Thyroid disease in pregnancy

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Pregnancy is a time of dynamic hormonal interactions between the mother and the fetus via transplacental passage of thyroid hormone. In addition, there are established gestational changes in thyroid function that render the diagnosis of thyroid disease during pregnancy more challenging. Due to increases in serum thyroid-binding globulin, the pregnancy reference ranges for total T4 and T3 are 1.5 times the nonpregnant ranges. However, binding protein changes and volume dilution affect automated FT4 assays lowering gestational reference ranges compared to nonpregnant norms. In the first trimester, due to hCG thyroidal stimulation, maternal serum TSH levels may decreased nonpregnant reference range; serum TSH levels then rise as pregnancy progresses and newer data demonstrates the upper gestational limit is higher than 2.5 or 3mIU/L as in prior guidelinesand closer to 4.0mIU/L. Euthyroid women with thyroid autoimmunity are at higher risk for miscarriage and preterm delivery related to the autoimmunity rather than to alterations in thyroid function but whether LT4 therapy ameliorates this is unknown. During pregnancy, the incidences of subclinical (SCH) and overt (OH) hypothyroidism are 2% and 0.5% respectively. Recent cohort studies have investigated adverse effects associated with maternal subclinical hypothyroidism. A recent meta-analysis of these studies reported increased rates of pregnancy loss, preterm delivery, abruption and breech presentation but results may be confounded by maternal autoimmunity and the definition of SCH. Gestational and infant cognitive outcomes were not found to be improved in 2 recent randomized controlled trials (RCT) evaluating screening strategies, where the most common detected maternal thyroid disorder was SCH. In addition, a recent multicenter RCT of LT4 for maternal SCH diagnosed in the early 2nd trimester did not show any benefit. These studies may all be criticized because the intervention, LT4, was administered too late in gestation to potentially prevent harm given that that maternal thyroid hormone is present in the fetus at 6 weeks gestation. For LT4 treated hypothyroid women, dosage requirements increase during gestation and decrease postpartum. Thyroid function should be regularly monitored during pregnancy. Graves' disease complicates 0.2% of pregnancies. Both antithyroid drugs (ATD), PTU and methimazole (MMI) cross the placenta, potentially compromising fetal thyroid hormone production and resulting in goiter. In addition, although both are associated with organogenesis defects, those related to MMI are more severe. Therefore, during the 1st trimester, cessation of ATD should be considered with careful TFT monitoring for hyperthyroid women on low dose ATD who may be in remission. Otherwise PTU is recommended for the first 16 weeks gestation until completion of organogenesis. Neonatal thyroid function is inversely correlated with maternal ATD dosage and directly correlated with maternal thyroid hormone levels. The therapeutic target for maternal Graves' during pregnancy is actually "subclinical hyperthyroidism" and women should receive the lowest ATD dose to maintain their serum total T4 at the upper pregnancy reference range or the FT4 at the upper nonpregnant reference range. Fetal and neonatal thyrotoxicosis complicates 1% of pregnancies in women with active or treated Graves' disease, even after I-131 ablation, via transplacental passage of thyroid stimulating immunoglobulins. Serum TSH receptor antibody (TRAb) should be checked early in pregnancy and if positive, at the end of the 2nd trimester. If levels are 3x>NL, a fetal ultrasound should be obtained to screen for fetal thyrotoxicosis. When treating a pregnant woman with thyroid disease, the clinician must continually be aware that there are two patients, the mother and the fetus.

abs #2

Severe hypercalcemia in pregnancy - beating the odds

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Background

Primary hyperparathyroidism (PHPT) during pregnancy is a rare condition that is under recognized. Moderate to severe hypercalcemia is associated with considerable maternal and fetal morbidity and mortality. Rates of up to 3.5 fold increase in miscarriage have been reported[1.] Pregnancy loss often occurs in the second trimester.

We describe a case of a woman who presented in early third trimester with severe pancreatitis due to PHPT. Usual localisation studies, appropriate in pregnancy, failed to locate a parathyroid adenoma and subsequent cervical exploration and parathyroidectomy failed to cure the hypercalcemia. We discuss the localisation studies that can be used in pregnancy and management challenges of PHPT in pregnancy.

Case Presentation

In August 2017, a 35-year-old primigravida at 27^{+5} weeks gestation was transferred from a private hospital, for surgical opinion, with 24-hour history of nausea, vomiting and epigastric pain, radiating to the back. Past medical history was significant for cholecystectomy. Soon after presentation, she deteriorated. Acute pancreatitis with a lipase of 739 U/L was diagnosed and she was transferred to intensive care unit (ICU) with acute respiratory distress syndrome. Investigation excluded hypertriglyceridemia and hyperglycaemia. Abdominal ultrasound demonstrated oedematous pancreas and MRI excluded biliary tree dilatation or choledocholithiasis. Plasma adjusted calcium on admission was 2.70 mmol/L and ionised calcium was 1.43 mmol/L. As pancreatitis improved, plasma adjusted calcium rose and after a week, the calcium was 3.3 with a concurrent parathyroid hormone (PTH) of 9 pmol/l. An obstetric ultrasound scan (USS) showed normal growth parameters and SD (systolic/diastolic) ratio, but amniotic fluid index (AFI) was in the 5th centile. Calcium level peaked at 3.50mmol/L (**Fig 1**). The hypercalcemia did not respond to intravenous hydration, averaging 4 litres a day. Repeat obstetric USS showed oligohydramnios with AFI 2.5th centile, precluding the use of frusemide. An USS of the neck showed an abnormal bulky thyroid gland containing multiple, mostly solid isoechoic nodules with increased vascularity without microcalcification, no parathyroid adenoma was identified. MRI of the neck showed no definite parathyroid adenoma. Multiple nodules were seen within the thyroid gland. A 4D CT of the neck with contrast also failed to show any adenoma in the neck or the superior mediastinum. FNAC of the thyroid nodules revealed colloid nodules.

A multidisciplinary team meeting concluded neck exploration and parathyroidectomy was preferred. Use of pre-operative salmon calcitonin had little effect. At 31+ 4 weeks gestation, a cervical and mediastinal exploration with parathyroidectomy, cervical thymectomy and total thyroidectomy were performed. Left inferior and right superior parathyroid were removed and confirmed in frozen section. Left superior parathyroid was macroscopically normal and preserved. Right inferior parathyroid was not found. Intra-operative PTH after 15mins was 16pmol/L therefore total thyroidectomy performed. Histology confirmed L inferior parathyroid weighing 76mgs, right superior parathyroid weighing 280mgs, right inferior parathyroid attached to thyroid, at the right inferior pole. A repeat CT neck 2 days post–op failed to localise a parathyroid adenoma.

At 32^{+1} weeks gestation, an elective Caesarean section was performed, and a live normal neonate was delivered with APGAR 8 and 9. There was no neonatal hypocalcemia and she was managed with IV calcium through the umbilical vein which was ceased on day 8 (**Fig 2**). The baby's PTH was 0.3 at birth, which subsequently normalised. The patient received zoledronic acid infusion 2 days after delivery. A parathyroid sestamibi scan demonstrated a nodule in the anterior mediastinum, anterior to the aortic root, in keeping with an ectopic parathyroid adenoma (**Fig 3**). In February 2018, she underwent a video-assisted thoracoscopic surgery (VATS) procedure in private hospital, for removal of the mediastinal parathyroid adenoma. Two weeks post-op, she was noted to be short of breath at routine outpatient clinic follow up and was admitted for treatment of a chylothorax (**Fig 4**) with an intercostal catheter (ICC) . This was complicated by empyema requiring decortication. She has recovered well, has hypoparathyroidism with a normal PTH (2.3 pmol/L) and hypocalcemia (2.01 mmol/L) three months post op and remains on calcitriol 0.25mcg daily.

Discussion

Primary hyperparathyroidism is a common endocrine problem that is uncommon in pregnancy and under recognised[9]. PHPT in pregnancy is associated with hyperemesis, nephrolithiasis, pancreatitis and pre-eclampsia. When untreated, fetal complications include intrauterine growth retardation, low birth weight, premature delivery, stillbirth or neonatal tetany[2,3]. The diagnosis is confounded by the physiological adaptations in pregnancy. There is increased intestinal absorption of calcium to meet the fetal needs and maternal calcium stores. Maternal PTH level declines in pregnancy and PTH is inhibited by PTH-related peptide (PTHrP) produced by the placenta and due to increased 1,25 dihydroxyvitamin D from increased conversion of 25 hydroxyvitamin D by 1 a hydroxylase, expressed by the placenta. The prevalence of the condition is not entirely known. Fewer than 200 cases have been reported. The prevalence of ectopic parathyroids is even rarer and ranges from 6.3% to 16% in surgical series of patients operated for PHPT and up to 45% in patients who had re-exploration [4]. The prevalence of mediastinal parathyroid adenomas has been reported to be 6-30% [5].

PHPT in non-pregnant individuals runs a fairly benign course. However, in pregnancy it has serious implications on obstetric and fetal outcome, which is directly related to the severity of hypercalcemia. Pregnancy loss occurs most commonly at calcium levels of greater than 2.85mmol/L[1]. Hence at these levels, parathyroidectomy should be offered in the early second trimester. High maternal calcium level suppresses fetal PTH, which may lead to neonatal hypocalcaemia and in severe cases, tetany and seizures. The medical options to treat hypercalcemia in pregnancy are limited due to paucity of safety data. Aggressive hydration is not always sufficient as in our patient. Calcitonin does not cross the placenta and acts directly by inhibiting osteoclasts. However, its use is limited by tachyphylaxis and low efficacy. Cinacalcet (a calcimimetic agent) has been used in various cases where surgery was not possible, however it crosses the placenta and the long-term effects on the fetus is not known; the drug is poorly tolerated, and normalization of serum calcium is not achieved in most cases[6]. Bisphosphonates cross the placenta and the long-term effect on the offspring is not known, hence is not recommended in pregnancy. Surgery is the only effective cure.

Our patient's clinical presentation of primary hyperparathyroidism with pancreatitis, pregnancy status and mediastinal location of parathyroid adenoma is relatively rare. With retrospect, if parathyroid scintigraphy had been utilised in the pregnant state, the mediastinal parathyroid adenoma would have been recognised, altering the surgical approach. Sestamibi scintigraphy has been used safely in pregnancy in reported cases[7]. The dose to fetus can be estimated by a physicist to ensure safety; in our patient it was estimated to be below threshold for any tissue reactions or neurological effects[8]. There is a near immeasurable increased risk of developing a cancer following radiation exposure however is negligible compared to the natural incidence of cancer.

Learning points:

1. A high index of suspicion is needed to diagnose primary hyperparathyroidism in pregnancy. Any gravid female with hyperemesis, hypophosphatemia, nephrolithiasis, bone fractures and peptic ulcers should be screened with plasma adjusted calcium.
2. Parathyroidectomy is the definitive treatment for severe hypercalcemia and multidisciplinary team approach helps achieve the best outcome.
3. Sestamibi scintigraphy may be considered for preoperative localisation if USS fails.
4. Anticipation of hypocalcemia in the neonate at delivery and appropriate timely management is crucial.

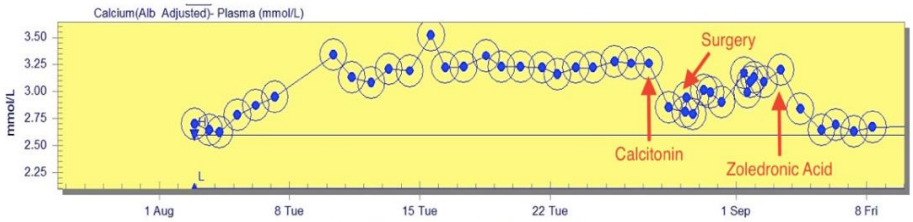

Fig 1: Mother's Plasma Adjusted Calcium

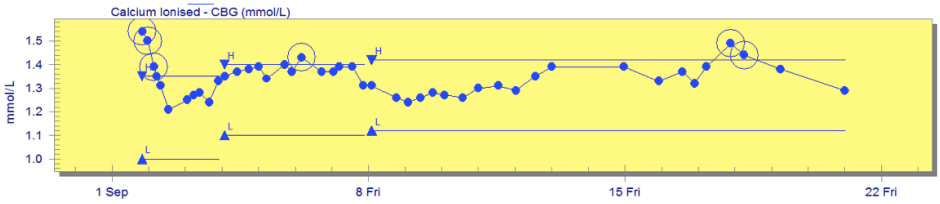

Fig 2: Baby's Ionised Calcium

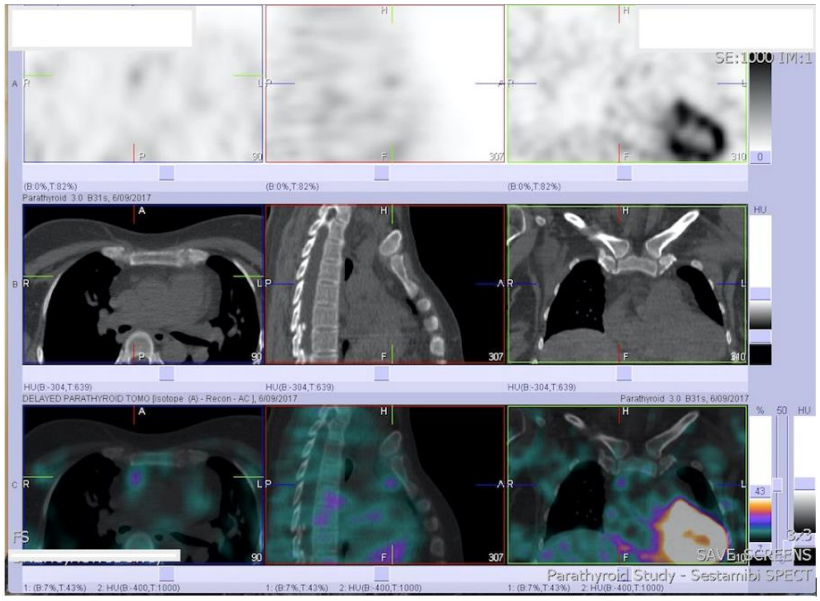

Fig 3: Sestamibi Scintigraphy

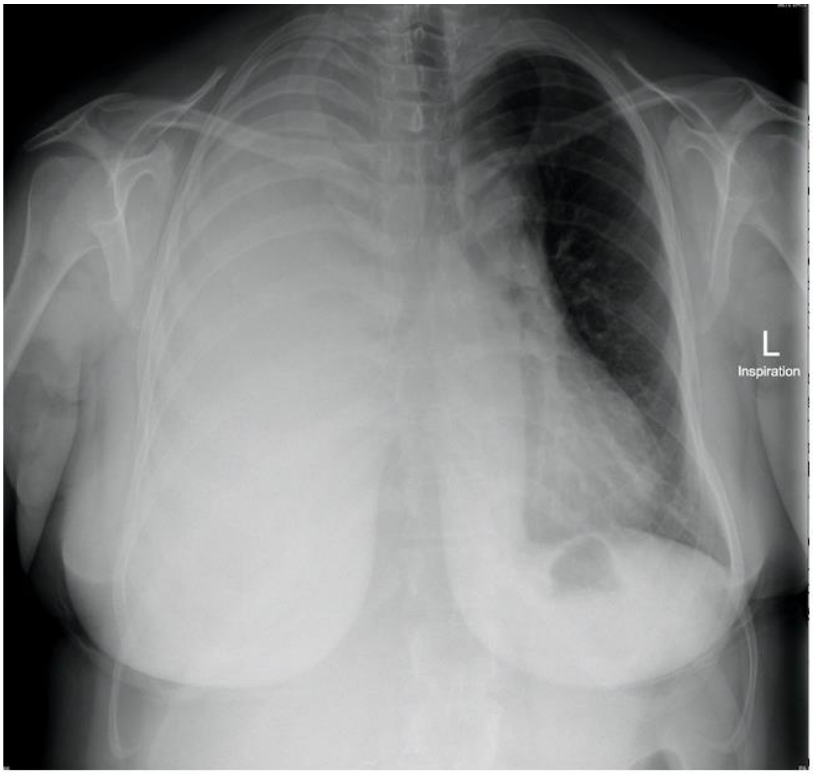

Fig 4: CXR Two Weeks Post VATS

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abs#3

A bony conundrum

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Case:

A 73-year-old woman was referred to the Endocrine Unit in 2017 for investigation of severe osteoporosis. She had multiple fragility fractures predominantly involving the femurs and ribs since 2006, with consistent hypophosphataemia and elevated alkaline phosphatase (ALP) levels. She suffered from chronic bony pain along her lower back and thighs. Treatment for her osteoporosis in the past 8 years included initial therapy with Zoledronic acid, followed by 18 months of Teriparatide and subsequently recommencement of Zoledronic acid. Antiresorptive therapy was then switched to Denosumab in July 2016. Other relevant medical history included lumbar spine osteoarthritis, nephrolithiasis and hiatus hernia on Esomeprazole 40mg daily. She was on Caltrate 600mg BD and was commenced on Sandoz phosphate in January 2016.

Further history revealed unintentional weight loss of 34kgs over 7 years. She had normal childhood development and had no family history of metabolic bone disorder. Menopause was in her late 40s and she had a brief trial of hormone replacement therapy. Adequacy of her dietary calcium intake was unclear.

There were no clinical signs of cortisol or thyroid hormone excess on examination and she had severe kyphoscoliosis.

Most recent pathology showed a normal renal function, serum calcium, magnesium and 25-hydroxy vitamin D (25OH D) levels. Phosphate level whilst on phosphate supplement was 0.78mmol/L (reference range (rr): 0.75-1.50). Parathyroid hormone (PTH) level was elevated at 27.1pmol/L (rr: 1.1-6.9). Serum 1,25 dihydroxy vitamin D (1,25 (OH)2 D) level was normal. Thyroid stimulating hormone was normal. Myeloma screen and coeliac serology were unremarkable. Past blood results demonstrated chronic hypophosphataemia over a 10-year period, with persistently elevated ALP and PTH. However, PTH was normal at one stage in 2010 (Table 1).

