isolates 1–9 are grouped in a single cluster, whereas outbreak isolate 10 is not within this cluster, suggesting that it is not part of the outbreak.

With regard to the three real case studies, Figure 2a shows the relatedness of eight isolates of *P. aeruginosa* obtained from the same adult CF patient during a 16-month period. It can be seen that no two isolates share a similar antibiogram profile with the 11 antibiotics tested during this period of time, indicating the presence of a heterogeneous population within this species. Figure 2b shows the relationship of 48 viridans-group streptococci to each other, based on measuring zone sizes (mm). Figure 2c shows the clustering of *S. typhimurium* in five family members, whereas contemporaneous isolates were not highly related at the linkage distance (0.0).

The relatedness of bacterial isolates is often assessed qualitatively by simple mental comparison of each respective organism's antibiogram against a common set of antibiotics. This analysis becomes increasingly difficult as the number of isolates examined increases, as well as when the number of antibiotics examined increases. Many laboratories perform antibiotic disk susceptibility beyond that required to aid therapeutic management, yet relatively few data are obtained from this additional effort. The generation of antibiotic susceptibility data is a primary function of any clinical microbiology laboratory. However, it is predominantly used for the clinical management of acute infection. In the absence of any further phenotypic or genotypic epidemiological subtyping scheme, it is often used to compare the relatedness of isolates based on similarities/differences in antibiotic susceptibility patterns. One limitation to exploiting this is the fact that an inability to perform clustering of antibiotic resistance in clinical pathogens in humans may be traced back to resistance mechanisms in environmental bacteria, and any factors likely to alter (upregulate) resistance in environmental organisms are of potential and eventual consequence to human pathogens.

This small study demonstrated the software's ability to generate dendograms that aid the interpretation of each dataset. Commercial availability of software analysis packages, such as the one examined in this study, allows its application presently in clinical microbiology laboratories attempting to interpret antibiogram information.

In conclusion, this software may be a useful adjunct in the interpretation of antibiogram data in clinical microbiology laboratories and an easy-to-use tool, thereby adding further value to the generation of antibiogram information.

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References

1 Goering RV. Pulsed field gel electrophoresis: a review of application and interpretation in the molecular epidemiology of infectious disease. *Infect Genet Evol* 2010; **10** (7): 866–75.

2 Underwood A, Green J. Call for a quality standard for sequence-based assays in clinical microbiology: necessity for quality assessment of sequences used in microbial identification and typing. *J Clin Microbiol* 2011; **49** (1): 23–6.

Hypoglycaemia due to autoimmune insulin syndrome in a 78-year-old Chinese man

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Autoimmune insulin syndrome (AIS) is a rare cause of hypoglycaemia in which a high titre of autoantibodies to endogenous insulin is formed in the absence of prior exposure to exogenous insulin, and was first described in 1973 by Hirata.[1] Epidemiologically, most of the reported cases come from Japan and it is a very uncommon disease in Caucasian population. No epidemiological data have been published regarding the exact incidence of AIS in the Chinese population and only a few case reports are found in the literature.[2–4] The exact aetiology is unknown but it is believed to involve an interplay of genetic predisposition and environmental factors.[5] Clinically, it is characterised by episodes of hypoglycaemia, elevated insulin level and the presence of circulating insulin autoantibodies. This study includes a case report of a Chinese patient with AIS to illustrate the relevant clinical and biochemical features.

A 78-year-old Chinese man was referred for management of recurrent fasting hypoglycaemic attacks presenting with confusion, sweating and hand tremor for one month. His past medical history included hypertension and chronic obstructive airways disease. Medication included prazosin, salbutamol inhaler, terbutaline and theophylline. He had no history of diabetes mellitus and denied use of exogenous insulin or oral hypoglycaemic agents. Physical examination was unremarkable and his vital signs were stable.

During hospitalisation, he was documented to have symptomatic hypoglyacaemia with plasma glucose in the range 1.1–2.1 mmol/L. The simultaneous insulin level during hypoglycaemia was in the range 131–1688 miu/L and C-peptide level was 0.27–0.43 nmol/L. Simultaneous serum growth hormone, cortisol and thyroid function tests were all normal.

Other laboratory studies were significant for mild hypochromic microcytic anaemia (haemoglobin: 11.3 g/dL [reference interval: 13.0–19.0 g/dL), which was subsequently confirmed to be due to underlying β-thalassaemia trait. He had renal impairment (plasma creatinine: 152 μmol/L [85–133 μmol/L]). Liver function was normal. Plasma globulin level was elevated (68 g/L [24–36 g/L]) with albumin:globulin ratio of 0.47. Serum protein electrophoresis revealed a generalised increase in immunoglobulins but no paraprotein band.

