ID: 15-0090; October 2015
DOI: 10.1530/EDM-15-0090

Rare association of insulin autoimmune syndrome with ankylosing spondylitis

Nishant Raizada[1], S H Rahaman[1], D Kandasamy[2] and V P Jyotsna[1]

Departments of [1]Endocrinology and Metabolism [2]Radiology, All India Institute of Medical Sciences, New Delhi, India

Correspondence should be addressed to V P Jyotsna
Email
vivekapjyotsna@gmail.com

Summary

Insulin autoimmune syndrome (IAS) is a rare cause of hyperinsulinemic hypoglycaemia, which is known to occur in association with the use of sulfhydryl-containing drugs and autoimmune disorders. We describe a patient with hitherto an unreported association of IAS with ankylosing spondylitis. We have also performed and described a simplified method of polyethylene glycol (PEG) precipitation of an insulin bound antibody in the serum.

Learning points:

- IAS should be considered in differential diagnosis of endogenous hyperinsulinemic hypoglycaemia.
- Ankylosing spondylitis can be associated with IAS apart from several other autoimmune diseases.
- Very high serum insulin levels (100–10 000 μU/ml) are frequently seen in IAS.
- When faced with very high serum insulin before suspecting insulinoma, it is advisable that PEG precipitation of serum be done to identify antibody bound insulin.
- A clinical suspicion of IAS can avoid expensive imaging and unnecessary surgery in affected patients.

Background

Insulin autoimmune syndrome (IAS) is a rare condition that presents with hyperinsulinemic hypoglycaemia. Several autoimmune diseases, notably Graves' disease, are associated with IAS. This case highlights the association of IAS with ankylosing spondylitis, which to the best of our knowledge has not been reported earlier.

IAS is typically characterized by very high serum insulin levels, most of which is actually antibody-bound insulin. The precipitation of this bound insulin with polyethylene glycol (PEG) provides indirect evidence of anti-insulin antibody. This simple test can be done in any laboratory. The results may prevent the search for an insulinoma and instead guide the clinician toward a diagnosis of IAS.

Case presentation

A 49-year-old male presented to us with complaints of episodic sweating and palpitations for the last 3 months. These episodes occurred predominantly in the fasting state although he had several episodes in the postprandial period as well. The symptoms disappeared after eating food. He had gained 3.5 kg in the last 3 months. There was no history of diabetes mellitus and he had never used oral hypoglycaemic agents or insulin. He was a known hypertensive for the last 15 years and was taking telmisartan and amlodipine for control of blood pressure. He also had a history of inflammatory backache for the last 15 years. There was no history of use of any sulfhydryl-containing drugs. There was history of ankylosing spondylitis in his father and brother.

On examination, he was obese (BMI 25.9 kg/m^2). He had acanthosis nigricans. His blood pressure was 130/80 mmHg. There was no flexion deformity of the cervical spine. The chest expansion was 3.5 cm. The modified Schobers test was positive and lateral spine flexion was reduced. There was no evidence of sacroiliac joint tenderness. There was no clinical evidence of peripheral arthritis or enthesitis.

Investigation

His laboratory investigations were notable for elevated erythrocyte sedimentation rate (169 mm/h) and fasting blood glucose, 42 mg/dl. HLAB-27 was positive. Serum rheumatoid factor was negative and antinuclear antibodies were normal. Pelvic radiographs showed no evidence of sacroiliitis. Magnetic resonance imaging (MRI) of the spine and sacroiliac joints showed evidence of sacroiliitis and involvement of the lumbar spine consistent with ankylosing spondylitis (Fig. 1A, B, C, D and E).

He developed spontaneous hypoglycaemia during his hospital stay with blood glucose being 36 mg/dl. The corresponding serum insulin levels were >1000 μU/ml and C-peptide was 16.37 ng/ml. He had several spontaneous hypoglycaemias during his hospital stay, all of which revealed hyperinsulinemic hypoglycaemia with serum insulin levels >1000 μU/ml in all samples. An extended oral glucose tolerance test with 75 g glucose was performed, which revealed a paradoxical response, i.e., 0, 1, 2, 3, 4 and 5 h plasma glucose 36, 198, 227, 185, 113 and 39 mg/dl with corresponding serum insulin above 1000 μU/ml each time.