Table 1: Pathology results from 2007 to 2017

Test	2007	2010	2011	2013	2015	2017	Units	Normal range	
Na	141	-	140	142	135	140	mmol/L	135	145
K	4.7	-	4.0	3.9	4.2	4.5	mmol/L	3.5	5.2
Creatinine	74	-	43	48	49	54	µmol/L	45	90
eGFR	-	-	>90	>90	>90	>90	mL/min/1.73m^2		>90
ALP	234*	-	316*	176*	300*	101	U/L	30	110
Corrected Calcium	2.15	2.26	2.35	2.25	2.29	2.22	mmol/L	2.10	2.60
Mg	-	-	0.83	0.77	0.74	0.85	mmol/L	0.70	1.10
PO4	0.54*	0.16*	0.25*	0.43*	0.33*	0.78	mmol/L	0.75	1.50
PTH	-	5.4	14.8*	-	11.8*	27.1*	pmol/L	1.1	6.9
25OH D	-	-	81	79	70	48	nmol/L	31	107
TSH	1.59	-	-	-	0.9	1.3	mU/L	0.27	4.2
Serum CTx	-	-	-	-	-	75	ng/L		<1000
1,25 (OH)2 D	-	-	-	-	-	120	pmol/L	60	200

CT chest to pelvis done a year ago showed no evidence of malignancy and parathyroid sestamibi scan in 2009 was normal. BMD scan in 2015 showed osteoporosis at left femoral neck (T-score: -4.2) and right total hip (T-score: -4.3). Z-scores were below the expected range for her age (Table 2). A bone scan in 2016 showed multiple fractures of varying ages with no metastatic bone disease.

Table 2: Bone mineral density scan in 2015

Site	Bone Mineral Density	Units	T-score	Z-score
Lumbar (L2-L4)	0.95	g/cm^2	-2.1	-1.2
L femoral neck	0.49	g/cm^2	-4.2	-3.1
R total hip	0.48	g/cm^2	-4.3	-3.7

Her recurrent fractures, hypophosphataemia and elevated ALP were consistent with hypophosphataemia-induced osteomalacia. Differential diagnosis included hereditary hypophosphataemia, renal phosphate wasting and Fanconi's syndrome. Esomeprazole was switched to Ranitidine due to suspected PPI-mediated decreased intestinal phosphate absorption. 24-hour urine chemistry was performed. In the context of normal renal function, satisfactory 25OH D and normal 1,25 $(OH)_2$ D, fractional excretion of phosphate was inappropriately high whilst on and off phosphate replacement at 45% and 44% respectively (rr:5-20), indicating renal loss of phosphate (Table 3a and 3b). Tubular reabsorption of phosphate was low at 57% (rr: 82-95). Disease manifestation in adulthood and no family history of osteoporosis made familial hypophosphatemia an unlikely diagnosis. The absence of glucosuria, proteinuria and hypercalciuria were not consistent with Fanconi's syndrome. Normal PTH in 2010 with hypophosphataemia made hyperparathyroidism less likely to be the cause of phosphate wasting.

Table 3a: 24hr urine chemistry whilst on phosphate supplement

Test (on phosphate supplement)	Values	Units	Normal range	
Urine Creatinine	7.6	mmol/24h	6.0	18.0
Urine Glucose	<1.2	mmol/24 h		<2.8
Urine Protein	133	mg/24h		<150
Urine Calcium	1.5*	mmol/24h	2.5	7.5
Urine Phosphate	46.3*	mmol/24h	12.9	42.0
Serum Phosphate	0.78	mmol/L	0.75	1.50
Fractional excretion of phosphate	45*	%	5	20

Table 3b: 24hr urine chemistry whilst off phosphate supplement

Test (off phosphate supplement)	Values	Units	Normal range	
Urine Creatinine	4.4*	mmol/24h	8.8	17.6
Urine Calcium	2.3*	mmol/24h	2.5	7.5
Urine Phosphate	13*	mmol/24h	16	48
Serum Phosphate	0.55*	mmol/L	0.75	1.50
Fractional excretion of phosphate	44*	%	5	20

Fibroblast growth factor-23 (FGF-23) level was markedly raised at 140 U/L (rr:10-54), in keeping with FGF-23 mediated hypophosphataemia. Ga-68 DOTATATE-PET showed a moderate to intensely DOTATATE-avid lesion at the right lateral mass of the atlas (Figure 1a and 1b), suspicious for a somatostatin receptor positive malignancy. Her biochemistry and imaging were consistent with tumour-induced osteomalacia (TIO), likely due to a benign mesenchymal tumour localising to the right lateral atlas.

Figure 1a: Ga-68 DOTATATE PET showing focal moderate to intense dotatate accumulation at the right lateral mass of the atlas

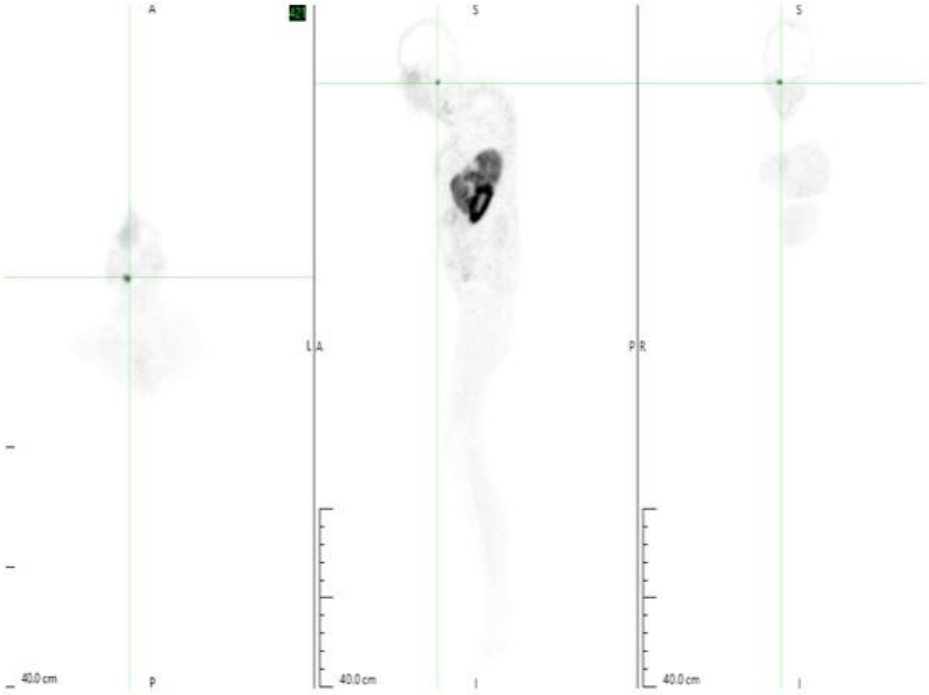
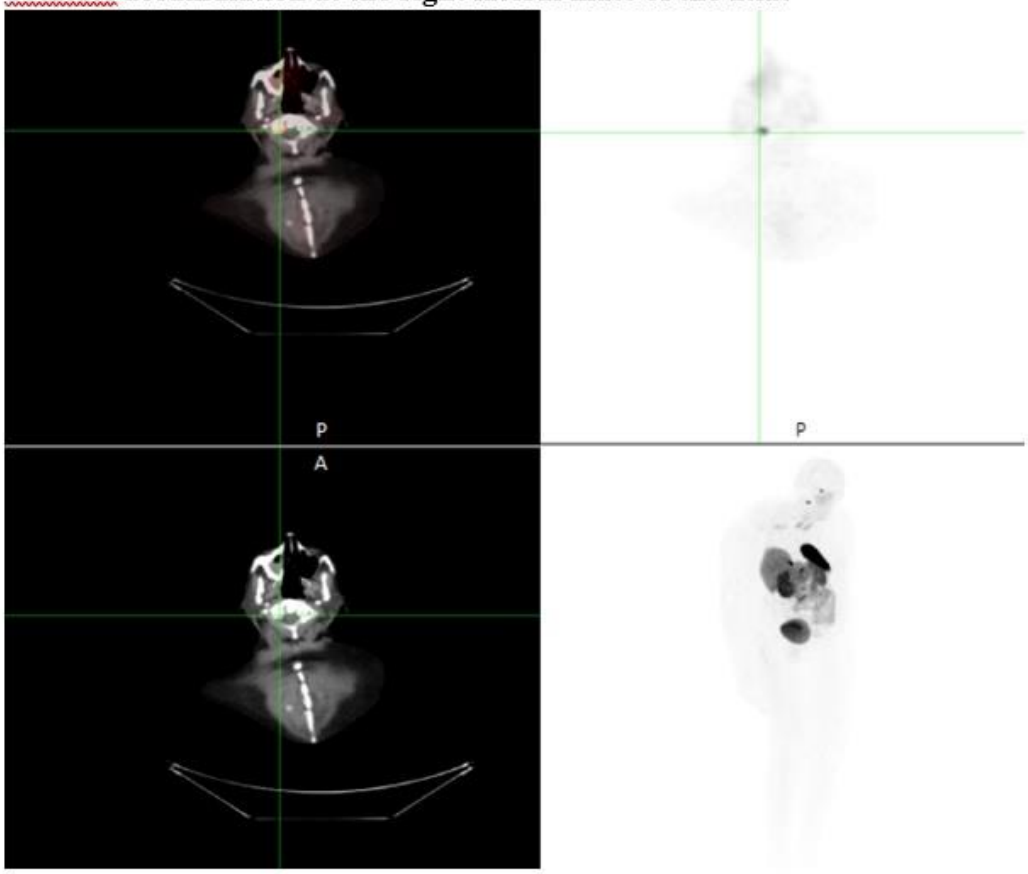


Figure 1b: Ga-68 DOTATATE PET showing focal moderate to intense dotatate accumulation at the right lateral mass of the atlas



Her extensive kyphoscoliosis and the tumour location made it difficult for it to be biopsied or operated on. Patient elected for medical therapy only and did not undergo further investigations given her comorbidities. Her management included commencement of Calcitriol, cholecalciferol and continuation of phosphate supplement. Antiresorptive therapy was discontinued. She was followed-up in the Endocrine clinic 6-monthly and since the normalisation of serum phosphate level with phosphate supplement, there had not been any further insufficiency fractures.

Discussion:

Tumour-induced osteomalacia (TIO) is a rare metabolic bone disorder that is usually associated with benign mesenchymal tumours. These tumours are usually located in the lower extremity long bones, craniofacial region and the upper extremities.[1,2] Its prevalence is unknown.[2] Patients often present with years of non-specific symptoms including progressive bone aches, muscle weakness, multiple fractures and in some cases, weight loss. These symptoms can mimic other neurological, rheumatological or orthopaedic conditions.[1,2]

The characteristic metabolic derangements in TIO is hypophosphataemia caused by impaired renal phosphate reabsorption. Additional tests including low to normal 1,25 (OH)2 D levels, elevated ALP levels and normal calcium and parathyroid hormone levels can be helpful in assisting the diagnosis of TIO. PTH levels are occasionally elevated, reflecting secondary hyperparathyroidism due to low 1,25 (OH)2 D caused by elevated FGF-23.[1,2]

The tumours cause osteomalacia in TIO by the production of phosphatonins, commonly FGF-23, an important regulator of phosphate homeostasis. FGF-23, a peptide hormone synthesized by osteoblasts and osteocytes, binds to proximal renal tubule where 80% of filtered phosphate is reabsorbed, resulting in the reduction of type-2a and 2c sodium-phosphate co-transporter and a consequent decrease in phosphate reabsorption.[1] FGF-23 also inhibits the expression of alpha1-hydroxylase hence reducing the production of 1,25 (OH)2 D and intestinal phosphate absorption.

The assessment of suspected TIO in a patient with hypophosphataemia and symptoms of osteomalacia should begin with measurements of serum calcium, ALP, PTH, 25OH D, 1,25 (OH)2 D and urine phosphate, creatinine, calcium, glucose and amino acids. Renal phosphate wasting can be established by measurement renal tubular absorption of phosphate. FGF-23 is a useful diagnostic tool and is elevated in most patients with TIO. It is essential to rule out hereditary causes of hypophosphataemic syndromes such as X-linked hypophosphataemia and autosomal dominant hypophosphataemic rickets, both of which are also FGF-23 dependent phosphate wasting disorders.[1] Differential diagnosis should also include Fanconi's syndrome, a disorder of the proximal renal tubules, leading to impaired phosphate reabsorption and hypophosphataemia. The key discriminating factor is that the plasma FGF-23 level is low in Fanconi's syndrome and high in TIO.[1-3]

Functional testing is preferred over anatomical imaging.[4] Many TIO tumours express somatostatin receptors that regulate its secretory activity. The use of Ga-DOTANOC PET/CT and Ga-DOTATATE PET/CT have been explored to localise TIO. These scans utilise the use of modified octreotide molecules that have increased affinity for somatostatin receptors 2 and 5. FDG PET/CT has also been used, however it lacks specificity. Anatomical imaging that can be performed includes MRI, either localised if prior functional imaging has identified the tumour or whole body if being used as the initial imaging modality.[4,5]

The gold standard treatment is tumour resection with a wide margin to ensure complete tumour removal and to prevent recurrence. Late recurrence has been shown to occur in <5% of TIO cases. After resection, there is a dramatic resolution of the biochemical disturbances, with normalisation of serum phosphate and FGF-23 levels within 5 days.[1]

In TIO cases where the tumour cannot be localised or if medical therapy is preferred, phosphate supplementation to correct the ongoing renal losses and calcitriol supplementation to enhance phosphate reabsorption from the renal tubules and gastrointestinal tract may be used. PTH suppression induced by Calcitriol also helps to minimise phosphate loss. For refractory cases, octreotide therapy or cinacalcet may be considered.[1-3]

Learning points:

- TIO is a rare paraneoplastic syndrome characterised by hypophosphataemia and osteomalacia.
- Ga-68 DOTATATE PET is the imaging modality of choice.
- The treatment of choice for TIO is surgery.
- Medical therapy includes phosphate supplement and calcitriol.
- Not all insufficiency fractures are due to osteoporosis.

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abs#4

What's in a nAME? A case of severe hypokalaemia

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Case report

A 72 year old woman presented to the emergency department with a 2 day history of severe leg weakness and myalgia. She reported feeling generally unwell for 2 weeks prior with poor appetite and fatigue. Approximately 1 month prior, her General Practitioner had prescribed sitagliptin to improve her glycaemic control (HbA1c 7.6%). Her past medical history was significant for type 2 diabetes mellitus, hypertension, dyslipidaemia and gastro-oesophageal reflux disease. Her regular medications included sitagliptin 100mg daily, insulin glargine 50 units nocte, perindopril 5mg daily, rosuvastatin 10mg daily, duloxetine 30mg daily, esomeprazole 30mg daily and colecalciferol 25microg daily.

On examination, she was alert and orientated to time, place and person. Initial vital signs included temperature 37.2 degrees, pulse 75 beats per minute, blood pressure 145/70 mmHg, respiratory rate 18 breaths per minute and SaO2 98% on room air. Her cardiovascular, respiratory and gastrointestinal examinations were unremarkable. Lower limb neurological examination revealed normal tone, normal reflexes but asymmetric left sided moderate weakness (3/5) affecting hip flexion/extension, knee flexion/extension and ankle dorsiflexion/plantarflexion and abnormal sensation in the L2/3 dermatomes.

An initial diagnosis was made of lumbar radiculopathy due to compression of L2/3 nerve roots.

However, these results then became available:

Test	RR	Result (27/3/2018)
Sodium	135-150 mmol/L	145 mmol/L
Potassium	3.5-5.0 mmol/L	**1.9 mmol/L**
Chloride	100 – 109 mmol/L	**92 mmol/L**
Bicarbonate	22 – 32 mmol/L	**44 mmol/L**
Anion gap	7 – 17 mmol/L	12 mmol/L
Urea	2.7 – 8 mmol/L	3.1 mmol/L
Creatinine	50 – 100 µmol/L	86 µmol/L
Glucose	3.2 – 5.5 mmol/L	6.0 mmol/L
Calcium	2.10 – 2.60 mmol/L	2.22 mmol/L
Phosphate	0.75 – 1.50 mmol/L	0.74 mmol/L
Albumin	34 – 48 g/L	**28 g/L**
Magnesium	0.70 – 1.1 mmol/L	0.87 mmol/L
Creatine kinase	0 – 150 IU/L	**3594 IU/L**
CRP	<8.0 mg/L	30 mg/L
ECG		Sinus rhythm, **prolonged QTc 557 ms, prolonged PR 200 ms**, no U waves

Figure 1: Initial biochemistry

The patient was subsequently diagnosed with "1) Severe hypokalaemia with ECG changes ?cause; 2) Rhabdomyolysis ?cause ?statin-related; 3) left leg pain/numbness ?nerve root compression ?myositis. Initial management included transfer to ICU for intravenous fluids, magnesium and potassium replacement and cessation of rosuvastatin. Despite aggressive potassium replacement (oral effervescent potassium 100mmol and intravenous potassium chloride 180 mmol/12 hours), the patient remained mildly hypokalaemic (K+ 3.1 mmol/L) and hypertensive (193/78 mmHg).