Other immune markers (ANA, RF, C3, C4, ANCA) were all negative. Coombs' test was negative. Thyroid function tests were normal and serum antithyroglobulin and antimicrosomal antibodies were both negative. Imaging of the abdomen (CT and MRI) showed no evidence of an insulinoma.

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A polyethylene glycol precipitation and recovery study was performed and the blood insulin level decreased from 131 miu/L to 7.9 miu/L, which suggested a diagnosis of autoimmune insulin syndrome. Initially the patient was treated with octreotide but this failed to prevent hypoglycaemic episodes. Subsequently he was given frequent three-hourly meals both in hospital and at home after discharge, which significantly decreased the frequency of the hypoglycaemic attacks. Steroid or other immunosuppressive therapy was not initiated and the hypoglycaemia resolved.

Autoimmunity is a well-recognised pathogenic mechanism in many endocrine diseases (e.g., Graves' disease and type I diabetes mellitus). In these autoimmune diseases, autoantibodies have been shown to be involved in the pathogenesis. Hypoglycaemia is an important clinical condition because it is associated with serious morbidity and can be fatal if untreated. It is characterised by Whipple's triad: i) symptoms consistent with hypoglycaemia; ii) low plasma glucose concentration; and iii) relief of symptoms after plasma glucose level is corrected to normal. It is most commonly caused by drugs used to treat diabetes, including insulin and sulphonylureas. Other causes include insulinoma, liver, renal or heart failure, severe sepsis, hormone deficiency and metabolic disease.

The aetiology of AIS, like many other autoimmune diseases, is not well understood. Genetic predisposition related to an association with certain areas of the major histocompatibility complex has been suggested. Studies from Japan have shown a striking association with HLA-DR4, in particular with the DRB*0406 allele.[6,7] This may explained the extremely low prevalence of IAS among Caucasians, in whom the prevalence of DRB1*0406 is low.[8] It should be noted that AIS is commonly associated with various rheumatological diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis) and AIS can actually be the presenting symptom.[9] In the reported cases in China, up to 68% were associated with Graves' disease.[9] In the case reported here, the presence of Graves' disease was excluded by normal thyroid function and absence of an elevated thyroid autoantibody titre. Haematological diseases, including multiple myeloma, monoclonal gammopathy and Hodgkin's disease, are also reported to be associated with this condition. A number of drugs have been implicated in the causation or triggering of AIS. In particular, drugs that contain sulphydryl groups (e.g., methimazole) are well documented to be related to AIS in the Japanese case series.[10]

Patients with AIS present with episodes of late post-prandial hyperinsulinaemic hypoglycaemia. However, fasting hypoglycaemia, as in the current patient, has also been reported.[11] Paradoxically, blood glucose level may rise to above the diagnostic cut-off for diabetes mellitus following a meal or oral glucose tolerance test.

In most patients with AIS, the clinical course is benign, with hypoglycaemia showing improvement or resolution within six months, even without treatment with immunosuppressants. The most effective intervention is small, frequent meals to prevent post-prandial hypoglycaemia. Sweets should be avoided, except during attacks of hypoglycemia. If a drug is suspected to be the precipitating cause, the offending drug should be stopped. Immunosuppression with corticosteroid, plasmapheresis, acarbose, octreotide, diazoxide, and also partial pancreatectomy, have all been tried with variable results.[5,12-15]

The mechanism whereby insulin autoantibodies induce hypoglycaemia has been well studied.[16,17] After a meal, endogenous insulin is released from pancreatic β-cells, as is the case in normal individuals. However, the insulin autoantibodies bind a portion of the secreted insulin so that the free and active form in plasma decreases, which explains why some patients actually have hyperglycaemia after an oral glucose tolerance test, and are misdiagnosed as having diabetes mellitus. As plasma glucose level drops, so does endogenous insulin secretion and total insulin concentration. The insulin previously bound to autoantibodies is then released, resulting in a surge of free insulin and the clinical manifestation of hypoglycaemia.

The differential diagnosis of AIS from other causes of hyperinsulinaemic hypoglycaemia is important. Insulinoma was initially considered in the patient reported here; however, extensive imaging studies, including CT and MRI, failed to demonstrate a tumour mass in the pancreas. Also, the insulin value is rarely greater than 100 miu/L in cases of insulinoma. Surreptitious insulin injection is another important factor to consider. The C-peptide level during a hypoglycaemic episode due to exogenous insulin should be suppressed, while in insulinoma and AIS it should be normal or even elevated. Insulin autoantibody titre is elevated in the serum of all patients with AIS; however, the assay is not available in the authors' hospital.

