leading reasons for hypoglycemia in Japan due to hyperinsulinemia [4]. IAS has been reported to be related with specific HLA-DR4 regions, especially DRB1\*04:06 [5]. Some infections (mumps, measles, hepatitis C) and some drug administrations (methimazole, propylthiouracil, alpha-lipoic acid, sulfhydryl-containing drugs) were found to

be possibly associated with IAS progression [6, 7]. If one of these predisposing factors is found to be responsible for developing IAS, getting rid of these factors may be useful. IAS may be accompanied by some other autoimmune diseases like Graves, systemic lupus erythematosus and rheumatoid arthritis [5, 8].

Insulin has a short half-life, about 2–5 minutes; while half-life of C-peptide is 20–30 minutes [9]. Aforementioned predisposing factors may result in insulin antibody generation. These antibodies are mostly presented with IgG type. Seropositivity of insulin autoantibody in blood promotes binding most of the secreted postprandial insulin and creates macroinsulin complex. Thus, elimination of insulin delays and half-life of insulin prolongs. Bioavailable insulin is the unbound free insulin. When the insulin-antibody complex splits, then a huge amount of bioavailable insulin comes out, creating hy-



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poglycemia [1]. However, data about the stability of this complex is unclear. Sometimes it does not dissociate.

IAS is characterized by nonketotic hypoglycemia (if the complex dissociates) with high serum insulin, normal C-peptide levels, and insulin antibody positive. Molar ratio of C-peptide: insulin is  $<1$  in IAS [1, 6]. Presence of macroinsulin is confirmed by polyethylene glycol (PEG) precipitation and gel filtration chromatography.

## CASE REPORT

A 69-year-old male patient was admitted to the hospital with overtiredness. Physical examination was normal. His body mass index was  $29.75 \text{ kg/m}^2$  indicating an impaired glucose metabolism. His medical history included a diagnosis of migraine and lumbar disc herniation. Family history did not give special information about his complaints. Some laboratory tests were ordered to screen diabetes mellitus, hypothyroidism and acute infection. Laboratory findings of the first blood sample are presented in Table 1. Unexpectedly high insulin levels (252 mU/L) were remarkable. Other results were normal.

After the first visit, fasting plasma glucose levels were screened for a while. However, glucose levels were normal. About three months later, some other tests were ordered again. Fasting insulin was 229 mU/L and anti-insulin antibody was positive (45.8%) (Table 2).

After a month, the patient attended his practitioner again and some laboratory tests were performed on the third blood sample (Table 2). Insulin result of the second sample was also unexpectedly high. The third sample was analyzed and then treated with PEG. Insulin level fell after PEG precipitation. The discrepancy between fasting glucose, insulin and C-peptide made us think that this might be due to macroinsulinemia.

The same sample, which had an initial insulin level of 187 mU/L, was reanalyzed for insulin after PEG precipitation and the result was 33.2 mU/L. Recovery was 17.75% which meant 82.25% of the insulin was precipitated. Fasting C-peptide result was 3.12 ng/mL. C-peptide: insulin molar ratio was 0.79. Glycated hemoglobin (HbA1c) result was reported as 6.1%.

All analyses were performed on Roche Cobas 8000 modular system (Cobas c702 and Cobas e801) (Mannheim, Germany). Chemistry tests were analyzed by using spectrophotometric method. Insulin and C-peptide tests were analyzed by electrochemiluminescence immunoassay (ECLIA) method by using original Roche

**TABLE 1.** Test results and reference ranges of the first sample

Test	Result	Reference range
Hemoglobin (g/dL)	14.7	12–16
Glucose (fasting) (mg/dL)	75	74–106
Creatinine (mg/dL)	0.9	0.7–1.2
AST (U/L)	17	0–40
ALT (U/L)	13	0–41
Na (mmol/L)	142	136–145
K (mmol/L)	4.59	3.5–5.1
TSH (mIU/L)	1.57	0.45–3.86
Free T4 (ng/dL)	1.28	0.93–1.71
CRP (mg/L)	1.82	0–5
Insulin (fasting) (mU/L)	252	2.6–24.9
C-peptide (fasting) (ng/mL)	3.25	1.1–4.4

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Na: Sodium, K: Potassium; TSH: Thyroid stimulating hormone; Free T4: Free thyroxine; CRP: C-reactive protein.

Elecsys reagents. HbA1c analysis was performed by high-performance liquid chromatography (HPLC) method (boronate affinity chromatography, Premier Hb9210, Trinity Biotech, Ireland).