Further investigations:

Test	27/3	28/3	29/3	30/3	31/3	1/4	2/4	3/4
Maximum Blood pressure (mmHg)	**145/70**	**193/78**	**155/75**	**150/70**	**160/80**	**170/80**	130/70	**160/90**
Potassium	**1.9**	**3.1**	**3.0**	3.8	**2.9**	3.7	3.9	4.3
Aldosterone (60 -980 pmol/L)		**41**						**<30**
Renin (7 – 50 µIU/L		**<2**						**<2**
TSH (0.5 – 4 mIU/L)		2.12						
fT4 (10-25 pmol/L)		15						
24 hour urinary free cortisol (10-150 mmol/24 h)				**427**				
Late night salivary cortisol (<3 nmol/L)							<8	<8

Figure 2: further investigations

Endocrinology was consulted on 4/4/18 to help clarify if there was an endocrine cause for the hypokalaemia, hypertension and metabolic alkalosis, and whether this could be due to Cushing's syndrome.

Further history from patient revealed that she had no recent nausea, vomiting, diarrhoea, unintentional weight loss, use of diuretics/laxatives/corticosteroids, easy bruising or poor wound healing. Up until 1 month ago, she had eaten multiple bananas per day, but had been advised by her General Practitioner to cut down on her fruit intake due to high sugar content. As the Endocrinologist was walking out of the room, she asked one last question, a long shot: "Do you eat liquorice?" The patient replied, "Well yes! I import it from Holland as a powder called Zwart Wit Zout. I love it! I've eaten it for years," After translating the Dutch ingredients in Zwart Wit Zout, the consultant confirmed that it contained liquorice root extract. A diagnosis was made of apparent mineralocorticoid excess syndrome due to chronic excessive liquorice ingestion presenting with severe hypokalaemia, hypertension and rhabdomyolysis. The patient was counselled regarding cessation of liquorice (much to her despair), potassium supplements and perindopril were ceased and spironolactone was commenced for hypertension. The patient was discharged on 8/4/18 with normotension (blood pressure 130/70 mmHg) and normokalaemia (3.7 mmol/L) and will be followed up in the Endocrine Clinic.

Discussion

The syndrome of apparent mineralocorticoid excess (AME) or pseudoaldosteronism is an uncommon cause of hypokalaemia, metabolic alkalosis and hypertension. This can be easily distinguished from primary aldosteronism by a suppressed aldosterone and suppressed renin, suggestive of non-aldosterone mediated mineralocorticoidism. (1) Causes of this include Cushing's syndrome, chronic liquorice ingestion and rare genetic disorders including AME and Liddle's syndrome. (2)

Liquorice is obtained from the root of *Glycyrrhiza glabra* and has been used for centuries for a variety of purposes. Most commonly, it is used as a sweetener or flavouring agent in confectionary, teas and chewing tobacco and less commonly used as a colouring agent in alcoholic beverages. (3) It has been used historically to treat a number of ailments including cough, peptic ulcer disease and chronic viral hepatitis. (4, 5) Recently, the body building community has reported using liquorice as a supplement due to purported androgenic features. (6)

The mineralocorticoid-like properties of liquorice were first reported in 1946 by Revers, a Dutch doctor, who noted that patients prescribed liquorice extract for peptic ulcer disease commonly developed peripheral oedema due to salt and water retention. (1) Indeed, in the 1950s Molhuysen & Card intentionally exploited this property in patients with Addison's disease, with variable success. (7) It was not until 1968 when Conn identified that the mineralocorticoid effects of liquorice were not aldosterone-mediated with the advent of renin and aldosterone assays. (1) Subsequently, Stewart et al. proposed that glycyrrhetinic acid, the main active component of liquorice, inhibited 11β-hydroxysteroid dehydrogenase 2 (11HSD2) in a dose-dependent fashion, preventing the conversion of cortisol to inactive cortisone. This potentiated the effects of cortisol on the mineralocorticoid receptor which, given the much higher concentrations of cortisol compared to aldosterone, resulted in clinically significant hypokalaemia, metabolic alkalosis, hypertension, and suppression of aldosterone production. (8)

There have been numerous case reports of severe hypertension, hypokalaemia and rhabdomyolysis caused by chronic ingestion of glycyrrhetinic acid. Risk factors for this phenomenon include pre-existing hypertension, the use of potassium-excreting

diuretics, female gender and older age, most of which were present in this case. (9) Recommended management involves cessation of the liquorice-containing substance, potassium replacement and use of spironolactone as a mineralocorticoid receptor antagonist. However, it should be noted that the clinical effects of glycyrrhetinic acid may persist for several weeks following cessation of the offending agent, and it may even take several months before normalisation of the renin-angiotensin-aldosterone system occurs. (8) The Scientific Committee on Food recommends daily glycyrrhetinic acid consumption of less than 100mg, which is similar to the critical dose predicted by a simulated pharmacokinetic pharmacodynamics study. (10)

In conclusion, glycyrrhetinic acid causes dose dependent inhibition of 11HSD2, which can result in pseudoaldosteronism and clinically significant hypertension, hypokalaemia and metabolic alkalosis. Suppression of aldosterone and renin can be used to easily distinguish this from other disorders. Clinicians should be aware of this important interaction and enquire about various forms of liquorice consumption in the right clinical context.

Lessons learned

1. Suppression of aldosterone and renin can be used to distinguish pseudoaldosteronism from primary aldosteronism in the setting of hypokalaemia, metabolic alkalosis and hypertension
2. Chronic natural liquorice ingestion can cause severe hypokalaemia, hypertension and rhabdomyolysis
3. Take a detailed history of diet and complementary/alternative medicines – natural liquorice can come in any forms

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abs#5

Another case of Oligomenorrhoea and Metabolic Syndrome

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Another Case of Oligomenorrhoea and Metabolic Syndrome.

Introduction

Oligomenorrhoea is a common endocrinological symptom. One of the predominant conditions causing this is polycystic ovarian syndrome (PCOS). Some other causes of oligomenorrhoea include hypothalamic hypogonadism, prolactinomas, genetic and chromosomal abnormalities such as Turners syndrome.

PCOS is common, affecting 5-10% of women and is one of the leading causes of infertility[1]. The diagnosis of PCOS requires the presence of two out of three features; clinical or biochemical evidence of hyperandrogenism, oligomenorrhoea and sonographic features of polycystic ovaries[2]. It is associated with the metabolic syndrome and patients often have co-existing obesity, hypertension and insulin resistance.

We present a case of oligomenorrhoea and metabolic syndrome referred to our general endocrine service.

Case Report

Miss J is a 29 year old female referred with a likely diagnosis of polycystic ovarian syndrome with oligomenorrhoea and some hirsutism. She had multiple features of metabolic syndrome with central obesity, hepatic steatosis, type 2 diabetes and hypertension. Miss J struggled with weight loss despite dietician input and regular exercise and was intolerant of metformin and statins.

Miss J had late onset puberty and longstanding oligomenorrhoea. Breast development began at the age of 12. She underwent menarche at the age of 16 and had an irregular menstrual cycle, no more than 3-4 times a year. Pubarche reportedly occurred at age 16. Oral contraceptive pills were trialled but were contraindicated due to migraines with aura or worsening hypertension. In recent years, intermittent cyclical progesterone has resulted in withdrawal bleeds with increasingly infrequent spontaneous bleeds.

Miss J had a BMI of 35kg/m^2 and her weight was stable. She denied having significant hirsutism or acne. Her blood pressure was 154/100 prior to starting amlodipine, a calcium channel blocker. Her liver function tests have been deranged since 2008, and are progressively worsening with a current GGT of 305U/L (<50U/L) and ALT 73U/L (<45U/L). An ultrasound done previously confirmed hepatic steatosis. Glucose intolerance was first diagnosed in 2012 with an HbA1c of 42mmol/mol (<40mmol/mol). Lipid profile has been abnormal since 2012 with mildly elevated cholesterol, triglyceride and LDL levels at 6.6mmol/L, 4.4mmol/L and 3.6mmol/L respectively.

At the age of 15, she required a laparotomy for ovarian cyst torsion. Her ovaries were left intact. In 2008, at the age of 20, she presented with left iliac fossa pain and was found to have complex cyst measuring 6x3.7x4.8cm in the Pouch of Douglas. The endometrium was noted to be thin and regular, the ovaries were well visualised and appeared normal in size and morphology. A subsequent scan 4 months later showed spontaneous resolution of the cyst, with an endometrium measuring 10mm. The left ovary was normal with a volume of 7cc; right ovary had normal appearances with a volume of 3cc. A repeat transabdominal USS in 2016 did not adequately visualise the pelvic organs including the endometrium. The ovaries were thought to have been identified and measured 2.2cc in volume on the left and 1.4cc on the right.

Gonadotropins were measured in 2012; FSH was 3.3iu/ml, LH 1.8iu/ml, oestradiol 171pmol/L, prolactin 59mIU/L testosterone 0.9nmol/L. Based on all the above features she was given a provisional diagnosis of polycystic ovarian syndrome in 2012 at age 24.

Examination in clinic was not consistent with polycystic ovarian syndrome and instead revealed features of androgen deficiency. Breasts were small and consistent with tanner stage 4. There was no evidence of virilisation and tanner stage 3 pubic hair. She had sparse axillary hair with no clinical hirsutism or acne. She had short stature with a height of 157.5cm and weight 86kg. Her midparental height is estimated to be about 164cm. She had some abnormal physical features with down slanting eyes, broad chest with widely spaced nipples, wide carrying angle and a congenital absent left 4th metacarpal. She had multiple skin tags on her neck and back, some abdominal striae that were not pigmented. No heart murmurs were detected. A previous x-ray had shown a mild Madelung deformity of the right wrist but this was not obvious clinically.

Investigations showed a FSH of 15IU/L (3-10IU/L *follicular phase*), LH 7 IU/L (2-8IU/L *follicular phase*), oestradiol 81pmol/L (>150pmol/L), progesterone 0.2nmol/L, DHEA 6.5umol/L (1.9-11.0umol/L), testosterone 0.4nmol/L (0.0-1.8nmol/L), 17OH progesterone <1.0nmol/L (<9.0nmol/L).

Initial cytogenetic investigations of 30 peripheral blood lymphocytes identified an abnormal 45X karyotype. Given her mild phenotype and normal appearing ovaries on imaging, more detailed cytogenetic analysis was requested with FISH analysis and increased cell count analysis. This identified a second cell line with two copies of X centromere, confirming the diagnosis of a mosaic Turner syndrome with approximately 5% mosaicism.

Discussion

Turner Syndrome is a common sporadic disorder that occurs in 1 in 2500 live born girls. It is caused by the loss of all or part of the second sex chromosome. 45X complete monosomy affects about half of Turner patients, 20-30% have mosaicism and the remainder have structural abnormalities of one of the X chromosomes[3].

The clinical phenotype is highly variable with short stature and ovarian dysgenesis being the most common features. It is associated with a wide range of clinical manifestations including cardiovascular and renal abnormalities, autoimmune conditions and metabolic syndrome. The diagnosis is often made during childhood due to short stature or delayed puberty. However patients can be diagnosed from the prenatal period till adulthood when they present with premature ovarian failure.

The Italian Study Group for Turner Syndrome described the incidence of spontaneous puberty, sexual development and menarche in 522 patients[4]. They found that 16.1% of all patients experienced complete spontaneous puberty whilst a further 10.9% had arrested puberty. When reviewed by karyotype, spontaneous complete puberty occurred in 40.6% of the mosaicism group X-monosomy/X-without structural abnormalities, 28.6% in those who had one structurally abnormal X chromosome

compared with 9.2% in the X-monosomy group. 84 patients in their cohort developed spontaneous menarche, with at least a third having a regular menstrual cycle pattern regardless of karyotype[4].

Turner syndrome is routinely diagnosed on karyotyping of 30 blood lymphocytes which is adequate to exclude mosaicism of 10%. This does not detect low level mosaicism or tissue mosaicism[5]. Molecular genetic methods, such as FISH, are able to detect the presence of a second cell line where standard karyotype may not. Y chromosome probes are also used to detect the presence of Y chromosome material which if present, increases the risk of gonadoblastomas[5,6].

For the patient in our case, the presence of normal appearing ovaries on previous imaging was suggestive of at least tissue mosaicism hence further analysis was requested. If Y chromosome material had been detected, gonadectomy would have been indicated.

Take Home Messages:

- phenotype for Turner syndrome is wide and needs to be considered in a patient with oligomenorrhoea as 30% may have some spontaneous puberty, 10% achieve menarche and 2-5% are fertile.

-pubertal development, sex hormones and ultrasound evaluation of uterine volume and ovarian morphology are all important considerations in the diagnostic work up.

- consider FISH analysis or increased cell count for karyotypes if high suspicion of mosaicism

- It is important to exclude the presence of Y chromosome material due to risk of gonadoblastomas.

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abs#6

A curious case of severe premenopausal hyperandrogenism

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Introduction

This is an unusual case of severe premenopausal hyperandrogensism with a diagnostic challenge involving differentiating ovarian and adrenal source of excess androgen.

Case presentation

A 41 year old female patient presented to endocrine outpatient clinic for review of longstanding severe hirsutism and oligomenorrhoea. She had a total testosterone level of 11nmol/L (reference range <2.0). She had not been reviewed by an endocrinologist prior to this presentation.

The patient went through menarche age 9 years. She was subsequently told at age 10 years that she had polycystic ovarian syndrome (PCOS), took the oral contraceptive pill (OCP) between the ages of 11 and 18 years and after ceasing this was amenorrhoeic for 3 years. During her 20's she commenced on fertility treatment with clomiphene then underwent laparoscopic ovarian diathermy followed by ovulation induction which was aborted due to the risk of ovarian hyper-stimulation syndrome. She did not ever achieve pregnancy. Her last period was 4 years prior to the time of review and she had a total of two menstrual periods in 10 years. Hirsutism began early. She began to wax her face at age 12 years and subsequently went on to have multiple sessions of laser hair removal. However, she continued to shave her chest and arms. There had been no worsening of hirsutism recently.

On examination, her weight was 87.3kg, BMI 30. She had hirsutism with moderate hair on her abdomen and back.

Initial investigations: TABLE 1

Investigation	Result	Reference range	Time	Date
Testosterone	15.1nmol/L	(<2.0)	09:00am	21.3.17
FAI	33.90%	(1-5%)	09:00am	21.3.17
SHBG	45nmol/L	(12-137)	09:00am	21.3.17
17-hydroxyprogesterone	14.7nmol/L	(Follicular0.3 - 3.3 nmol/L)(Luteal 3.0 – 14.3 nmol/L)	09:00am	21.3.17
		(Luteal 3.0 – 14.3 nmol/L)	09:00am	21.3.17
DHEAS	3.5umol/L	(2-11.1)	09:00am	21.3.17
Androstenedione	6.6nmol/L	(1.7-16.4)	09:00am	21.3.17
LH	19.7IU/L		09:00am	21.3.17
FHS	5.1IU/L		09:00am	21.3.17
Progesterone	1.6nmol/L		09:00am	21.3.17
Oestradiol	433pmol/L		09:00am	21.3.17
Short synacthen test 0 minutes			08:50am	31.3.17
17-OHP	11.8nmol/L			
Cortisol	233nmol/L			
Short Synacthen test			09:50am	31.3.17
60 minutes				
17-OHP	48.1nmol/L			
Cortisol	726nmol/L			
Testosterone	13.2nmol/L	(<2.0)	08:50am	31.3.17
ACTH	4nmol/L		08:50am	31.3.17
Prolactin	253mIU/L	(110-560)		6.4.17
TSH	1.1mIU/L	(0.4-3.5)		09.1.17
24hr Urinary free cortisol	123nmol/L	(<270)		22.3.17

Gynaecological ultrasound showed a complex cystic lesion in the left ovary measuring 19mm with no sinister features and both ovaries were enlarged. The findings were not classical of PCOS.

The rise in 17-OHP was suggestive of an adrenal source of hyperandrogenism with non-classical congenital adrenal hyperplasia (NCCAH) the main differential. The LH/FSH ratio was elevated, but PCOS was thought to be a less likely source of androgen in the clinical context.

Dexamethasone 0.25mg nocte was commenced and there was an initial fall in testosterone to 9.8nmol/L. 17-OHP remained at 11.9nmol/L although at that time progesterone increased to 17.4nmol/L and there can be cross reactivity with assays. The response was short-lived. The patient did not resume menses and her testosterone increased to 14.3nmol/L within one month. The dexamethasone dose was increased to 0.5mg nocte with suppression of morning ACTH to 0.8nmol/L. The testosterone level decreased again, this time from 15.2nmol/L to 2.6nmol/L. The 17-OHP remained variable but decreased to 4.4nmol/L. Ovarian hormone profiling showed oestrogen and progesterone rising and falling in a cyclical fashion. Despite this, no menstruation occurred (TABLE 2).

Then, without warning or change in dexamethasone dose, testosterone levels increased to 10nmol/L with 17-OHP increasing to 11.3nmol/L (TABLE 2). Repeat ultrasound scan of the ovaries showed no change in the cystic lesion or ovarian size. The patient was commenced on combined oral contraceptive pill (evonorgestrel/ethinylestradiol) and testosterone fell to 3nmol/L for several months (TABLE 2). It became clear that the adrenals were not the primary source of androgen excess and dexamethasone was weaned.

