Discordant thyroid function tests—beware of albumin variants

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Familial dysalbuminaemic hyperthyroxinaemia (FDH) is a benign euthyroid condition caused by albumin variants with increased binding affinity for thyroxine (T4). FDH falsely elevates T4 by common indirect/analogue methods confounding patient diagnosis.[1,2] Uncommonly, FDH may be present in a patient with Graves' disease, as seen in the case below, making interpretation of tests used for monitoring difficult.

Case report

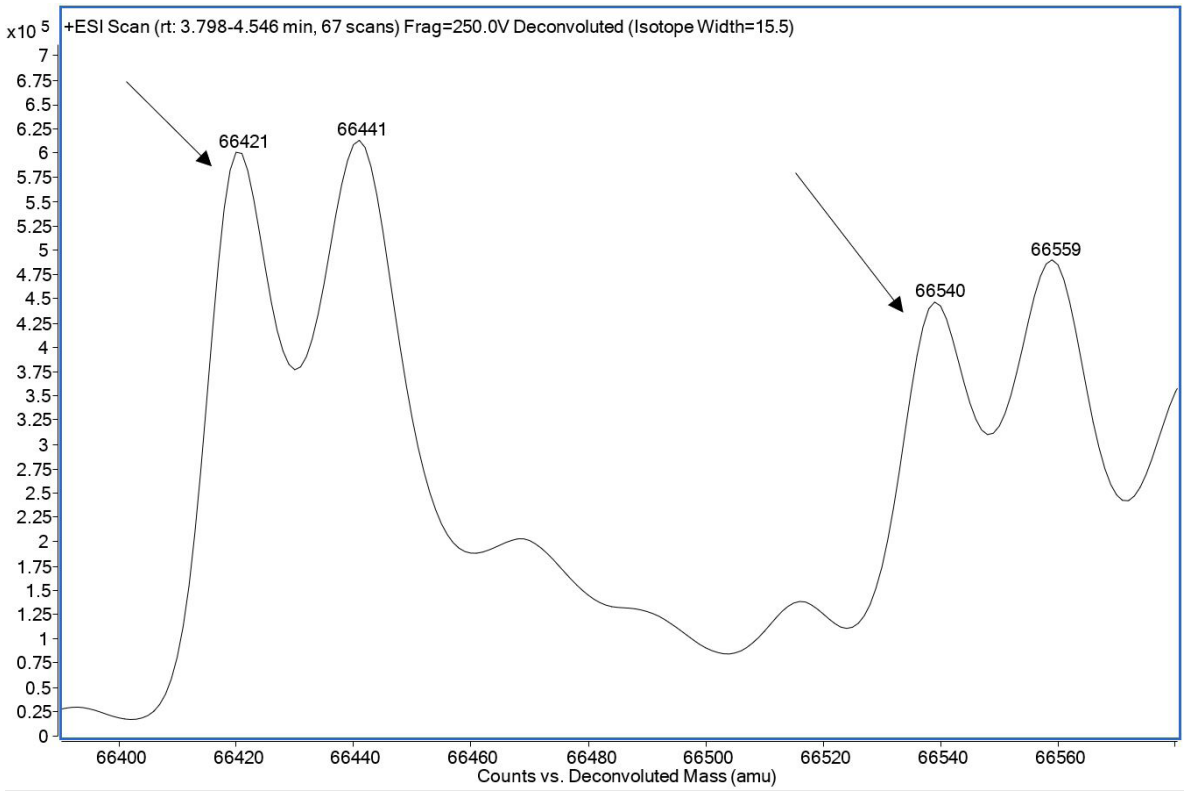
A 24-year-old Afghan woman, G1P1, was referred with hyperthyroidism in April 2022 following sinus tachycardia during labour, which persisted following delivery despite fluids and labetalol. She had three presentations with hyperemesis gravidarum requiring anti-emetics and intravenous fluids in the first 14 weeks of pregnancy. No thyroid function tests (TFTs) were performed during pregnancy. Community bloods 3 years earlier (October 2019) demonstrated free thyroxine (FT4) >77pmol/L (reference interval [RI] 7–16), free triiodothyronine (FT3) 31.6pmol/L (RI 3.6–6.5) and thyroid stimulating hormone (TSH) <0.01mU/L (RI 0.3–5.0) (Table 1). She denied symptoms of thyrotoxicosis and was unaware of her previous abnormal result. It was unclear why these results were not followed up at the time. Clinically, she was mildly thyrotoxic, with a small diffuse goitre and no thyroid eye disease. Initial TFTs were thought somewhat atypical for Graves' disease with discordance between FT4 and FT3 levels. TSH receptor antibodies were positive at 3.1U/L (RI <2U/L) consistent with a diagnosis of Graves' disease. Carbimazole was commenced, increasing to 15mg per day. Thyroid hormone levels increased in May 2022, requiring a further

Table 1: TFT result comparison.