C-peptide levels were normal, C-peptide: insulin molar ratio was  $<1$  and anti-insulin antibody was positive. Since more than 80% of the insulin was precipitated with PEG treatment, this case was evaluated as macroinsulinemia. However, the third sample was analyzed by using other manufacturers without PEG precipitation. Abbott Architect i2000 (CLIA) (IL, USA) gave an insulin result of 59 mU/L (reference range: 6–27) while Beckman Coulter Access DXI (CLIA) gave 21.8 mU/L (reference range: 1.9–23). Insulin result without PEG precipitation by Roche Cobas 8000 system was much higher compared to these results.

Subsequent tests were performed, as IAS may be accompanied by some connective tissue diseases and other autoimmune diseases [1, 5]: Anti-nuclear antibody (ANA): Negative, Extractable Nuclear Antigen Antibodies (ENA) Panel: Negative, Cytoplasmic Neutrophil Antibodies (ANCA): Negative, Serum protein electrophoresis: Normal (no apparent monoclonal protein).

Informed consent was obtained from the patient.

## DISCUSSION

IAS is caused by insulin antibodies resulting in formation of macroinsulin complex in the circulation. Macroinsu-

**TABLE 2.** Test results and reference /clinical decision ranges of the second and third samples

Test	Result	Reference range/Clinical decision limit
<b>2<sup>nd</sup> sample</b>		
Insulin (fasting) (mU/L)	<b>229</b>	<b>2.6–24.9</b>
Anti insulin antibody (%)	45.8	>8.2%, Positive
<b>3<sup>rd</sup> sample</b>		
Insulin (fasting) (mU/L), (pmol/L)	<b>187, 1298.6</b>	<b>2.6–24.9, 18.1–172.9</b>
Insulin (fasting) (mU/L), (after PEG precipitation)	<b>33.2</b>	<b>2.6–24.9</b>
Recovery (%)	<b>17.75</b>	<b>&gt;60%, Negative</b>
C-peptide (fasting) (ng/mL), (pmol/L)	3.12, 1033	1.1–4.4, 364.2–1456.8
C-peptide:insulin molar ratio	0.79	<1 (for IAS)
HbA1c (%)	6.1	4–6
ANA	Negative	Negative
ENA Panel	Negative	Negative
ANCA	Negative	Negative

IAS: Insulin autoimmune syndrome; PEG: Polyethylene glycol; ANA: Anti nuclear antibody; ENA: Extractable nuclear antigen antibodies; ANCA: Cytoplasmic neutrophil antibodies.

linemia causes reporting high insulin levels depending on the performance of the immunoassay. These patients present with normal C-peptide levels that do not correlate with concurrent insulin results. Macroinsulinemia can be shown by PEG precipitation or gel filtration chromatography. Gold standard method to confirm macrohormones or macroenzymes is gel filtration chromatography [1].

In this case, we used PEG precipitation to confirm macroinsulinemia. We used different immunoassays to determine the free insulin. Roche Cobas immunoassay seems to bind both the free insulin and insulin-antibody complex, while Abbott Architect and Beckman Coulter Access immunoassay seem to bind free insulin mostly. Because insulin analysis of the sample after PEG precipitation with Roche Cobas system gave more compatible results with the naive sample analyzed by Abbott Architect and Beckman Coulter Access systems. Although American Diabetes Association (ADA) published a report about standardization of insulin immunoassays, there is still a gap in analysis of total or free insulin [10].

The epitope recognized by the antibody used in immunometric tests may differ. Capturing insulin antibodies used in Roche Cobas system recognizes A7-A10 region of the A-chain of insulin while the Ruthenium labeled antibody recognizes the C-terminal region of B-chain of insulin [11]. Since Abbott Architect and Beckman Coulter Access analyze free insulin but not

insulin-antibody complex, it may be difficult to screen macroinsulinemia and IAS with these systems. Because these systems give a higher C-peptide: insulin molar ratio. Thus, IAS cases can be easily detected with Roche Cobas systems. On the other hand, Abbott Architect and Beckman Coulter Access systems seem to be more practical in detecting bioavailable insulin.

IAS may be associated with some drugs and medications. Pantoprazole was reported to be one of these drugs [12]. Our patient has been using pantoprazole either. This might be the reason for hyperinsulinemia in this case.

In conclusion; the discrepancy between high fasting insulin, normal fasting C-peptide, normal fasting glucose requires further investigation, especially when the serum insulin antibody titer is high. Clinicians should consult a laboratory specialist in these cases for correct decision-making. Otherwise, this may yield to misdiagnosis (such as insulinoma) or unnecessary interventions and treatments [1]. This kind of interference may be more critical, considering the other macroenzymes or macrohormones. Unlike macroamylasemia or macroprolactinemia, remnant insulin may cause serious metabolic effects like hypoglycemia. PEG precipitation is a simple and practical method to investigate such cases. The limitation of this study was that we could not perform gel filtration chromatography to confirm these results.

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