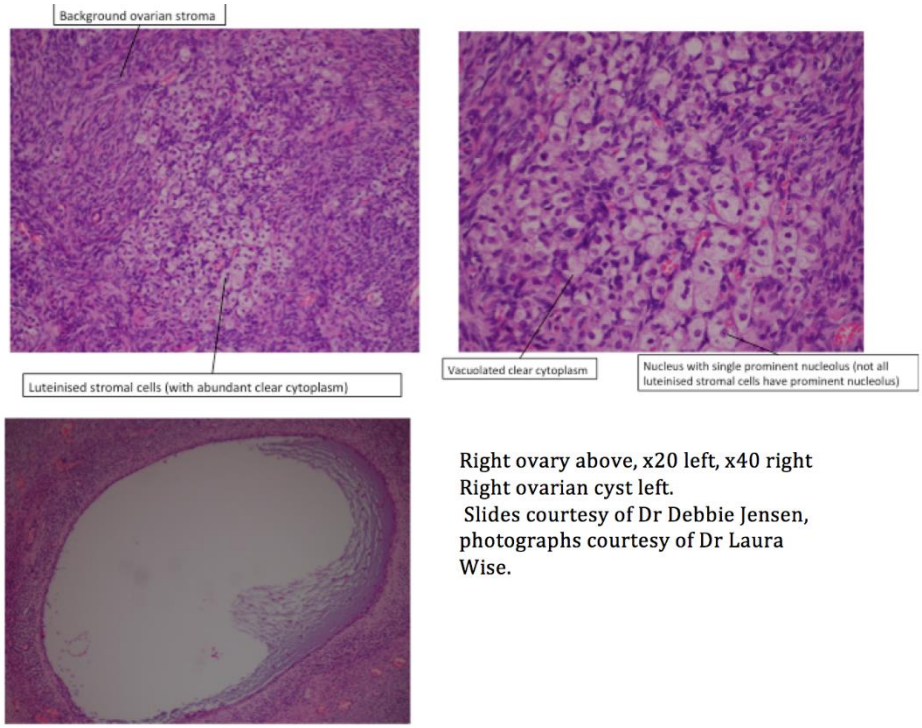
TABLE 2

Date	31/3/18	6/4/17	24/4/18	8/5/17	10/5/17	23/5/18	2/6/17	8/6/17	7/7/17	9/8/17	31/8/17	14/9/17	28/9/17	October	12/10/17	1/3/18	7/5/18
Medication		Dex 0.25mcg			Dex 0.5mg					OCP				Weaned from dex		Ovariectomy	
Testosterone	13.2		9.8	14.3		15.2	3.9	2.6	9.6		12.8	3.6	2.9		6.1		0.5
DHEAS	3.7			2.1		1.3	1.2	1.1	0.9		0.9	1.1	1.3		1.1		2.7
17-OH progesterone	11.8		11.9	10.2		11.2	10.2	4.4	11.3		9.5	4.6	3		7		1.9
Progesterone	0.9		17.4	1.1		5	30.4	1.7	13.4		1.1	5.2	<0.3		0.4		<0.3
Oestradiol	198		265	138		255	251	<100	196		188	176	<100		<100		277
LH	8.5		8	8.3		7.8	4.5	4.1	9.3		9.7	4	3.3		4.7		3.3
FSH	4.9		5.8	6.1		3.3	2.7	4.9	5.3		5.2	2.4	3		3.4		2.4
ACTH	4					1.1	0.8	1.1							1.2		

Due to the overwhelming suspicion of an ovarian source of androgen excess, the cystic complex on ovarian ultrasound scan and the long term risk of endometrial malignancy from unopposed oestrogen excess over many years, the patient proceeded to hysterectomy and bilateral salping-oopherectomy with oestrogen implant inserted at time of surgery.

Microscopic histopathology showed enlarged ovaries with no clear definition the of the medulla and cortex. The stroma contained small aggregates of luteinised stromal cells scattered singularly in the small nests in the background. There were a few cystic luteinised follicles present in both ovaries. No malignant changes. (FIGURE 1)

FIGURE 1



Right ovary above, x20 left, x40 right
Right ovarian cyst left.
Slides courtesy of Dr Debbie Jensen, photographs courtesy of Dr Laura Wise.

The patient was diagnosed with ovarian hyperthecosis. Following the operation she had normalisation of testosterone level to 0.5nmol/L, proving the majority of the excess androgens were of ovarian origin. Basal 17-OHP reduced to 1.9nmol/L post-surgery also suggesting an ovarian origin.

Discussion

Hyperandrogenism is a common presentation in endocrine practice. Diagnostic evaluation involves identifying the specific androgens involved, the organ of origin and pathogenesis underlying the excess production. History, examination, laboratory studies, dynamic testing (ACTH stimulation and dexamethasone suppression) and imaging are used to differentiate between adrenal and ovarian sources[1,2].

The challenge in this case is the presence of elevated 17-OHP and yet a clear ovarian source of hyperandrogenism. One of the key points is the evidence of an ACTH-stimulated rise of 17-OHP and evidence of some dexamethasone responsiveness prior to ovariectomy (which would normally indicate an adrenal source of androgen) however there was normalisation of testosterone and 17-OH progesterone following ovariectomy, suggesting an ovarian source.

This raises the question: is this evidence of a concurrent diagnosis of non-classical congenital adrenal hyperplasia or is this a rare manifestation of ovarian hyperthecosis/PCOS with ACTH-stimulated ovarian production of 17-OHP and elevated androgens that showed at least partial responsiveness to dexamethasone?

Ovarian hyperthecosis can be a severe variant of PCOS[3]. It is a histological diagnosis. In the premenopausal patient population it is a rare condition with few case reports in the literature and the prevalence is unknown[4]. It is characterised by luteinised thecal cells in the ovarian stroma, separate from the follicles, with accompanying stromal hyperplasia.

The main differential diagnosis for the case patient was non-classical congenital adrenal hyperplasia (NCCAH). The widely accepted standard for diagnosis is ACTH-stimulated 17-OHP levels >30nmol/L, however there have been studies suggesting that there is a false positive rate in the diagnosis of NCCAH when genetic testing was performed in women with hyperandrogenism and stimulated 17-OHP >30nnmol/L on ELISA [5,6]. The proposed mechanism is adrenal hyperresponsiveness to ACTH in the setting of hyperandrogenism due to PCOS or idiopathic hirsutism, although in the majority of these cases 17-OHP did not exceed 30nmol/L[6,7,8]. The potential of assay error must also be considered. The case patient had a high basal 17-OHP and a convincing elevation of 17-OHP to ACTH stimulation, yet the basal 17-OHP normalised after ovariectomy, suggesting a non-adrenal source. Ovarian hyper-responsiveness to GnRH agonist stimulation resulting in elevated 17-OHP has been demonstrated in a PCOS population suggesting dysregulation of the steroidogenic enzyme pathway at the level of the ovary[9].

We propose that the ACTH-stimulated elevation of 17-OH progesterone was ovarian in origin. On review of the literature, no case reports were identified that demonstrated an ovarian source of ACTH-responsive 17-OHP and hyperandrogenism in the setting of PCOS or ovarian hyperthecosis. Supporting this hypothesis is the normalisation of 17-OHP post-ovariectomy. The next step to confirm this would be to repeat ACTH-stimulation testing post-operatively. Ideally, immunohistochemical ACTH-receptor staining would be performed on the ovarian tissue to confirm the presence of an ACTH-mediated pathway.

Learning points:

1. The diagnostic challenge involved in determining adrenal versus ovarian source of excess androgen and 17-OHP in this rare presentation of severe hyperandrogenism
2. That consideration needs to be given to the possibility of dual pathology, disease mimics and false positives in unusual cases
3. We propose this is a novel case of an ovarian source of ACTH-stimulated 17-OH progesterone with some dexamethasone responsiveness

abs#7

Are you heading the wrong weigh?

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Case:

Mr TH, aged 19 years, was referred to Endocrinology clinic with 6 months of decreased libido, dry skin, lethargy, cold intolerance and low mood.

He reported intentional weight loss from 110kg to 66kg over 20 months with calorie counting and increased exercise. He received no medications and denied anabolic steroid use. He denied headaches, anosmia or visual disturbance. He reported normal puberty with cessation of longitudinal growth, and denied testicular or head trauma. He had never been sexually active. He has a diagnosis of Asperger's syndrome.

BMI was 21.5kg/m^2, blood pressure 96/59mmHg, and heart rate 42 beats/min. He appeared pale, with scant facial hair. He had no eunuchoid features, and voice pitch was normal. Testicular volume (25 ml), scrotal hair and penile length were normal. Visual fields were full.

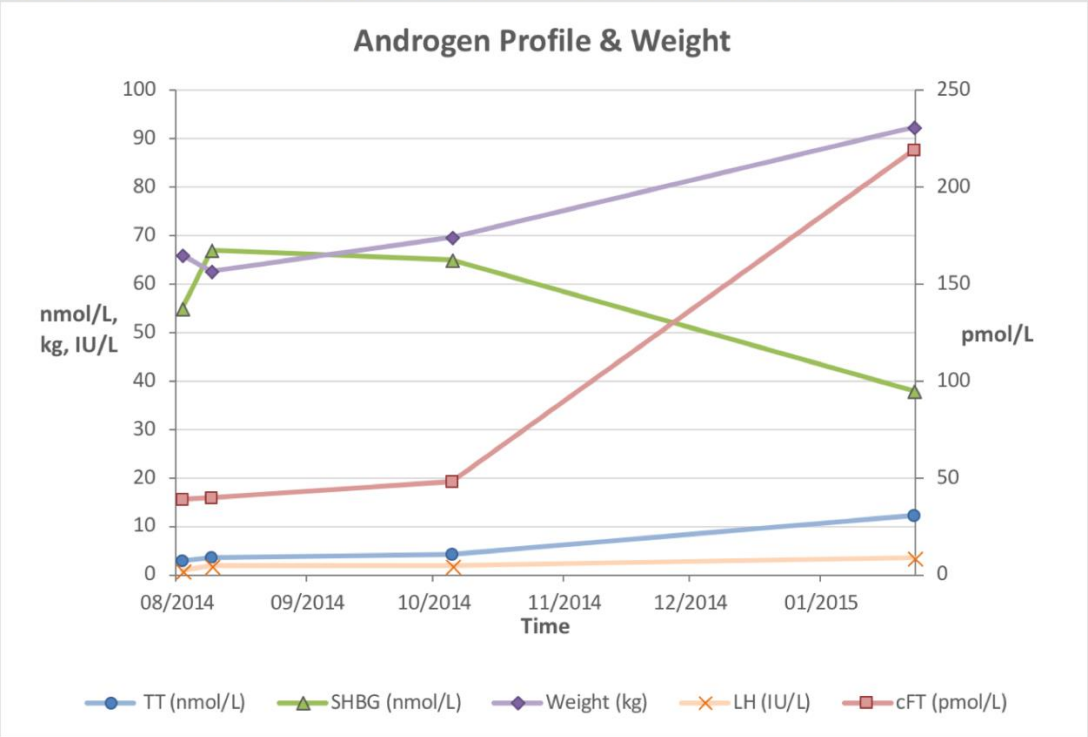
Biochemistry demonstrated hypogonadotropic hypogonadism (HH) on repeated testing, elevated morning cortisol and reduced insulin-like growth factor 1 (IGF-1) and triiodothyronine (Table 1). He had mild normocytic anaemia and leucopaenia. MRI pituitary was normal. Given the relatively short history of hypogonadism, bone densitometry was not performed.

He had no evidence of anorexia nervosa or body dysmorphic disorder, and readily agreed to gain weight. Over 5 months he gained 30kg and reached 92.5kg (BMI 29.5kg/m^2) with near normalisation of his reproductive hormones with increased testosterone (12.1nmol/L), LH (3.5IU/L), free testosterone (219pmol/L) and decreased SHBG (38nmol/L). (Figure 1). Vital signs and hypogonadal symptoms normalised.

Table 1. Initial Laboratory Studies

Test	07/08/14 0848	14/08/14 0850	10/10/14 1204	Units	Range
FSH	2	2	2	*IU/L*	1 – 8
LH	**1**	2	2	*IU/L*	2 – 8
Total Testosterone	**3.0**	**3.5**	**4.1**	*nmol/L*	8 – 30
SHBG	55	**67**	65	*nmol/L*	17 – 66
Calculated Free Testosterone	**39**	**40**	**48**	*pmol/L*	170 – 500
Cortisol	**758**	**771**	350	*nmol/L*	110 – 550
Growth Hormone	**12.5**			*ug/L*	<5
IGF – 1	**11.2**			*nmol/L*	16.6 – 55.6
TSH	5.28		2.02	*mIU/L*	0.35 – 5.5
Free T4	13.2		13.7	*pmol/L*	9 – 25
Free T3	**2.8**		**3.5**	*pmol/L*	4.7 – 7.2
Hb	**127**			*g/L*	130 – 180
WCC	**3.6**			*X10^9/L*	4 – 11
Platelets	180			*X10^9/L*	150 – 450
Albumin	50	49		*g/L*	35 – 50
25OH Vitamin D		62		*nmol/L*	>50
Iron	18.7			*umol/L*	10 – 30
Transferrin	**1.65**			*g/L*	2.1 – 3.8
Saturation	45			*%*	16 – 60
Ferritin	258			*ug/L*	20 – 300
Vitamin B12		681		*pmol/L*	>180
RBC Folate		1837		*nmol/L*	>450

Figure 1. Changes in body weight and biochemical gonadal axis parameters over time.



Discussion:

While the male gonadal axis is more resistant to stressors than the female axis, male starvation is an underrecognised cause of acquired HH. Disordered eating, gonadal dysfunction, and osteoporosis is not just a female athletic triad. With pressure on bodily appearance, male starvation and anabolic steroid use (not uncommonly for cosmesis) are increasing in prevalence[1]. Starvation-associated HH requires differentiation from organic HH and anabolic steroid use.

1) Male anorexia/overtraining as an underrecognised condition

Excessive weight loss and exercise can depress the gonadal axis via inhibition of pulsatile gonadotropin-releasing hormone (GnRH) secretion, a defence mechanism to prevent reproduction during states of energy deficit[2]. Women are more susceptible because of the greater energy demands of pregnancy. The phenomenon in men is underappreciated, in part due to the absence of specific hypogonadal features akin amenorrhoea in women, analogous to the delayed recognition of hyperprolactinaemia.

Although eating disorders have a female preponderance, 30% diagnosed with DSM-5 "Avoidant & Restrictive Food Intake Disorder" are male[3]. Still, the literature on male starvation is limited to case reports[4,5,6] (Table 2). The largest contemporary series (four male anorexics) demonstrated varying degrees of HH, hypercortisolaemia, non-thyroidal illness syndrome, bradycardia, hypothermia and erectile dysfunction[4]. Concomitant excessive exercise cause stress-induced fragility fractures[6]. Differences in the chronicity and extent of calorie restriction, baseline weight, exercise intensity and genetic susceptibility (*see below*) may influence the degree of hormonal suppression.

Table 2. HH cases reported in the contemporary literature *(blank denotes values not reported)*

Case Reports	**Skolnick et al[4]**					**Hunt et al[5]**		**Rigotti et al[6]**	
Characteristic	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Range**	**Case 5**	**Range**	**Case 6**	**Range**
Age (years)	20	24	23	20		27		22	
Nadir BMI (kg/m^2)	12.9	12	13	18	*20 – 25*	13.3	*20 – 25*	13	*20 – 25*
Weight loss (kg)	20	64	15.8	59		68.1		11	
Weight loss velocity (kg/month)	-6.8	-5.3	-7.9	-9.8		-5.2		-0.2	
TT (ng/dL)	88		198	614	*249 – 836*	58.04	*241 – 827*	18	*300 – 1100*
LH (IU/L)			1.4	6.3	*2.4 – 12.6*	0.29	*1.5 – 9.3*	1.3	*3 – 18*
FSH (IU/L)			3.1	1.9	*4.7 – 21.5*	0.85	*1.4 – 18.1*	6.7	*5 – 25*
Cortisol (µg/dL)	25.1	217	18	18.6	*2.3 – 19.4*	35.43	*4.2 – 22.4*	16.2	*<5*
ACTH (pg/mL)	24		21	8	*0 – 46*	26	*6 – 43*		
IGF-1 (ng/mL)	<25		147		*83 – 344*				
GH (ng/mL)			1.33	0.6	*<5*			24	*<5 µg/L*
TSH (µIU/mL)	2.6	1.28	0.54	2	*0.27 – 4.2*			2.8	*0.5 – 3.5 mU/L*
FT4 (ng/dL)	0.8	0.7	0.62	1.72	*0.9 – 1.8*	0.81	*0.89 – 1.76*	7.7	*4 – 12 µg/dL*
FT3 (pg/mL)	1.8	1.1	0.2		*1.8 – 4.6*	1.56	*2.3 – 4.2*		
PRL (ng/mL)	11.5		17.7		*4 – 15.2*			6.3	*<10 µg/L*

Endurance training lowers testosterone levels and impacts male fertility and bone mineralisation. During intensive military training courses, healthy male soldiers demonstrated significant decreases in total and free testosterone levels (>70-80% decrease from baseline) along with increases in SHBG (>50% from baseline), which normalise after recuperation[7].

Clues to male starvation/overtraining-associated HH include consistent pituitary tests such as a sick euthyroid picture, stress-induced increase in serum cortisol, and low IGF-1 due to acquired growth hormone (GH) resistance.

2) Differential diagnosis (organic HH, anabolic use)

Functional HH is a diagnosis of exclusion. Congenital HH is generally excluded by evidence of pubertal development and acquired organic HH by a targeted assessment. Previous or current anabolic steroid use with an synthetic androgen not detected by testosterone assays is an important differential, particularly in young body-conscious males. Apart from history, SHBG, HDL and haemoglobin values can be useful in differentiating starvation- and anabolic-associated HH. Energy deficit is associated with increased SHBG, exercise-associated increases in high-density cholesterol (HDL) and mild anaemia (due to nutritional and androgen deficiencies). In contrast, anabolic steroids supress SHBG and HDL, while haemoglobin is typically high, due to erythropoietic androgen actions.