He underwent dual phase computed tomography, endoscopic ultrasonography and contrast enhanced MRI of the abdomen, which did not reveal any pancreatic lesion.

In view of very high serum insulin levels, we suspected IAS in this patient. Accordingly we measured serum-free insulin levels after precipitation of serum with PEG (6000). To study the effects of PEG precipitation on serum insulin levels, we performed insulin assays in both the serum and the PEG precipitated serum. We also took serum from a healthy control and subjected it to a similar analysis. To further support our findings, we re-eluted the pellet formed after PEG precipitation, and an insulin assay was performed in the sample.

PEG precipitation method

Twenty microliters patient serum was mixed with an equal volume of 25% PEG 6000 solution in 40 μM phosphate buffer and the sample was vortexed for 10 s. Mixture was allowed to stand in crushed ice for 5 min. After this, the sample was centrifuged at 10 000 ***g*** for 2 min. The supernatant was taken and used for insulin assay by an electrochemiluminescence assay (Roche).

To study the effect of PEG precipitation on serum insulin levels, we performed a serum insulin assay before and after PEG precipitated. We also took serum from a healthy control and subjected it to a similar analysis. To further support our findings, we treated the pellet formed after PEG precipitation with phosphate buffer with pH adjusted to 3.0. After mixing on a vortex mixer for 10 s, this sample was again centrifuged and supernatant was

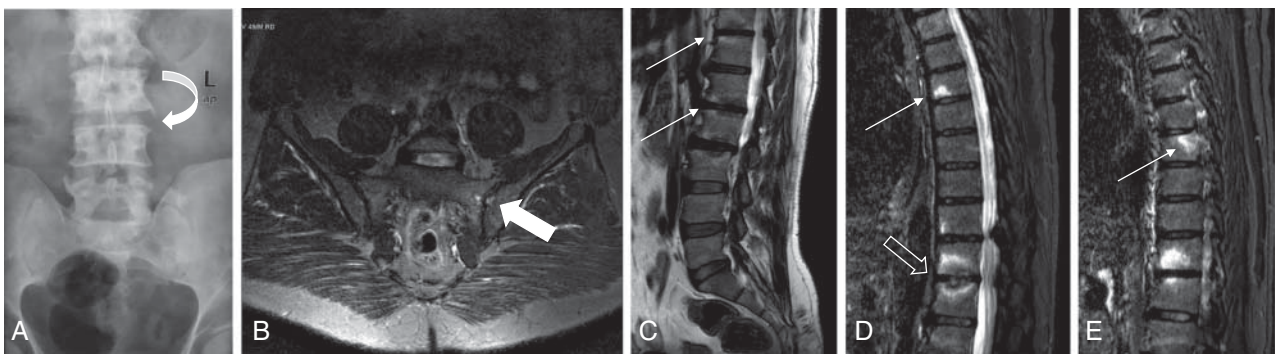

Figure 1

Anteroposterior radiograph of lumbosacral spine with bilateral joints (A) show thin marginal syndesmophytes (curved arrow). T2 weighted non-fat saturated sequences (B and C) show focal left sacroiliitis (thick arrow) and hyperintensity involving the anterior corners at multiple levels (thin arrow), which is known as shiny corner sign. T2 weighted fat saturated sequences (D and E) also show shiny corners (thin arrow) and endplate changes (outlined arrow).

Table 1 Polyethylene glycol precipitation of serum for free insulin estimation.

	Patient (μU/ml)	**Control** (μU/ml)
Serum insulin	>1000	49.6
Serum insulin after PEG precipitation (free insulin)	37.06	45.8
Bound insulin (re-extracted from PEG pellet)	>1000	3.4

taken. The pH was readjusted to 7.0 before an insulin assay was performed in the supernatant.

Although we found that the free insulin level in the patient serum was 37.06 mU/ml, bound insulin levels were >1000 mU/ml. In contrast, the amount of bound insulin in the control serum was only 3.4 mU/ml (Table 1).

Insulin binding nature of the protein was confirmed by a second assay by a commercial laboratory, which revealed an insulin IgG antibody value of >300 U/ml (normal range <12 U/ml).