In conclusion, AIS is a rare but important cause of hyperinsulinaemic hypoglycaemia and should be suspected in all patients with unexplained hypoglycaemia with insulin levels >100 miu/L and unsuppressed C-peptide level. The clinical course of AIS is mostly benign and correct diagnosis may prevent unnecessary invasive investigation or even pancreatectomy.

References

1 Hirata Y. Insulin autoimmune syndrome (in Japanese). *Nihon Rinsho* 1973; **31** (7): 2227–31.

2 Benson EA, Ho P, Wang C, Wu PC, Fredlund PN, Yueng RT. Insulin autoimmunity as a cause of hypoglycemia. *Arch Intern Med* 1984; **144** (12): 2351–4.

3 Ma WY, Won JG, Tang KT, Lin HD. Severe hypoglycemic coma due to insulin autoimmune syndrome. *J Chin Med Assoc* 2005; **68** (2): 82–6.

4 Chen CH, Huang MJ, Huang BY *et al.* Insulin autoimmune syndrome as a cause of hypoglycemia – report of four cases. *Changgeng Yi Xue Za Zhi* 1990; **13** (2): 134–42.

5 Lupsa BC, Chong AY, Cochran EK, Soos MA, Semple RK, Gorden P. Autoimmune forms of hypoglycemia. *Medicine (Baltimore)* 2009; **88** (3): 141–53.

6 Uchigata Y, Kuwata S, Tokunaga K *et al.* Strong association of insulin autoimmune syndrome with HLA-DR4. *Lancet* 1992; **339** (8790): 393–4.

7 Uchigata Y, Tokunaga K, Nepom G *et al.* Differential immunogenetic determinants of polyclonal insulin autoimmune syndrome (Hirata's disease) and monoclonal insulin autoimmune syndrome. *Diabetes* 1995; **44** (10): 1227–32.

8 Uchigata Y, Hirata Y, Omori Y, Iwamoto Y, Tokunaga K. Worldwide differences in the incidence of insulin autoimmune syndrome (Hirata disease) with respect to the evolution of HLA-DR4 alleles. *Hum Immunol* 2000; **61** (2): 154–7.

9 Qing Y, Zhou JG, Yuan G. Systemic lupus erythematosus presenting as hypoglycaemia with insulin receptor antibodies and insulin autoantibodies. *Lupus* 2009; **18** (5): 457–9.

10 Uchigata Y, Hirata Y, Iwamoto Y. Drug-induced insulin autoimmune syndrome. *Diabetes Res Clin Pract* 2009; **83** (1): e19–20.

11 Taylor SI, Barbetti F, Accili D, Roth J, Gorden P. Syndromes of autoimmunity and hypoglycemia. Autoantibodies directed against insulin and its receptor. *Endocrinol Metab Clin North Am* 1989; **18** (1): 123–43.

12 Redmon B, Pyzdrowski KL, Elson MK, Kay NE, Dalmasso AP, Nuttall FQ. Hypoglycemia due to an insulin-binding monoclonal antibody in multiple myeloma. *N Engl J Med* 1992; **326** (15): 994–8.

13 Schlemper RJ, Uchigata Y, Frolich M, Vingerhoeds AC, Meinders AE. Recurrent hypoglycaemia caused by the insulin autoimmune syndrome: the first Dutch case. *Neth J Med* 1996; **48** (5): 188–92.

14 Suzuki K, Hirayama S, Ito S. A case of a non-insulin dependent diabetic patient with regular spontaneous hypoglycemic attacks, which were due to insulin-binding antibodies induced by human insulin therapy. *Tohoku J Exp Med* 1997; **182** (2): 163–73.

15 Wasada T, Eguchi Y, Takayama S, Yao K, Hirata Y, Ishii S. Insulin autoimmune syndrome associated with benign monoclonal gammopathy. Evidence for monoclonal insulin autoantibodies. *Diabetes Care* 1989; **12** (2): 147–50.

16 Redmon JB, Nuttall FQ. Autoimmune hypoglycemia. *Endocrinol Metab Clin North Am* 1999; **28** (3): 603–18, vii.

17 Dozio N, Scavini M, Beretta A *et al.* Imaging of the buffering effect of insulin antibodies in the autoimmune hypoglycemic syndrome. *J Clin Endocrinol Metab* 1998; **83** (2): 643–8.