3) Pathophysiology and susceptibility factors, including possible genetic determinants

GnRH pulsatility is essential for gonadal axis function and determined by genetic, hormonal and environmental factors. In rats, acute starvation-induced suppression of the pituitary-testicular axis can be reversed by pulsative administration of GnRH[8]. KNDy (kisspeptin/neurokinin B/dynorphin) neurons in the mediobasal hypothalamus are major upstream regulators of GnRH neuron activity, integrating metabolic cues from the periphery (e.g. leptin *see below*) to align energy stores with reproductive potential. Kisspeptin infusions increase LH pulsatility in women with hypothalamic amenorrhoea and in obese men with low testosterone, suggesting that both energy deficit and excess lead to central gonadal axis dysregulation by similar mechanisms[8]. In chronically starved, markedly underweight (< 40kg) men, the LH response to GnRH can be blunted, suggesting an additional pituitary-testicular defect, which is reversible with weight gain[6].

Circulating leptin, a marker of fat reserves, is low in nutritionally-deplete women with HH and replacement restores gonadal axis function through stimulation of GnRH secretion via kisspeptin[8]. In healthy men, administration of a replacement dose of recombinant leptin prevented the starvation induced suppression of the gonadal axis, suggesting leptin plays similar roles in men[9]. Thus, the starvation-associated leptin decline signals energy deficits to the hypothalamus, with subsequent gonadal axis suppression intended to limit procreation and promote survival.

Interestingly, a case-control study of women with functional hypothalamic amenorrhoea reported, compared to eumenorrheic controls, an increased frequency of mutations in genes known to cause congenital GnRH deficiency[10]. This suggests that such mutations may contribute to the variable susceptibility to hypothalamic amenorrhea, linking rare genetic variants to a common multifactorial disease.

Key Learning Points:

- Starvation/overtraining are important causes of functional HH in men.
- Functional HH due to energy deficit is a diagnosis of exclusion, and important clues can be used to distinguish it from functional hypogonadism due to (covert) anabolic steroid use, an important differential in body-conscious young men.
- Pathophysiology involves genetic, hormonal and environmental factors including reduced leptin signalling in KNDy neurons.
- HH is usually reversible with energy restoration, which may require a multidisciplinary approach (including psychiatry and dietician involvement).

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abs#8

Slow K, Special K, low K, oK?

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Hypokalemia, often as an incidental finding, but sometimes associated symptoms including paralysis, not infrequently confronts the practising endocrinologist. Although many cases will reflect a secondary response to a range of systemic insults a number of causes are relatively specific to the endocrine system. Intrinsic disorders of renal tubular function including the inherited eponymous syndromes of Bartter and Gitelman, may provide a diagnostic challenge particular when the later first presents in adulthood. Insights from renal physiology and genetics provides both therapeutic insights and, in some cases, diagnostic certainty.

In hypokalemia associated with hypertension, the endocrinologist will be drawn to the possibility of aldosterone-induced sodium retention with potassium loss in the distal nephron but other possibilities, including specific monogenetic aetiologies in which aldosterone levels are not increased, need at least to be considered. That being said, it can be argued that up to 10% of hypertension is caused by aldosterone excess, often absent of frank hypokalemia. Recent developments in the genetics, diagnosis and treatment of primary aldosteronism will be discussed including the case for screening.

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abs#9

Is ping pong bad for your bones?

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Case

A 40-year-old female was referred for management of osteoporosis following a minimal trauma fifth metatarsal fracture whilst playing ping pong. She had a normal menstrual cycle, no gastrointestinal symptoms or symptoms of hyperthyroidism or hypercalcaemia, no history of eating disorders, excessive exercise or bone offensive medications. Bone mineral density (BMD) showed lumbar spine Z-score -3.0 and femoral neck Z-score -2.4. She had gained 4kg of weight over the preceding year with subtle increased central weight distribution, mild proximal weakness and mood irritability. Past medical history included endometriosis and two previous successful pregnancies, the first achieved with in-vitro fertilisation. Medications included cholecalciferol and calcium supplementation. Clinical examination revealed height 1.70m, weight 57.8kg, BMI 20.0, blood pressure 110/70mmHg, mild facial plethora, minimal proximal muscle weakness and but no thin skin, bruising or abdominal striae.

Clinically she did not appear cushingoid but a secondary osteoporosis screen revealed hypercortisolism (Table 1), as evidenced by failure of cortisol suppression following 1mg dexamethasone and elevated 24-hour urine cortisol and midnight salivary cortisol on three occasions. Adrenocorticotrophic hormone (ACTH) was persistently elevated. A dexamethasone-corticotrophin hormone (CRH) test was consistent with Cushing's syndrome (Table 2). There was partial cortisol suppression (>50%) following administration of 8mg dexamethasone however there was no significant ACTH or cortisol responses to CRH stimulation (Table 3). Pituitary MRI revealed a 14x14x12mm enhancing lesion appearing distinct from the pituitary gland encapsulating the right ICA and invading the right cavernous sinus (Figure 1). CT brain did not show calcification or bone invasion suggestive of meningioma (Figure 2). Baseline visual testing was normal. Given an elevated ACTH and lack of response to CRH stimulation, ACTH interference from heterophile antibodies was excluded with interference studies. The differential diagnosis included ACTH-secreting pituitary macroadenoma, and due to the lack of response to CRH, a meningioma with concomitant ectopic ACTH-production. Pseudocushings had been excluded. Due to diagnostic uncertainty, bilateral inferior petrosal sinus sampling (BIPSS) with CRH stimulation was performed and revealed extraordinarily high ACTH levels (pre-CRH) with right sided localisation (Table 4), consistent with an ACTH-secreting pituitary macroadenoma.

The peri- and post-BIPSS course was complicated by headache, nausea and cranial nerve deficits including a partial third nerve palsy, partial right sixth nerve palsy, sensation loss in the ophthalmic division of the fifth cranial nerve and post-ganglionic Horner's syndrome, consistent with cavernous sinus syndrome. Post-BIPSS CT (Figure 3) was not consistent with pituitary apoplexy. Post-BIPSS MRI (Figure 4) revealed an increased size of the macroadenoma, measuring 22x20x18mm with new susceptibility weighted image (SWI) blooming suggestive of intratumoural haemorrhage or cavernous sinus thrombosis. The patient was treated conservatively with dexamethasone and prophylactic doses of anticoagulation.

Follow-up progress MRI 10 days' post-BIPSS showed a decreased size of the macroadenoma (8x11x11mm), with reduced SWI signaling, favouring a resolving cavernous sinus thrombosis (Figure 5). Post-procedure laboratory investigations (Table 5) demonstrated remission of hypercortisolism. We hypothesis that BIPSS cannulation in a patient with Cushing's disease (CD) was complicated by cavernous sinus thrombosis and pituitary macroadenoma infarction, resulting in extraordinarily high ACTH levels on BIPSS, followed by remission of hypercortisolism. The patient has commenced cabergoline, has had complete resolution of neurological deficits and continues monitoring for active CD.

Discussion

Osteoporosis in a 40-year-old female with BMI 20 warrants secondary work up including assessment of hypercortisolism despite lack of clinical features. ACTH-dependent CS accounts for approximately 80% of endogenous CS, and includes ACTH secreting pituitary adenomas (CD), ectopic ACTH production, and CRH-producing tumours. The majority of ACTH-dependent CS are microadenomas (<10 mm), with macroadenomas (>10mm) occurring in 13-15% of cases (1, 2). Differentiating pseudocushings and then pituitary from ectopic sources of excess ACTH can be challenging due to overlap in presentations (1).

A pituitary lesion more than 6mm on MRI in the presence of biochemical evidence of CD has 96% specificity for the diagnosis of CD (3). However, MRI imaging is limited by the sensitivity of identifying sub-centimeter ACTH-secreting microadenomas and by the high prevalence (10-20%) of non-functioning pituitary incidentalomas and cannot be completely relied upon (4).

In CD, ACTH and cortisol usually respond to CRH stimulation, whereas ectopic ACTH secreting tumours do not. A response to CRH administration of >20% increase in cortisol and >50% increase in ACTH from baseline has a sensitivity of 91% and specificity of 95% and sensitivity of 86% and specificity of 95% respectively for diagnosis of CD. Patients with macroadenomas may have a lower ACTH response to CRH which may account for false negative results in our patient (1).

The diagnostic accuracy of the high-dose DST is lower than the CRH test and its usefulness has been challenged (1,3,4). Following 8mg dexamethasone, morning cortisol levels are suppressed >50% in 86% of patients with CD and 31% of patients with ectopic ACTH secretion. A stricter criteria of >80% suppression results in correctly identifying all patients with ectopic secretion but reduces sensitivity for CD from 85 to 50% (5). The accuracy is not improved by combining the high dose DST with the CRH test (1).

BIPSS is the gold standard to confirm ACTH excess of pituitary origin. A central-to-peripheral ACTH maximal ratio ≥2 in basal conditions and ≥3 following CRH simulation is strongly suggestive of pituitary ACTH hypersecretion (3). Sensitivity and specificity are enhanced by CRH stimulation and are approximately 96% and 100% respectively (3, 4). Potential complications of BIPSS include brainstem infarction, haemorrhage, deep venous thrombosis, cranial nerve palsies and cavernous sinus thrombosis. Serious neurological complications are rare (3, 4).

Interference with the ACTH immunoassay by heterophile antibodies can produce false positive results and complicate the diagnosis of apparent ACTH-dependent CS. The prevalence of heterophile antibodies varies widely (1-80%) and may occur in up to 3% of specimens tested. Heterophile blocking tubes (Scantibodies™), measurement of ACTH using an alternative method, and analysis of serial sample dilutions may reveal heterophile antibody interference (6).

Pituitary apoplexy is rare with an estimated prevalence between 0.6%-9.1% (7). The risk of apoplexy increases with increasing adenoma size (8). ACTH-secreting adenomas are usually microadenomas and pituitary apoplexy in these patients is rare (2,9). Corticotroph macroadenomas are uncommon and apoplexy in these tumours has been reported but is not well described (10). Pituitary apoplexy can occur spontaneously or in the setting of precipitating factors including hypertension, anticoagulation, dopamine agonist therapy or anterior pituitary testing (8,9). TRH and LH-RH are the most common hormonal stimulants associated with apoplexy (8). There is one report of apoplexy following CRH administration in a patient with CD in the literature (9), but to our knowledge apoplexy has not been reported following cavernous sinus thrombosis secondary to BIPSS cannulation. Pituitary apoplexy generally occurs within hours of stimulant exposure (9).

Management of apoplexy includes high-dose glucocorticoids and neurosurgical decompression. Conservative management may be appropriate if neurological signs and symptoms remain stable (7,9). The rate of remission of hypercortisolism following apoplexy in CD is high, however relapses can occur more than seven years later and long-term follow-up is required to detect hypercortisolism or hypopituitarism (2).

Key learning points

1. It is important to consider all secondary causes of osteoporosis despite the absence of clinical features
2. Investigation and differentiation of CS and diagnosing CD can be challenging
3. Cavernous sinus thrombosis and pituitary infarction as a rare complication of BIPSS
4. Remission of CD in the setting of pituitary apoplexy

Table 1. Relevant baseline laboratory investigations

Investigation	Value	Reference Range
Corrected Calcium (mmol/L)	2.42	2.25-2.65
Ionised calcium (mmol/L)	1.21	1.18-1.30
Phosphate (mmol/L)	0.7	0.8-1.5
25-hydroxy vitamin D (nmol/L)	85	>49
Parathyroid hormone (pmol/L)	84	14-72
TSH (pmol/L)	2.7	0.40-4.00
FT4 (pmol/L)	16	10-23
LH (U/L)	4	
FSH (U/L)	9	
Oestrogen (pmol/L)	270	
Progesterone (nmol/L)	5	
GH (mU/L)	1	
IGF-1 (nmol/L)	29	9.8-35
Prolactin (ug/L)	9	<20
Random cortisol (nmol/L)	490, 590, 570	220-660
24 hour urine free cortisol (nmol/day)	760, 1000, 1010	80-590
24 hour urine extracted free cortisol (nmol/day)	140, 300, 270	
Midnight salivary cortisol (nmol/L)	8, 11, 16	<8
ACTH (ng/L)	65, 71	5-50
8mg dexamethasone suppression test (nmol/L)	400 (baseline) to 182	<80% of baseline
1mg dexamethasone suppression test (nmol/L)	490 to 260	<50nmol/L
Hba1c (%)	5.1	4.3-6.0

Table 2. Dexamethasone- CRH test

Time	ACTH (ng/L)	Cortisol (nmol/L)	24 hour urine cortisol and cortisone (nmol/24h)		Post CRH
			Cortisol (ref: 10-120)	Cortisone (ref: 40-340)	
Day 1 0800	85	392			
Day 1 1600	56	278			
Day 2 0800	61	389			
Day 2 1600	61	336			
Day 2			448	730	
Day 3 0030	64	373			
Day 3 0800	77	426			
Day 3 1600	52	229			
Day 3			652	980	
Day 4 0800	59	224			
Day 4 1600	52	162			
Day 4			193	434	
Day 5 0745	85	300			-15
Day 5 0758	60	310			-10
Day 5 0758	42	305			-5
Day 5 0805	73	273			-1
Day 5 0810	79	257			+5
Day 5 0825	80	286			+15
Day 5 0840	53	287			+30
Day 5 0855	75	265			+45
Day 5 0910	70	238			+60
Day 5			100	446	

0.5mg dexamethasone was administered every 6 hours from 1200hours on day 3 until 0600hrs on day 5
1mcg/kg CRH was administered on Day 5

Table 3. CRH test

Time (mins)	ACTH (ng/L)	Cortisol (nmol/L)	Plasma free cortisol (nmol/L)	Cortisol LC/MS/MS (nmol/L)
-60	89	456	22	403
-15	82	444	25	372
+15	87	471	23	378
+30	83	459	23	388
+45	56	406	23	371
+60	77	420	19	360
+90	70	446	20	355
+120	78	353	16	318

CRH stimulation test reference ACTH >50% rise in ACTH

Table 4. BIPSS

Time (min)*	Right ACTH (ng/L)	Left ACTH (ng/L)	Periphery ACTH (ng/L)	PPR# (Right)	PPR (Left)
-2	254630	9100	4300	59.2	2.11
0	169954	13710	3100	54.8	4.4
2	186413	10105	3400	53.9	3.0
5	48477	18573	3200	15.1	5.8
10	43981	5100	3100	14.2	1.6
15	35062	4000	2800	12.5	1.42

*Time since CRH injection (1uq/kg)
PPR: petrosal sinus to peripheral gradient

Table 5. Post BIPSS laboratory results		
Investigation	**Result**	**Reference range**
24 hour urine cortisol (nmol/day)	73	10-120
Salivary cortisol (nmol/L)	1.8 nmol/L	0-12
Cortisol (nmol/L)	382	
ACTH (ng/L)	56	10-50
IGF-1 (nmol/L)	36	9.8-35
FSH (U/L)	5.2	
LH (U/L)	3.5	
Oestradiol (pmol/L)	79	
Prolactin (mU/L)	66	
PTH pmol/L	5.4	1.0-7.0