Treatment

A diagnosis of ankylosing spondylitis with IAS was made. The patient was advised to follow a 1800 kcal diet containing 40% carbohydrate divided into six meals a day. Simple sugars were excluded from the diet. The patient stopped having symptomatic hypoglycaemias after the above treatment.

Outcome and follow-up

The patient has been on follow-up for the past year. No episodes of hypoglycaemia have recurred so far.

Discussion

IAS refers to a combination of spontaneous hypoglycaemia, hyperinsulinemia and insulin-binding antibodies in a patient who has never been exposed to exogenous insulin (1). IAS is a rare syndrome that was first reported in Japan by Hirata. Later on, IAS emerged as the third most common cause of spontaneous hypoglycaemia (excluding insulin or oral hypoglycaemic drug use) in Japan (2). By 1994, 197 cases of IAS had been reported in Japan (3). However, IAS is less commonly reported in western countries.

In IAS, insulin antibodies develop without prior exposure to exogenous insulin. The patients often have underlying autoimmune disorders predisposed to the development of IAS. It is unclear why only some of these patients develop anti-insulin antibodies. Certain HLA class II alleles are associated with the development of these antibodies. In Japanese patients, an association with HLA haplotypes DRB1*0406/DQA1*0301/DQB1*0302 has been found (4). The HLA-DRB1*04:03 allele in Caucasians (5) has also recently been associated with IAS. While in the majority of the cases, no precipitating cause of the development of insulin autoantibody can be found, 43% of cases were found to be taking drugs that were sulfhydryl compounds (3). Notably, the use of carbimazole or methimazole for Graves' disease has been associated with IAS and the symptoms resolve when the drug is withdrawn. The other drugs associated with IAS include α-mercaptopropionyl glycine, glutathione, penicillamine, tiopronin, captopril, D-penicillamine and gold thioglucose. More recently, α-lipoic acid has been implicated in several cases of IAS (5) (6). The sulfhydryl group may interact with disulfide bonds in the insulin molecule rendering it more immunogenic (7).

Among the autoimmune conditions associated with IAS, Graves' disease is the most common. Systemic lupus erythematosus (8), rheumatoid arthritis (9) (10), antineutrophil cytoplasmic antibodies-associated glomerulonephritis (11), viral hepatitis C (12), alcoholic cirrhosis (13), polymyositis (14) and systemic sclerosis (15) are other reported conditions associated with IAS. The association of ankylosing spondylitis and IAS has not been reported in the literature and to the best of our knowledge, this is the first reported case of this association.

The mechanism by which insulin antibodies cause hypoglycaemia is unclear. Most likely, the insulin antibodies bind the secreted insulin and release the bound insulin intermittently. Thus, an inappropriate release of insulin leading to hypoglycaemia can occur (16).

IAS usually affects middle-aged individuals. The typical presentation is that of post-prandial hypoglycaemia, although fasting hypoglycaemia can also occur. Autonomic symptoms are more common but neuroglycopenic symptoms can occur. Seizures and even coma have been reported in IAS (17). The condition is often misdiagnosed as insulinoma or exogenous insulin use (18).

The laboratory features are notable for very high serum insulin levels ranging from 100 to 100 000 μU/ml (19). The high levels are due to the antibody-bound insulin but may represent interference by insulin antibody with the assay antibody. Free serum insulin levels can be estimated by PEG precipitation of insulin antibodies.

PEG has been used to separate free antigen from antibody-bound antigen (20). Most of the immunoglobulins are precipitated at 20% PEG, while smaller antigens like insulin are quite soluble at this concentration of PEG. PEG has been used as a precipitating agent in several immunological methods to separate free- and antibody-bound peptide hormones (21).

The technique of PEG precipitation as described above is simple and can easily be performed in the laboratory. In cases of suspected IAS, marked differences between the serum total insulin and free insulin is suggestive of this disorder.

Insulin antibody levels are elevated in IAS, and on further characterisation the antibodies are usually polyclonal IgG in nature. The antibodies may have high affinity/low capacity and low affinity/high capacity insulin-binding sites with the latter type of antibodies more often causing hypoglycaemia. C-peptide levels may be normal or elevated. Insulin antibodies may interfere with C-peptide assay as well (20). On oral glucose tolerance test, initial hyperglycaemia with late hypoglycaemia is noted, and the corresponding insulin response is that of a sustained peak and delayed decline (22).