Figure1. Baseline MRI pituitary

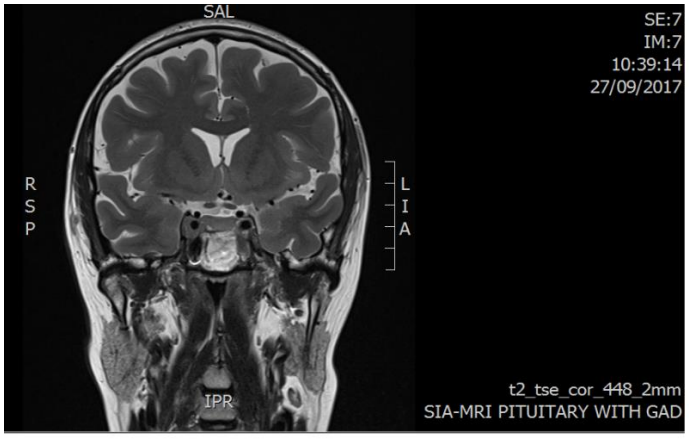


Figure2. Baseline CT pituitary

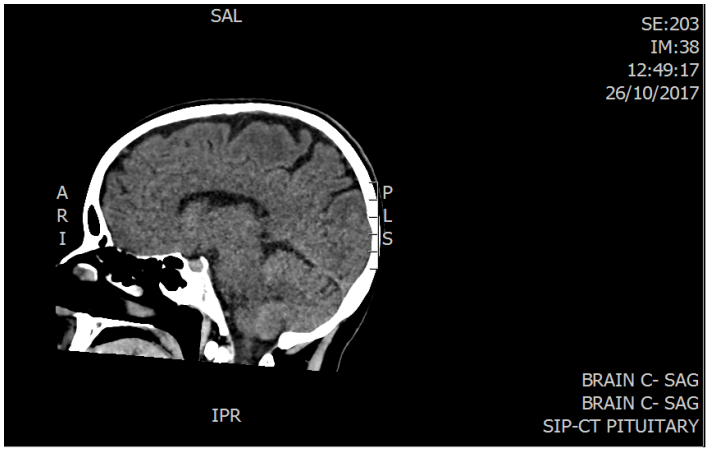


Figure3. Post-IPSS CT

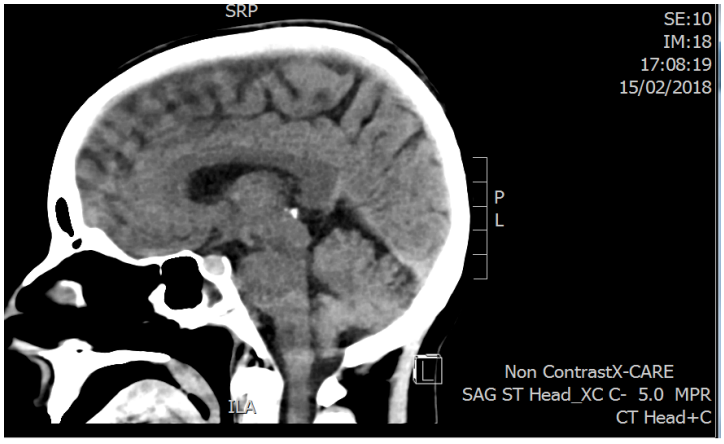


Figure4. Post-IPSS MRI pituitary

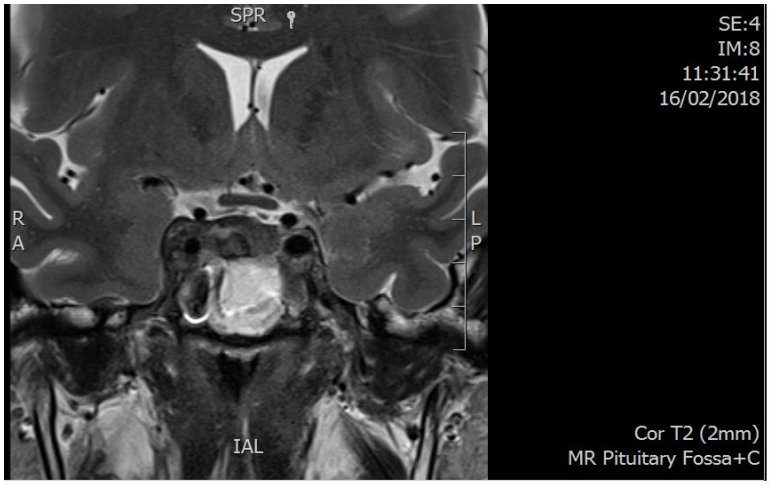
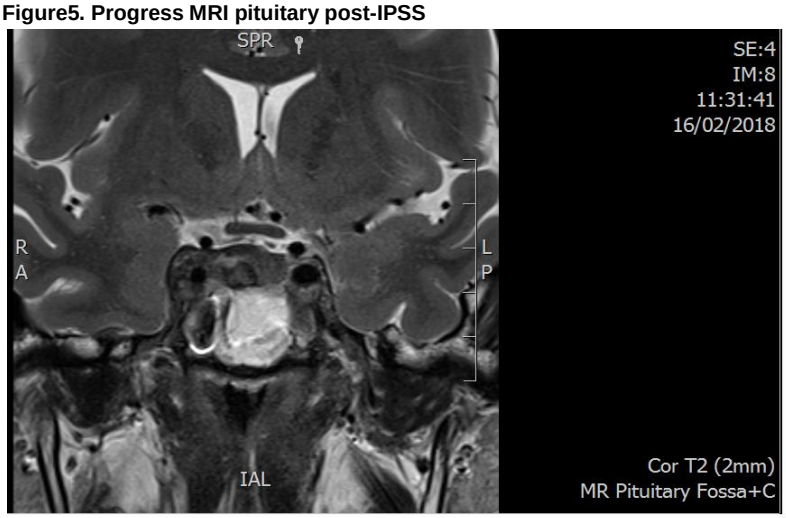


Figure5. Progress MRI pituitary post-IPSS



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abs#10

Thyroid conundrum, a test for a test.

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Introduction:

Measurements of thyrotropin and of free thyroxine and triiodothyronine are widely used diagnostic methods for thyroid function evaluation. However, some serum samples will demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Several case reports have described the presence of such interferences resulting in reported abnormal concentrations of thyroid hormones inconsistent with the patient's thyroid state[1]. Misdiagnosis, subsequent inappropriate treatment, and adverse consequences for the patient can result from interferences yielding false results[2-4] .

Automation of immunoassays has allowed the rapid measurement of serum hormone levels and other analytes, aiding in the accurate diagnosis of disease. However, endogenous antibody interference in immunoassays can yield false results. Heterophile antibodies (HAb) are human poly-specific antibodies targeted against animal antigens, the most common being human anti-mouse antibodies (HAMA). Heterophilic antibody interference against reagent antibodies has been well documented, and other interfering antibodies have also been reported[5-7].

Although interferences have been well documented. The streptavidin-biotin interaction provides an efficient and convenient method to manipulate assay components and is currently used in several immunoassay platforms. To date, there have been limited reports in the literature of interference from endogenous or nonspecific anti-streptavidin antibodies; however, such antibodies would potentially affect multiple diagnostic platforms.

We report results for a patient who presented with spurious thyroid function test and was diagnosed with thyrotoxicosis due to autonomous toxic nodule. Subsequent assessment showed abnormal thyroid function results in discordance with his clinical picture. His workup revealed interference from a non-specific streptavidin immunoglobulin.

Case study:

A 68 year old man presented to the emergency department with rapid atrial fibrillation in the absence of an infective or ischaemic precipitant. On examination, he was normotensive, but tachycardic with an irregular heart rate of 100 beats/minute. Oxygen saturation was normal and he was afebrile. Cardio-respiratory examination was normal and his ECG indicated atrial fibrillation with no acute ischaemic changes. Examination of the thyroid demonstrated asymmetrical enlargement of the left hemithyroid, without clinical evidence of retrosternal extension or ophthalmopathy.

His past medical history included type 2 diabetes, a haemochromomatosis carrier state (with H63D heterozygous mutation), osteoarthritis and gastroesophageal reflux disease. His initial thyroid function tests demonstrated severe thyrotoxicosis with a suppressed TSH of <0.02 mIU/L (NR 0.27-4.2mIU/L), FT4 >100 pmol/L (NR 12-22pmol/L) and FT3 of T3 23.8 pmol/L (NR 3.0-7.8pmol/L) with negative TRAb < 1.0 U/L (NR < 1).

A technecium-99 labelled thyroid scintigraphy scan demonstrated increase uptake in the lower pole of the left thyroid lobe with almost complete suppression of the remainder of the thyroid, consistent with a large left sided autonomous nodule. A thyroid ultrasound showed multiple thyroid nodules of increased vascularity, the dominant nodule measuring 4.1 cm maximally. The patient was subsequently discharged on carbimazole at a dose of 15 mg TDS and referred for outpatient follow up. Progress TFT results showed in Table 1.

Table 1. Progress thyroid function results using Roche Elecsys assay.

Roche Date	TSH (0.27-4.2mIU/L)	T4 (12-22pmol/L)	T3 (3.0-7.8pmol/L)	Carbimazole Dosage
11/12/15	<0.02	>100	22.2	15mg TDS
08/01/16	1.09	40	25.1	15mg BD
04/03/16	4.79	36.2	22.2	15 mg BD

The discrepancies between free thyroxine and TSH suggested the possibility that one or more of the laboratory results did not accurately represent his biologic status as he was clinically hypothyroid. Hence, sample was repeated using a Nonspecific Antibody Blocking Tube (NABT) (Table 2), however, results remained unchanged. Rheumatoid factor was negative at 13 mIU/L (RR <15 mIU/L) and there was no history of biotin use.

Carbimazole was ceased for one week and a repeat TFT done using a different platform (abbott Architect) showed similar results with a TSH of 29.8 mIU/L, FT4 of 36.2 pmol/L and FT3 of 22.2 pmol/L. In addition, TSH dilution did not show linear changes.

Hence, the sample sent for assessment using non-streptavidin base platform and results shown in Table 3. To identify the cause of interference, another sample was sent to Roche Germany to be preincubated with streptavidin microparticles. Further testing concluded that the interference was due to non-specific streptavidin immunoglobulin.

Table 2. Antibody Binding tubes (Scantibody)

Roche Elecsys	**TSH (0.27-4.2mIU/L)**	**T4 (12-22pmol/L)**	**T3 (3.0-7.8pmol/L)**
Neat Sample	0.61	>100	26.2
Post-Scantibody incubation	0.62	>100	25.4

Table 3.

Use ruthenium-streptavidin **Don't Use ruthenium-streptavidin**

Repeat TFT	**Roche**	**Abbott**	**Advia Centaur**
TSH (mIU/L)	0.57	3.23	3.02
T4 (pmol/L)	> 100	12.1	13.08
T3 (nmol/L)	26.2	4.6	5.04

Discussion:

There are many causes of interference in immunoassays causing erratic patient results.[8,9] These erroneous results potentially lead to unnecessary, expensive and possibly harmful investigations and treatment. A method-specific interference due to anti ruthenium antibodies in the Roche free thyroxine (fT4) and free triiodothyronine (fT3) assays has been described previously[10,11]. However, few case reports reported the impact of anti- streptavidin antibodies interference[12,13] and cause falsely elevated TSH. Streptavidin is produced by Streptomyces avidinni; it is unknown in what circumstances it could lead to an immunization. Unlike biotin, this interference is endogenous, therefore non-transient[13]. In the cases reported[12,13]. the interfering substance will have a more pronounced effect in competitive assay, and a less-remarkable effect on a sandwich assay: for example, FT3 and FT4 concentrations will be overestimated.

In our case report, the diagnosis with underlying primary thyroid disorder was a masking factor at the beginning of treatment; however the subsequent thyroid function results indicated a lack of the usual balance between the hormone and its regulating factor. The patient's specimen demonstrated interference across manufacturer platforms, affecting all streptavidin-mediated diagnostic assays tested. Platforms and assays that do not employ streptavidin did not demonstrate nonlinear dilution or elevated competitive and suppressed sandwich results.

One notable element of this case is that the interference caused reciprocal changes in TSH and T4. It is important for the clinical laboratory to recognize that a single interference can yield opposite effects in different assays, depending on whether a sandwich or competitive format is used. A traditional algorithm for investigating immunoassay interference from species heterophiles or human anti-mouse antibodies would have failed to resolve these results because the interference was not fully removed by Heterophile blocking. This case also demonstrates the value of having diverse immunoassay platforms available to aid in the investigation of problematic or suspicious results, as interference against common laboratory assays.

Although it appears a streptavidin antibody is a rare occurrence, this interference may easily go undetected and a seroprevalence study may be indicated to evaluate the magnitude of this problem.

Conclusion:

Despite progress in immunoassay technologies, the problem of unwanted interference has yet to be overcome. Critical analysis of the hormone results, together with an open and permanent communication between laboratory and clinical staff, remain the best strategy to avoid clinical mismanagement due to unsuspected interference. Given the striking effect of streptavidin antibody on thyroid function laboratory tests and the possibility of interference for an extended time, a cautioning of the patient is necessary and to provide the information on the potential effect of this antibody for future medical examinations, and avoid the risk of future inappropriate treatment or investigations.

The incidence of thyroid disease, and the frequency with which patients have their thyroid status assessed, has driven much attention and information on the streptavidin- biotin separation interference. However, this interference is not restricted to thyroid testing. If FT4, TSH and FT3 assays seem particularly prone to this interference, PTH and phosphocalcic examination is another field where these pitfalls are worrying[14,15,16].

abs#11

A grave injustice

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Case report

We present the case of a 71 year old woman with an almost 30 year history of Graves' disease (GD). Whilst her initial presentation and treatment followed the typical course, more recently she has developed more unusual complications of thyroid dermopathy and acropachy, which are severe and debilitating. The rarity of these complications and lack of proven treatments has led to difficult management decisions.

Our 71 year old woman was diagnosed with GD in 1990 at the age of 53 years. Although not formally diagnosed with ophthalmopathy at the time, she reports proptosis at the time of diagnosis. She was treated with radioactive iodine, rendering her hypothyroid, and she has been on stable doses of thyroxine since that time. She is an ex-smoker of 10 pack years' duration, ceasing in 1990. Subsequent to her initial management, two significant and unusual complications have developed.

In 2002, ~10 years after her radioactive iodine treatment, significant Graves' ophthalmopathy (GO) developed (Figures 1a-b and 2), which was managed with a combination of intraorbital steroids and radiotherapy, along with high doses of systemic prednisolone. Cyclosporin was added as a steroid-sparing agent in 2004, with complications of her high dose, long-term glucocorticoid therapy including insomnia, proximal myopathy, hypertension and osteoporosis. Attempts to wean the cyclosporin and prednisolone resulted in a relapse. An application for rituximab was thus made to the Drug and Therapeutics committee in 2013, which was unsuccessful, based on limited evidence of efficacy. Her eye disease subsequently remained stable after initiation of timolol for secondary glaucoma from her thyroid eye disease and topical treatment for her anterior eye symptoms (Table 1).

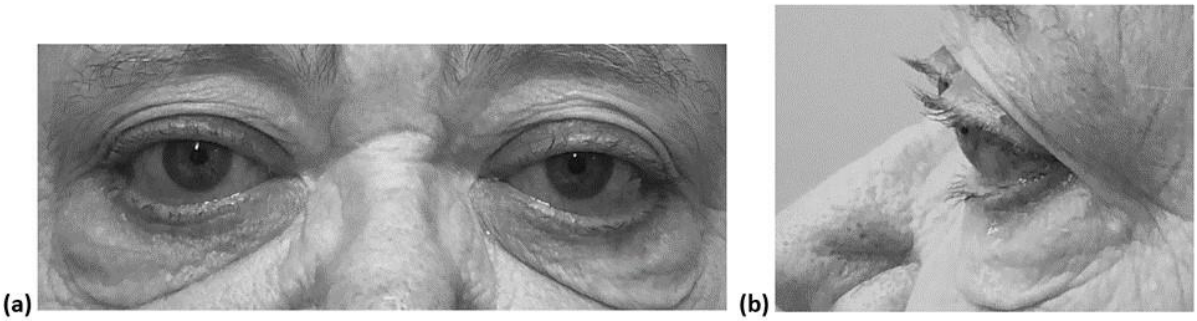

Figure 1 (a) Graves' ophthalmopathy with bilateral proptosis and lower lid retraction. **(b)** Proptosis on lateral view.

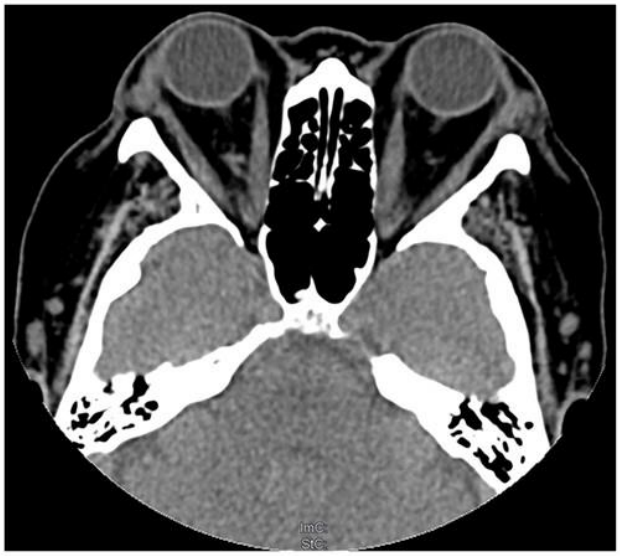

Figure 2 Computed tomography of orbits demonstrating bilateral symmetrical proptosis of 25mm, thickened extraocular muscles but no significant apical crowding or optic nerve compression, July 2013.

Date	V/A (best corrected)		IOP		C:D and VF		OCT (RNFL – microns)		CAS	Treatment
	RE	LE	RE	LE	RE	LE	RE	LE		
May 2013	6/6	6/6	16mmHg 21mmHg (upgaze)	16mmHg 24mmHg (upgaze)	0.6 Normal VF	0.7	71	73	2/7	Prednisolone and Cyclosporin Timolol and Polyvisc eye drops
July 2013	6/5	6/6	17mmHg 17mmHg (upgaze)	15mmHg 19mmHg (upgaze)	Normal VF		Details unavailable		0/10	
Nov 2016	6/6	6/6	15mmHg 20mmHg (upgaze)	15mmHg 20mmHg (upgaze)	0.6-0.7	0.5	71	75	0/10	Timolol and Polyvisc eye drops
March 2018	6/7.5	6/7.5	15mmHg	16mmHg	Normal VF		75	78	4/10	Prednisolone and Cyclosporin Timolol, Genteal and Polyvisc eye drops

Table 1 Ocular examination results over time on and off immunosuppressive agents
VA, visual acuity; IOP, intraocular pressures; C: D, cup to disc ratio; VF, visual fields; OCT, optical coherence tomography; RNFL, retinal nerve fibre layer; CAS, clinical activity score; RE, right eye; LE, left eye

Further complicating her GD, she developed severe, late-onset thyroid dermopathy. This appeared to be triggered by necrosis of the left first hallux following an ingrown toenail in 2014, which progressed to post-inflammatory lymphoedema. Conservative management with compression stockings and antibiotics failed, resulting in amputation of her left first toe in 2016. Progression of her skin disease, confirmed myxoedema on skin biopsy in 2017, worsened following surgical intervention, eventually involving both legs with evidence of acropachy and elephantiasic changes (Figure 3a-b and 4a-b). This became increasingly disabling with reduced mobility and restrictions on her daily activities. Poor mobility and significant falls risk has necessitated home modifications.