The clinical course of IAS is usually benign. The hypoglycaemia due to IAS is usually transient. The symptoms usually subside spontaneously in 3–6 months (3). In cases precipitated by drugs, withdrawal of the offending agent leads to a gradual decline in insulin antibody levels and remission.

The treatment options include frequently low carbohydrate small meals to reduce stimulus for insulin secretion and measures to reduce insulin antibody levels. Glucocorticoids and plasmapheresis have been tried to reduce insulin antibodies with variable results (23). More recently, corn starch has been reported to have a good response in a case of IAS (24). Other less commonly used options include diazoxide, octreotide and partial pancreatectomy (19).

The recognition of IAS is important as it can avoid unnecessary invasive investigations and surgery in patients presenting with hyperinsulinemic hypoglycaemia. The treatment is simple in most cases and usually spontaneous remission occurs. However, it is important to suspect this syndrome when the patient has an underlying autoimmune disease, is using sulfhydryl group-containing drugs or has unusually high insulin levels in the face of hypoglycaemia. The use of PEG precipitation of serum insulin antibody and the estimation of free insulin in the supernatant can be a useful method of confirming this rare condition.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Patient consent
Written informed consent has been obtained from the patient for publication of the submitted article and accompanying images.

Author contribution statement
N Raizada clinically managed the patient, conducted literature review and wrote the manuscript, S H Rahaman was involved in clinically managing the patient and literature review. D Kandasamy was involved in radiological diagnosis. V P Jyotsna was involved in clinical management and editing the manuscript.

References

1 Benson EA, Ho P, Wang C, Wu PC, Fredlund PN & Yueng RT 1984 Insulin autoimmunity as a cause of hypoglycemia. *Archives of Internal Medicine* **144** 2351–2354. (doi:10.1001/archinte.1984.00350220067015)
2 Takayama-Hasumi S, Eguchi Y, Sato A, Morita C & Hirata Y 1990 Insulin autoimmune syndrome is the third leading cause of spontaneous hypoglycemic attacks in Japan. *Diabetes Research and Clinical Practice* **10** 211–214. (doi:10.1016/0168-8227(90)90063-Y)
3 Uchigata Y, Eguchi Y, Takayama-Hasumi S & Omori Y 1994 Insulin autoimmune syndrome (Hirata disease): clinical features and epidemiology in Japan. *Diabetes Research and Clinical Practice* **22** 89–94. (doi:10.1016/0168-8227(94)90040-X)
4 Uchigata Y, Tokunaga K, Nepom G, Bannai M, Kuwata S, Dozio N, Benson EA, Ronningen KS, Spinas GA, Tadokoro K *et al*. 1995 Differential immunogenetic determinants of polyclonal insulin autoimmune syndrome (Hirata's disease) and monoclonal insulin autoimmune syndrome. *Diabetes* **44** 1227–1232. (doi:10.2337/diab.44.10.1227)
5 Gullo D, Evans JL, Sortino G, Goldfine ID & Vigneri R 2014 Insulin autoimmune syndrome (Hirata disease) in European Caucasians taking α-lipoic acid. *Clinical Endocrinology* **81** 204–209. (doi:10.1111/cen.12334)
6 Uchigata Y, Hirata Y & Iwamoto Y 2008 Drug-induced insulin autoimmune syndrome. *Diabetes Research and Clinical Practice* **83** e19–e20. (doi:10.1016/j.diabres.2008.10.015)
7 Taylor SI, Barbetti F, Accili D, Roth J & Gorden P 1989 Syndromes of autoimmunity and hypoglycemia. *Endocrinology and Metabolism Clinics of North America* **18** 123–143.
8 Rouabhia S, Ramanoelina J, Godmer P, Reach G, Dutel JL & Guillevin L 2003 Insulin autoimmune syndrome revealing systemic lupus erythematosus. *Annales de Médecine Interne* **154** 59–60.
9 Nishiya K, Tahara K & Hashimoto K 1997 Hypoglycemic syncope attack in a patient with rheumatoid arthritis. *Journal of Rheumatology* **24** 2052.
10 Le Dantec P, Veillard E, Guggenbuhl P, Perdriger A, Chales G & Pawlotsky Y 1996 Autoimmune hypoglycemia occurring in tiopronin-treated rheumatoid arthritis. *Revue du Rhumatisme* **63** 300–301.

11 Ka T, Moriwaki Y, Nakanishi T & Yamamoto T 2008 Insulin autoimmune syndrome in a patient with ANCA-associated glomerulonephritis. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* **19** 1–2.
12 Ruíz-Giardín JM, Cremades C, Romero J & Marazuela M 2002 Insulin autoimmune syndrome in a patient with viral hepatitis C. *Clinical Endocrinology* **57** 411–412.
13 Shah P, Mares D, Fineberg E, Pescovitz M, Filo R, Jindal R, Mahoney S & Lumeng L 1995 Insulin autoimmune syndrome as a cause of spontaneous hypoglycemiain alcoholic cirrhosis. *Gastroenterology* **109** 1673–1676. (doi:10.1016/0016-5085(95)90657-6)
14 Ichikawa K, Nishii Y, Hashizume K, Chino M, Nagasawa T, Suzuki S, Okada N, Kobayashi M, Koizumi Y, Arai M *et al.* 1992 A case of autoimmune insulin antibody syndrome associated with polymyositis, empty sella and apparent high urinary output of immunoreactive insulin. *Endocrinologia Japonica* **39** 307–313. (doi:10.1507/endocrj1954.39.307)
15 Herranz L, Rovira A, Grande C, Suarez A, Martinez-Ara J, Pallardo LF & Gómez-Pan A 1992 Autoimmune insulin syndrome in a patient with progressive systemic sclerosis receiving penicillamine. *Hormone Research* **37** 78–80. (doi:10.1159/000182286)
16 Dozio N, Scavini M, Beretta A, Sarugeri E, Sartori S, Belloni C, Dosio F, Savi A, Fazio F, Sodoyez JC *et al.* 1998 Imaging of the buffering effect of insulin antibodies in the autoimmune hypoglycemic syndrome. *Journal of Clinical Endocrinology & Metabolism* **83** 643–648.
17 Burch HB, Clement S, Sokol MS & Landry F 1992 Reactive hypoglycemic coma due to insulin autoimmune syndrome: case report and literature review. *American Journal of Medicine* **92** 681–685. (doi:10.1016/0002-9343(92)90787-C)
18 Arzamendi AE, Rajamani U & Jialal I 2014 Pseudoinsulinoma in a white man with autoimmune hypoglycemia due to anti-insulin antibodies: value of the free C-peptide assay. *American Journal of Clinical Pathology* **142** 689–693. (doi:10.1309/AJCPX56JQBJHUBGJ)
19 Redmon JB & Nuttal FQ 1999 Autoimmune hypoglycemia. *Endocrinology and Metabolism Clinics of North America* **28** 603–618. (doi:10.1016/S0889-8529(05)70090-6)
20 Creighton WD, Lambert PH & Miescher PA 1973 Detection of antibodies and soluble antigen antibody complexes by precipitation with polyethylene glycol. *Journal of Immunology* **111** 1219–1227.
21 Gerbilz KD & Kemmier W 1978 Method of rapid quantification and characterization of insulin antibodies. *Clinical Chemistry* **24/6** 890–894.
22 Eguchi Y, Uchigata Y, Yao K, Yokoyama H, Hirata Y & Omori Y 1994 Longitudinal changes of serum insulin concentration and insulin antibody features in persistent insulin autoimmune syndrome (Hirata's disease). *Autoimmunity* **19** 279–284. (doi:10.3109/08916939409071354)
23 Masuda A, Tsushima T, Shizume K, Shibata K, Kinoshita A, Omori M, Sato Y, Demura H, Ohashi H, Odagiri R *et al.* 1986 Insulin autoimmune syndrome with insulin resistant diabetes at the incipient stage prior to hypoglycemic attacks. *Journal of Endocrinological Investigation* **9** 507–512. (doi:10.1007/BF03346977)
24 Deguchi A, Okauchi Y, Suehara S & Mineo I 2013 Insulin autoimmune syndrome in a health supplement user: the effectiveness of cornstarch therapy for treating hypoglycemia. *Internal Medicine* **52** 369–372. (doi:10.2169/internalmedicine.52.7844)

Received in final form 20 September 2015
Accepted 24 September 2015