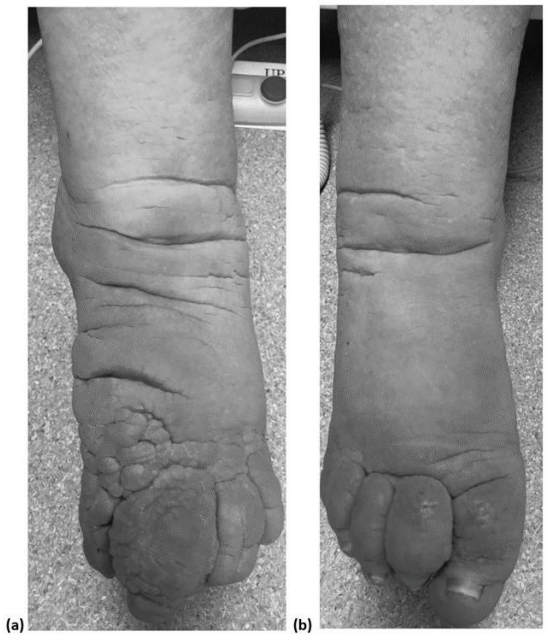

Figure 3 (a) Severe dermopathy with elephantiasic changes and acropachy of the left lower limb following amputation of the left first toe
(b) Progressive skin changes in the right lower limb

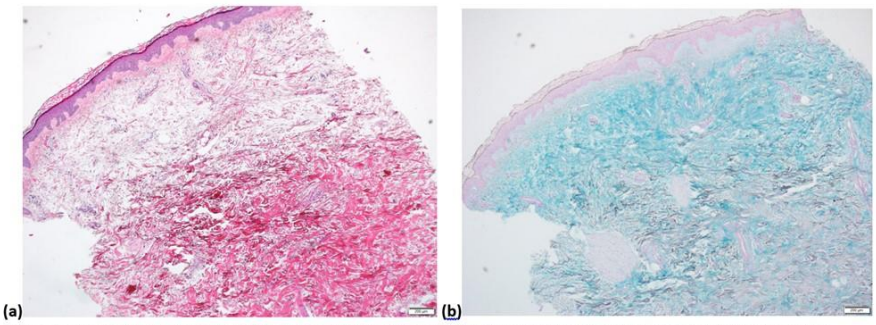

Figure 4 (a) Skin punch biopsy of left lower leg **(b)** Alcian Blue stains demonstrating marked increase in interstitial mucin within superficial and mid-dermis

Between 2013 – 2015 and 2015 - 2016, she was able to wean and cease her prednisolone and cyclosporin respectively. However, this again resulted in both a flare of her eye symptoms and further progression of her dermopathy, and both immunosuppressive agents were restarted in early 2018.

Throughout her disease course, high thyroid stimulating hormone receptor antibody (TRAb) levels have consistently been reported at > 40 IU/L. Dilution of a TRAb sample in 2018 yielded a result of 144 IU/L. Questions have thus been raised in regards to possible utility of plasmapheresis, rituximab and thyroidectomy in the management of her complications, which are associated with high TRAb levels. Widespread consultation has been sought and the current plan is for plasmapheresis prior to a total thyroidectomy in early June, with ongoing consideration of immunosuppression post-thyroidectomy, and potential radiotherapy

for the dermopathy. Additionally, serum collected for research will be sent to assay calsequestrin and collagen XIII antibodies, markers associated with ophthalmopathy.

Discussion

This case exhibited the complete triad of extra-thyroidal manifestations of GD in the typical order, initially displaying ophthalmopathy, followed by dermopathy and then acropachy, which is noted in literature to occur in less than 1% of patients.[1] These manifestations are usually associated with higher TRAb levels.[2, 3] GO is clinically relevant in up to 50% of patients with GD, of which 3-5% of those affected may have sight-threatening consequences.[3]

Administration of glucocorticoids is usually the first line treatment for GO but is associated with significant adverse effects, with reports of mortality and morbidity in those treated with intravenous glucocorticoids for GO at 0.6% and 6.5% respectively.[4] Furthermore, glucocorticoid effects are directed at reducing inflammation rather than the underlying pathogenicity.[4] Hence, other therapies including Rituximab have been reviewed as a potential agent for treating GO. Rituximab is a monoclonal antibody directed against the CD20 antigen on B-lymphocytes. In depleting B-cells, it not only directly targets their antigen presenting function but also prevents their progression to plasma cells and hence, production of thyroid autoantibodies. A recent meta-analysis evaluating the efficacy of Rituximab in GO revealed a statistically significant improvement in the clinical activity score but no significant change in other efficacy outcomes.[4] Further research is required before it can become a mainstream treatment option for refractory GO.[4]

The B-cell lymphocytic population that infiltrate thyroid follicles are postulated to be a major source of thyroid autoantibodies, with studies demonstrating higher levels of thyroid stimulating antibodies in thyroid vein blood over peripheral blood samples in patients with GO.[5] As there are common antigenic structures between thyroid follicles and retro-orbital adipose tissue fibroblasts, thyroidectomy could remove the source of the antibodies and potentially prevent progression of ophthalmopathy.[5] This theory was supported by a study by Nart et al whereby total thyroidectomy in patients with recurrent GD with orbitopathy resulted in significant improvement in the severity of eye disease with concurrent reduction in TRAb levels.[5]

While TRAb is well documented in its association with GO, there have been studies into other antibodies directed against collagen XIII and calsequestrin, antigens in orbital connective tissue and eye muscles respectively.[6] It is proposed that eyelid retraction and ocular myopathy results from antibodies against calsequestrin while congestive ophthalmopathy is an outcome of autoimmunity against collagen XIII in the cell membrane of orbital fibroblasts.[6]

Thyroid dermopathy occurs in 0.5 – 4.3% of GD patients, of whom 25% have acropachy.[3] Our case exemplifies the most severe form with elephantiasic changes, occurring in only 5% of those with dermopathy.[2] Current treatments include local and systemic corticosteroids along with compressive therapy, especially for severe cases.[7] Although milder cases will often resolve with or without intervention, more severe cases are usually refractory to treatment.[7] Unfortunately there is limited evidence for treatment options in severe cases. However, a recent case study demonstrated a significant clinical benefit from plasmapheresis and rituximab in a patient with severe refractory dermopathy, with an associated decline in TRAb levels.[1]

Thyroid dermopathy rarely develops without GO.[2,7] The presence of TSH-receptors in skin fibroblasts play a key pathogenic role in dermopathy, as in GO.[2] Furthermore, both manifestations are characterised by the accumulation of glycosaminoglycans in their respective tissues.[2] Hence, it is unsurprising that systemic therapy used for GO results in improvement of skin lesions.[2]

Our aim in this case is to reduce the TRAb load with plasmapheresis prior to removal of a major source of autoantibodies via total thyroidectomy. If further immunosuppression is required, rituximab has potential to provide benefit. Symptomatic improvement in GO and severe dermopathy would result in improved quality of life.

Discussion points

- Late-onset and severe complications can occur with a common clinical condition and can be debilitating
- An understanding of the pathogenesis of complications of Graves' disease could improve management
- Readily available treatment options for refractory Graves' ophthalmopathy and severe dermopathy are limited and require future research

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abs#12

The investigation and management of spontaneous hypoglycalemia is not always straightforward

Natasha Brown, Stephen Du Toit, John V Conaglen, Marianne S Elston

Mr DP, a 55 year old New Zealand European male, presented following a motor vehicle accident after an unremarkable night shift. He reported feeling uncoordinated when leaving work and had no recollection of getting into or driving his vehicle. Ambulance staff reported his capillary glucose was 2.3mmol/L. Despite 150ml of 10% intravenous dextrose and 32 g of oral glucose gel, on arrival at ED his venous blood glucose was still only 2.3mmol/L. DP denied any recent medication changes or alcohol consumption and had never received insulin or oral hypoglycaemic medications. There had been infrequent contact with his daughter-in-law, a type one diabetic, over the preceding weeks and no contact with other persons with access to insulin or insulin secretagogues. He had gained approximately five kilograms in weight over the previous year. Apart from one possible episode two weeks prior to presentation, he had been well with no other history of neuroglycopaenic or adrenergic episodes. Family history was notable only for bowel cancer, dementia and motor neurone disease with no significant endocrine or autoimmune disease in any relatives. Past medical history included significant cardiovascular and peripheral vascular disease, undefined teenage focal epilepsy (for which he had remained asymptomatic for many years without medication), essential hypertension, dyslipidaemia and previous lumbar spine surgery. Admission medications were: aspirin, clopidogrel, amodipine, quinapril, bisoprolol, atorvastatin, and ezetimibe. All medications had been used for more than one year and he took no supplements. With the exception of aspirin, all medications were stopped following admission.

Examination was largely unremarkable with the exception of mild left cervical lymphadenopathy, palmar erythema, an ejection systolic murmur and longstanding weak left radial pulse. Apart from soft tissue bruising he had sustained no injuries. There were no features to suggest a connective tissue disorder. With the exception of hypoglycaemia baseline observations were normal. As an inpatient when his venous glucose was 1.3mmol/L, the serum insulin (on both Roche and Beckman assays) and C-peptide levels were undetectable (Table 1). Renal and hepatic functions were normal and there was no evidence of pituitary or adrenal hormone insufficiency. Complete blood count demonstrated lymphopaenia suggesting possible haematological or autoimmune disease. There was no paraprotein and inflammatory markers were normal. Non-islet cell tumour was considered as an early possible diagnosis. A computerised tomography (CT) scan demonstrated low grade generalised lymphadenopathy but was otherwise unremarkable, as was an FDG-PET-CT. Repeatedly normal IGF-1 levels combined with a normal IGF-BP3 and hGH levels also made an IGF-2 secreting tumour unlikely. IGF-2 levels are pending.

Given the low grade lymphadenopathy, biopsy (FNA and excision biopsy) of two separate lymph nodes (axillary and cervical) was performed but demonstrated reactive changes only. Bone marrow aspiration and trephine were normal with no evidence of lymphoproliferative disease.

A strongly positive ANA titre (1:2560) and Ro52 were suggestive of an autoimmune cause. However ds-DNA was negative and with the exception of lymphadenopathy, lymphopenia and transient thrombocytopenia, there were no other features of systemic lupus erythematosus or other autoimmune disease. Given the absence of hyperinsulinemia anti-insulin antibodies were excluded. Suppressed beta-hydroxybutyrate levels were supportive of insulin-like activity and thus the presence of insulin receptor antibodies with competitive stimulatory effect was considered the most likely diagnosis. Sulfhydryl group-induced autoimmune hypoglycaemia was considered. At the time of admission the only sulfhydryl group-containing medication was clopidogrel (clopidogrel metabolite contains a sulfhydryl group). HLA typing was not consistent with a known genetic susceptibility to drug-induced autoimmune hypoglycaemia.

Recurrent hypoglycaemic episodes occurred (venous glucose down to 1.3mmol/L) despite a regular meal plan by the dietetic team and two-three hourly capillary glucose monitoring. DP also underwent a consented 48 hour period of observation to exclude surreptitious or malicious insulin administration. Recurrent hypoglycaemia continued throughout.

Owing to recurrent, severe hypoglycaemia, DP could not be fasted for greater than two hours and a OGTT could not be undertaken to exclude coexisting insulin resistance. Intravenous and oral glucose therapy was initially successful for emergency correction of hypoglycaemia. Glucagon was not administered. Despite regular oral intake, due to recurrent hypoglycaemia, nocturnal nasogastric feeding was required. Given the suspicion for an autoimmune aetiology, oral prednisone therapy was commenced whilst awaiting results of insulin receptor antibodies. Prednisone (60mg mane) resulted in a reduction in hypoglycaemic episodes and over three weeks, nasogastric feeding was successfully weaned and discontinued. Furthermore daytime caloric requirement dramatically reduced and DP was discharged on a regular (three times a day without snacks) meal regimen. A limited (16 hours) fast did not result in hypoglycaemia. Samples analysed by Centre de Recherche Saint Antoine in Paris, confirmed the presence of an immunoglobulin in the serum of DP capable of competitively binding the insulin receptor in vitro (Figure 2). Other potential treatments such as steroid-sparing medications, rituximab and plasma exchange were not utilised given the improvement with prednisone and patient preference. Prednisone therapy is currently being weaned in the outpatient setting.

Glucose (mmol/L)	2.3	1.3	2.7	2.6	2.2	*5.2*	2.2
Insulin (pmol/L)	<10	<10	<10	<10	<10	*29*	<10
C-peptide (pmol/L)	56	<30	118	<30	<30	*262*	85
Pro-insulin (pmol/L)							<13.5
Hydroxbutyrate (beta) mmol/L		0.37	<0.01	<0.01	<0.01	*<0.01*	<0.01
Comment	Following emergency correction					*20 minutes after meal*	

Table 1: Matched glucose, insulin and c-peptide, hydroxybutyrate (beta) and proinsulin levels.

Results

	Bound insulin (% of control without serum)
Date	**07/04/2018**
Without serum	**100**
control serum 1/5	**87**
patient serum 1/3	**23**
patient serum 1/5	**26**
patient serum 1/10	**35**
patient serum 1/20	**46**

Control without serum **77,1** pg bound insulin / mg proteins

Results

	Bound insulin (% of control without serum)
Date	**07/04/2018**
Without serum	**100**
control immunoglobulins 200ug	**103**
patient immunoglobulins 50ug	**84**
patient immunoglobulins 100ug	**74**
patient immunoglobulins 200ug	**41**
patient immunoglobulins 400ug	**39**

Control without serum : **45,7** pg bound insulin / mg proteins

Figure 1: Presence of Anti-IRAbs evaluated by their ability to inhibit the binding of radiolabelled insulin to its receptor in cells overexpressing the insulin receptor (CHO-IRA4) in the presence or absence of patient serum(Table 1) and post purification of immunoglobulins (table 2).

Discussion

Hypoglycaemia in a non-diabetic is uncommon and requires a systematic approach. It is imperative to first exclude critical illness and endocrine disease. Factitious hypoglycaemia should always be carefully excluded and the limitations of the insulin assay utilised needs to be considered (1). This case describes a patient with apparent spontaneous autoimmune hypoglycaemia secondary to stimulatory anti-insulin receptor autoantibodies, a rare presentation in this patient demographic, particularly given the absence of concomitant autoimmune disease (2). Insulin autoimmune syndrome (IAS) was initially described in 1972 by Hirata, in a Japanese man with hypoglycaemia and is most commonly reported in Japanese but rare cases in Caucasians have been reported (3). IAS is usually due to endogenous antibodies which bind insulin, however mixed cases with insulin receptor antibodies have also been reported and may mimic an insulinoma, although insulin levels are typically disproportionately high (3). A similar mechanism of hypoglycaemia may occur due to an insulin-binding paraprotein (4). IAS may be triggered in genetically susceptible individuals (5) by medications containing a sulfhydryl group with recent reports of clopidogrel-triggered IAS (6). Isolated agonist insulin receptor antibodies are extremely rare and may be associated with hypoglycaemia as in this case (7,8). Most are associated with other autoimmune disease (8). Treatment involves removal of any drug trigger which may result in spontaneous remission and use of immunosuppressive agents or plasmapheresis (9) although most reports are of single cases or small series and so there are no clear guidelines as to optimal treatment and duration.

To date the patient has responded to oral prednisone however other immunosuppressive therapies may be needed in the future.

Take home messages

- Hypoglycaemia in a non-diabetic is relatively uncommon
- Factitious hypoglycaemia needs to be excluded (newer insulins may not be detected by current platform assays)
- Autoimmune hypoglycaemia may occur due to antibody binding to insulin and/or the insulin receptor
- Autoimmune hypoglycaemia is more common in those with a genetic predisposition e.g. HLA-DR4 and those with other autoimmune disease
- Autoimmune hypoglycaemia may be triggered by exposure to sulfhydryl group-containing medications including clopidogrel and carbimazole

Special thanks to Pr.Corrine Vigouroux and the team at INSERM Centre de Recherche Saint Antoine for processing our samples in order to assess for the presence of Anti-IRABs

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abs#13

Too much of a good thing

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Introduction

This case describes a 60-year-old female with hyperinsulinaemic hypoglycaemia diagnosed as nesidioblastosis on selective arterial calcium stimulation test four years following sustained weight loss achieved with sleeve gastrectomy. While nesidioblastosis has been frequently reported following gastric bypass procedures it has not been reported following sleeve gastrectomy. This rare case increases our understanding of post-bariatric surgery noninsulinoma pancreatogenous hypoglycaemic syndrome and the impact of change in incretin secretion following sleeve gastrectomy. Further study of incretin secretion following sleeve gastrectomy is warranted.

Case Report

A 60-year-old lady was referred from her bariatric surgeon with symptomatic hypoglycaemia in the context of sleeve gastrectomy 4 years prior. Hypoglycaemic symptoms were first noted 18 months following sleeve gastrectomy with initial symptoms of , facial paraesthesia and palpitations, particularly at night. There was resolution of these symptoms following food intake and the episodes occurred infrequently every few months. However, by the time of referral the episodes were occurring daily with no relationship to food intake or activity and could occur day or night. She had one presentation to an emergency department following a syncopal episode which was attributed to vasovagal syncope. Fingerstick glucose measurement revealed several readings below 2.0mmol/L in the presence of symptoms. Initial biochemistry and oral glucose tolerance test (Table 1) arranged by the bariatric surgeon revealed hypoglycaemia to 1.8mmol/L, 2hours following an oral glucose load. Fasting proinsulin was noted to be elevated to 39.0pmol/L with concurrent fasting glucose level of 4.1mmol/L. Fasting insulin and c-peptide were normal. Post glucose load measurements of insulin, C-peptide and proinsulin were not requested.

Her background was significant for implanted pacemaker 5 years prior to her sleeve gastrectomy due to recurrent syncopal episodes and findings of a conduction block. Her weight prior to sleeve gastrectomy was 106kg. There was significant weight loss post operatively of 46kg in the first 12 months. She maintained a weight of 60- 65kg thereafter. She did not take any regular medications. There was no family history of endocrine tumours or diabetes.

A formal 72 hour fast was performed (Table 2). The fast was terminated at 32hours following symptoms consistent with hypoglycaemia and a fingerstick glucose measurement of 2.6mmol/L which was confirmed with a formal venous measurement of 2.5mmol/L. In the setting of hypoglycaemia, the pro-insulin level was inappropriately elevated suggestive of endogenous hyperinsulinism. Insulin, C-peptide levels and beta-hydroxybutyrate were within the normal limits. Sulphonylurea screening and insulin antibody testing were negative.

High resolution CT abdomen did not reveal a pancreatic lesion. There was no lymphadenopathy or any mass lesions and no liver lesions. Magnetic resonance imaging was unable to be performed due to an incompatible pacemaker. Gallium-68 Dototate PET did not reveal any octreotate-avid lesions.

During this time, there was increasing frequency and severity of neuroglycopaenic symptoms with reduced hypoglycaemic awareness. A trial of prednisolone did not provide any symptomatic benefit and was ceased after receipt of results showing undetectable anti-insulin antibody and evidence of increased proinsulin. Diazoxide was commenced and titrated to dose of 200mg three times a day with fewer documented hypoglycaemic episodes but with ongoing fingerstick glucose readings to <2 mmol/L but with no unconscious hypoglycaemia. She gained weight but only 4 kg over a 6-month period.

Selective arterial calcium stimulation testing (Figure1) revealed an increase in insulin secretion across multiple supply regions of the pancreas. The magnitude of insulin secretion was 4 to 6 times normal with a lack of regionalisation suggesting a diffuse process. A provisional diagnosis of nesidioblastosis was made and the woman has elected to proceed with total pancreatectomy which will be presented.

Discussion

As the prevalence of obesity and its related complication continues to increase worldwide, bariatric surgery is an important treatment option with beneficial effect on glycaemic control that is not explained by weight loss alone. The prevalence of bariatric surgery in Australia is rising, with over 22700 procedures performed in Australia in 2014-15 (1) with sleeve gastrectomy accounting for 52.5% of the total procedures performed.

Nesidioblastosis post sleeve gastrectomy has not previously been reported. Nesidioblastosis is a rare cause of hyperinsulinaemic hypoglycaemia and has been described in patients following Roux-en-Y gastric bypass procedure (2). Histologically, it is characterised by beta cell hypertrophy and neo-formation of islet cells from ductal epithelium. There is functional dysregulation of beta cells which causes hypoglycaemia, and this typically occurs in the post-prandial state as there is a retained physiological insulin response to meals.

There are several proposed mechanisms for hypoglycaemia following Roux-en-Y gastric bypass. A theory of accelerated carbohydrate delivery is supported by the observation that digestive bypass of glucose through a jejunal tube causes a significantly higher insulin and glucagon-like peptide 1 (GLP-1) peak compared to oral administration of the same quantity of glucose (3).

A second proposed mechanism is that changes in GLP-1 secretion lead to hyperinsulinism. Blockade of GLP-1 in patients with post-bariatric hypoglycaemia with exendin-9, a GLP-1 antagonist, has been shown to correct postprandial hypoglycaemia (4,5). Furthermore, mice models have demonstrated the role of GLP-1 in the activation and proliferation of beta cells by increased beta cell mass and insulin synthesis following administration of exendin-4, a GLP-1 agonist (6). However, previous studies investigating whether GLP-1 stimulates beta cell proliferation in human islet cells engrafted into mice models and in vitro have been inconclusive (7,8).

Immunohistochemical analysis of pancreatic islets in nesidioblastosis compared to controls show significant overexpression of insulin-like growth factor 2, insulin-like growth factor one receptor-α epidermal growth factor receptor and transforming growth factor β receptor 3, which may also contribute to development of nesidioblastosis (9).

Clearly, the incretin effect appears pivotal in post Roux-en-Y gastric bypass hypoglycaemia. Interestingly, exaggerated post-prandial insulin and GLP1 response comparable to those seen in post Roux-en-Y bypass patients have been demonstrated following sleeve gastrectomy (10). This is supportive that changes in the incretin milieu post bariatric surgery is not limited to gastric bypass procedures. Despite this, it is unclear why pathological hypoglycaemia has not been reported in gastric sleeve population – until now.

In this case, the presence of hyperinsulinaemic hypoglycaemia in the context of a previous bariatric procedure is highly suggestive of nesidioblastosis with the selective arterial calcium stimulation test suggestive of a diffuse process and normal imaging. Although no link between sleeve gastrectomy and nesidioblastosis has been described, there remains a plausible relationship between sleeve gastrectomy and nesidioblastosis secondary to the effect of GLP-1, both as an incretin for insulin secretion and as a growth factor for beta cell proliferation. The significant and sustained weight loss post – surgery in this patient suggests that sustained weight loss may reflect an exaggerated incretin response to sleeve gastrectomy as compared with others who slowly regain weight post sleeve gastrectomy.

Key Points

- The mechanism via which hypoglycaemia occurs following bariatric surgery appears to be related to increase in the secretion of incretin GLP-1 with subsequent changes in insulin secretion.
- Multiple cases of symptomatic hypoglycaemia from post Roux-en-Y gastric bypass nesidioblastosis has been reported, however, nesidioblastosis has not been previously reported following sleeve gastrectomy.
- Further studies are required to investigate the pathophysiology of nesidioblastosis following sleeve gastrectomy and long-term effects of bariatric surgery.
- The relationship between change in incretin secretion following sleeve gastrectomy and sustainability of weight loss requires further investigation.

Table 1

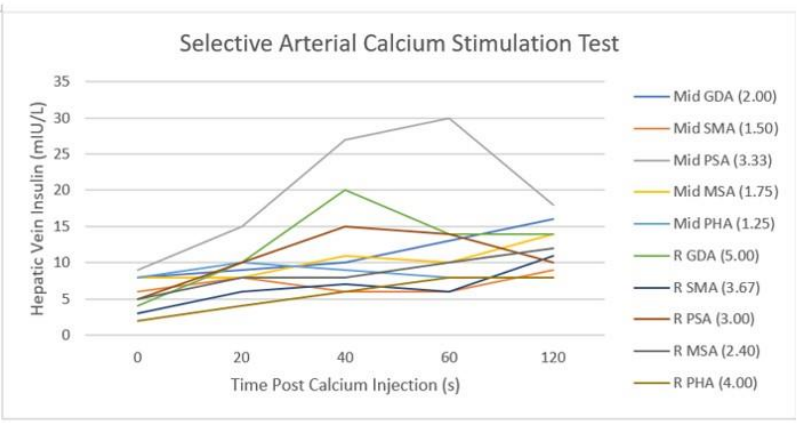
Oral Glucose Tolerance Test	Fasting	1 hour	2 hours	Ref Range
Glucose (mmol/L)	4.1	7.0	1.8	3.4 – 5.4
C peptide (ug/L)	1.0			0.8-3.4
Insulin (mU/L)	3.0			<10
Proinsulin (pmol/L)	39.0			3.6 -22
ACTH (pmol/L)	3.8			<10
Cortisol (nmol/L)	302			200-650

Table 2

Criteria	Diagnostic threshold *	Patient result
Formal glucose (mmol/L)	< 3.0	2.5
In the setting of glucose < 3.0		
Insulin (mIU/L)	> 3	2
C-peptide (nmol/L)	> 0.2	0.12
Pro-insulin (pmol/L)	> 5	10.8
Beta hydroxybutyrate (umol/L)	< 2700	2187
Glucose after glucagon (mmol/L)	Increase by > 1.4	+1.1

* Cryer PE et al: Evaluation and Management of Adult Hypoglycaemic Disorders: An Endocrine Society Clinical Practice Guideline, J Clin Endocrinol Metab 2009, 4: 709-28

Figure 1



Mid – middle hepatic vein, R – right hepatic vein
GDA – gastroduodenal artery, SMA – superior mesenteric artery, PSA – proximal splenic artery,
MSA – mid splenic artery, PHA – proper hepatic artery
Relative fold increase in hepatic vein insulin (rHVI) in brackets.

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abs#14

Sweet genes

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Case presentation

A 23-year-old man, DG, was referred to the endocrinology service for inpatient management of poorly controlled diabetes mellitus whilst admitted for incision and drainage of a perianal abscess.

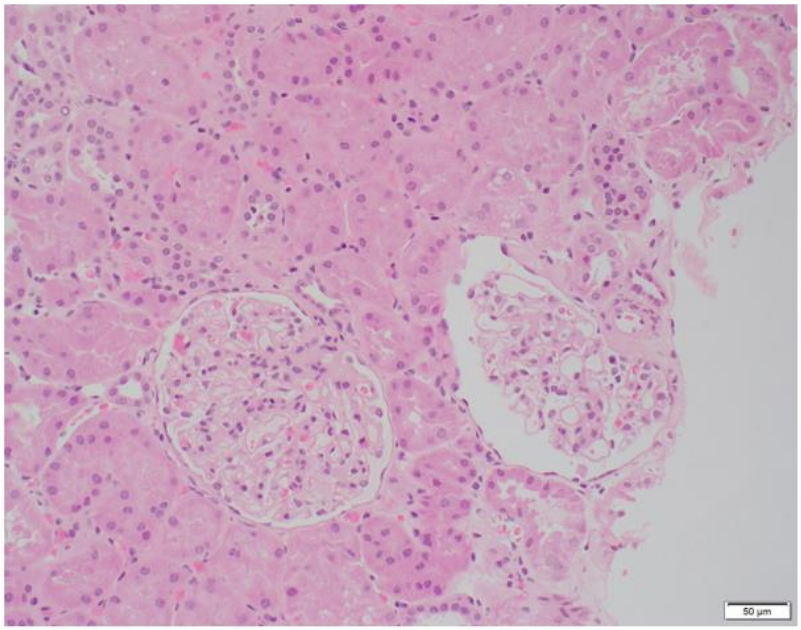
It would soon become apparent that this is not a run of the mill diabetes consult. On examination, he had dysmorphic features (Figure 1) including a long and narrow face, malar flush, reduced elbow extension, fixed flexion deformities of fourth and fifth fingers bilaterally, arachnodactyly, dolichostenomelia (height 166.5cm and arm span 176cm), and an umbilical hernia.

Figure 1 – Photograph of DG showing dysmorphic features including a long and narrow face, malar flush and reduced elbow extension

DG had a complex past history:

- Pancreatic agenesis with permanent neonatal diabetes mellitus and exocrine insufficiency
- Double outlet right ventricle, pulmonary stenosis, patent ductus arteriosus and ventricular septal defect
- Central hypogonadism and growth hormone deficiency
- Bifid left pelvicalyceal system, right hydrocele and right epididymal cyst
- Unexplained proteinuria (with minor segmental mesangial proliferation on renal biopsy; Figure 2)
- Neurocognitive impairment (as per neuropsychology assessment at age 18 years)

Figure 2 – DG's renal biopsy showing minor segmental mesangial proliferation. Haemotoxylin and Eosin (H&E) stain, magnified 200x.



A detailed family history (Figure 3) revealed that his brother, RG, had diabetes mellitus due to pancreatic hypoplasia, truncus arteriosus, bicuspid aortic valve, hypospadias and umbilical hernia. Their deceased father had a reportedly similar physical appearance to DG, atrial septal defect, umbilical hernia, and a positive result for diabetes on employment screening, although he did not seek medical attention for this. He died at age 45 from a myocardial infarction. Autopsy revealed severe triple vessel disease; the pancreas was presumed to have undergone autolysis. DG's deceased paternal grandmother also had a congenital heart defect and five other relatives had diabetes mellitus (Figure 2).

DG was keen to know if there was a genetic cause for his multisystem disorder. He also felt that the information could help his family, particularly his brother, RG, who had previously stated he would not have children as he did not want to pass on his pancreatic/cardiac disorders. His neonatal diabetes and positive family history was suggestive of monogenic diabetes, whilst his concomitant heart disease and the extant literature pointed specifically to a *GATA6* mutation. With DG's consent, Sanger sequencing of *GATA6* was arranged at the Exeter laboratory in the U.K., which has vast experience in monogenic diabetes.

Genetic testing revealed a heterozygous missense variant (Chr18:g.19761441T>C, c.1330T>C, p.Cys444Arg) in exon 4 of *GATA6*, a novel mutation not previously listed. This was classified as likely pathogenic (class 4 variant) in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines (1).

RG, as well as his mother (who also gave consent for testing of DG's deceased father's stored DNA) subsequently underwent genetic testing after genetic counselling, which revealed absence of the *GATA6* variant in his mother; presence of the *GATA6* variant in DG's deceased father; and presence of the *GATA6* variant in RG.

Discussion

This striking case of neonatal-onset diabetes due to a genetic cause only identified in adulthood is instructive in the general principles underlying clinical genetic testing in endocrinology.

Approach to genetic testing

Recognising a potential genetic disorder and accurately diagnosing it through the appropriate genetic test may pay dividends across the healthcare of an individual and their family. The decision to pursue genetic testing should be an index of how likely a disorder is to be genetic, multiplied by how likely a genetic result would be to alter management of the individual or their family. It should only be performed after comprehensive phenotyping using history, examination and preliminary investigations to determine the likelihood of finding a genetic disorder and identify candidate genes.

It is impossible to know all genetic disorders within endocrinology, but features such as young-onset and/or aggressive disease or the development of multiple rare disorders within an individual or one or more related rare disorders within multiple family members may be suggestive. A strong autosomal dominant family history of sulphonylurea-sensitive diabetes mellitus in lean family members with glycosuria immediately brings to mind monogenic diabetes due to a mutation in *HNF1A*. Monogenic diabetes due to *GATA6* mutations are far rarer but the phenotype of pancreatic agenesis, congenital heart disease and other congenital malformations should raise the suspicion of some sort of genetic disorder worthy of further evaluation.

Genetic spectrum of diabetes

Genetic forms of diabetes are often thought to be limited to the traditional forms of 'MODY'; however, diabetes may also be a component of syndromic disorders due to well defined genetic mutations. A critical clue in DG was the neonatal onset of his diabetes. Permanent neonatal diabetes is most commonly due to mutations in *KCNJ11, ABCC8, INS* and *GCK* (2). Neonatal diabetes specifically related to pancreatic agenesis arises from mutations in *PDX1, PTF1A, HNF1B, EIF2AK3, RFX6* and *GATA6* (3). Only the latter gene is currently implicated in the combined phenotype of pancreatic agenesis and congenital heart disease (4), and this allowed a successful single-gene analysis approach in the present case using Sanger sequencing.

GATA6 was first implicated in neonatal diabetes in 2011 with heterozygous loss-of-function mutations found in 15/27 individuals with pancreatic agenesis (4). Following multiple reports of *de novo* cases, only a handful of familial cases of *GATA6*-related pancreatic agenesis have been described and the present family represents one of the largest, illustrating an autosomal dominant, variably expressive pattern. The *GATA6* phenotypic spectrum includes neonatal-, childhood- and adult-onset diabetes, exocrine

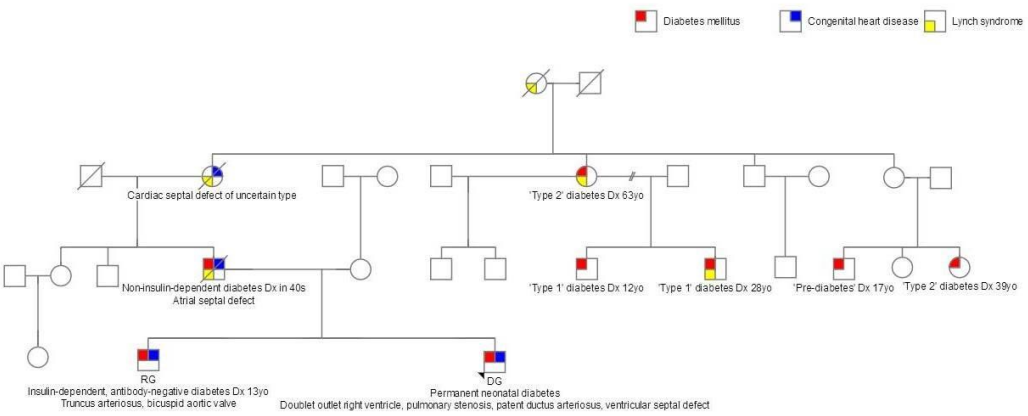
pancreatic insufficiency, pancreatic agenesis or hypoplasia, various cardiac malformations, gallbladder agenesis/biliary atresia, hypothyroidism, hypopituitarism and pituitary agenesis, intestinal malrotation, hernias, colonic perforation, structural kidney abnormalities, neurocognitive deficits and seizures (4–6). Other features are unique to this family. Dysmorphic facial features as observed in DG have not yet been described. Other than a single case report of proteinuria in an infant with a *GATA6* truncating variant (3) and another child with a *GATA6* deletion and structural renal abnormalities (6), renal findings as in DG have not been reported. It is tempting to speculate that *GATA6* may be implicated in both microscopic and macroscopic renal development. As *GATA6* overexpression in rat glomerular mesangial cells results in cell cycle arrest (7), loss-of-function *GATA6* variants could plausibly result in the mesangial cell proliferation observed on DG's biopsy.

Utility of genetic testing

The indications for genetic testing in endocrinology include: differentiation of clinical disorders (e.g. *CASR* testing to investigate familial hypocalciuric hypercalcaemia vs primary hyperparathyroidism where the urinary calcium creatinine clearance ratio is equivocal); disease monitoring (e.g. *RET* testing to diagnose MEN2 syndrome in patients with otherwise seemingly sporadic medullary thyroid cancer); therapeutic guidance (e.g. *HNF1A* testing to identify sulphonylurea-sensitive monogenic diabetes); prognostication (e.g. *GCK* testing to identify this benign form of monogenic diabetes); and predictive testing of family members (e.g. *MEN1* testing in individuals with clinical MEN1 syndrome). In addition, genetic testing with successful identification of the culprit mutation allows accurate family planning. Despite previously vowing to never have children because of the risk of passing on his disorder, the brother of RG is currently seeking reproductive advice regarding IVF with *GATA6* testing of embryos in order to have unaffected offspring. This would not have been possible without the recently obtained genetic result.

Take home messages:

1. Genetic diabetes should be suspected in patients presenting with syndromic features, multisystem congenital disease, neonatal onset diabetes and/or a suggestive family history
2. Recognition and identification of genetic diabetes may allow patients to better understand their disease, empower them and improve engagement with care
3. Identification of a genetic disorder may have important implications for family planning and reproduction



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