TFT	October 2019	April 2022	May 2022	October 2022	Reference interval
Roche					
TSH (mU/L)		<0.02		0.45	0.27–4.2
FT4 (pmol/L)		37		32	11–22
FT3 (pmol/L)		8.5		6.3	3.1–6.8
Beckman Coulter					
TSH (mU/L)	<0.01		<0.01	0.42	0.3–5.0
FT4 (pmol/L)	>77		67	31	7–16
FT3 (pmol/L)	31.6		9.1	6.1	3.6–6.5
TT4 (nmol/L)				281	78–157
FTI (nmol/L)				98	75–170
TT3 (nmol/L)				1.70	1.30–2.70

TFT = thyroid function test; TSH = thyroid stimulating hormone; FT4 = free thyroxine; FT3 = free T3; TT4 = total thyroxine; FTI = free thyroxine index; TT3 = total T3

Figure 1: Electrospray ionisation time-of-flight mass spectrometry (ESI-TOF-MS) deconvoluted albumin mass spectrum showing the expected major albumin isoform (66,441 Da) and cysteinylated derivative (66,559 Da), as well as variant masses for each component (66,421 and 66,540 Da; annotated by arrows).



dose increase to 25mg per day. Subsequently, in October 2022, TFTs measured on Roche e801 showed TSH had normalised (0.45mU/L [RI 0.27–4.2]). However, FT4 remained elevated (32pmol/L [RI 12–22]), while FT3 was normal (6.3pmol/L [RI 3.1–6.8]), raising the possibility of assay interference. Total T4 (TT4) was elevated on Beckman Coulter Access 2 (281nmol/L [RI 78–157]) but free T4 index (FTI) was normal (98 [RI 75–170]), as was total T3 (TT3) (1.70nmol/L [RI 1.30–2.70]).

Electrospray ionisation time-of-flight mass spectrometry (ESI-TOF-MS) identified the expected albumin isoform (66,441 Da) and cysteinylated derivative (66,559 Da) alongside variant albumin masses, approximately 19 Da fewer (Figure 1). Genetic sequencing confirmed heterozygosity for FDH variant NM_000477.5(*ALB*):c.725G>A, p.(Arg242His), also known as Arg218His by protein nomenclature.

Neonatal bloods for her daughter at day 5 showed FT4 >100pmol/L (RI 11–35), FT3 13.2pmol/L (RI 1.7–11) and TSH <0.02mU/L (RI 0.5–11), consistent with neonatal Graves' disease, despite relatively low maternal TSH receptor antibodies following delivery. It is likely that antibody levels may have been higher earlier in the third trimester. Treatment with carbimazole was commenced for 5 weeks, resulting in normalisation of FT3 and TSH but continued mild elevation of FT4. By 13 months of age, FT4 remained elevated (27pmol/L [RI 7–16]), with normal FT3 (7pmol/L [RI 3–10) and TSH (1.48mU/L [RI 0.3–5]).

Discussion

In patients with discordant TFTs showing elevated T4 or T3 but non-suppressed TSH, analytical artefacts should be considered. Discordant results on multiple platforms may represent FDH, transthyretin variants, thyroid hormone resistance, thyroid hormone autoantibodies or, rarely, TSH-secreting pituitary adenomas.[3,4] An ESI-TOF-MS method was developed locally to screen for FDH and transthyretin variants.[5] Arg218His is the most widespread FDH variant and has previously been detected

in Caucasian, Hispanic, Chinese, Korean and Anatolian Turkish patients.[3,5–10] A decrease in albumin mass of 19 Da is highly suggestive of Arg218His in combination with discordant TFTs. Genetic testing was undertaken for confirmation in our case, as this FDH variant has not previously been reported in Afghan patients.

Patients with FDH usually have normal TSH and high T4 and/or T3 depending on the variant, and no clinical symptoms of thyrotoxicosis. However, FDH may present alongside thyroid disease, as seen in our patient with Graves' disease. Identification of FDH in patients with Graves' disease requires high clinical suspicion. In the first instance, given clear evidence of Graves' disease, it was appropriate to treat for Graves' disease. However, with normalisation of TSH and FT3, the discordant pattern became clear, raising clinical suspicion for FDH.

FDH is inherited in an autosomal dominant manner. There was no known family history of hyperthyroidism for our case and the patient's mother and sister both had normal TSH and FT4. Her father was deceased. As such, it is likely she inherited FDH from her father. Given the high FT4 measured in the setting of normal TSH and FT3 for the daughter, it is likely she has also inherited the FDH variant. FDH can coexist with thyroid disease, complicating patient management when not diagnosed. Unfortunately, the daughter's elevated FT4 initially led her paediatrician to restart carbimazole unaware of her mother's FDH diagnosis. As such, it is recommended to identify FDH in first-degree relatives in order to prevent future misdiagnosis and inappropriate management from discordant TFTs.

Equilibrium dialysis mass spectrometry for FT4 or FTI calculated from TT4 and thyroid uptake by immunoassay appear unaffected by Arg218His, but these methods are not widely available in Australasia.[1] Although FTI was previously available at Canterbury Health Laboratories in Christchurch, the discontinuation of thyroid uptake reagent by Beckman Coulter has led to discontinuation of FTI reporting in New Zealand, though TT4 and TT3 remain available for specialist requestors for interference investigations. FT3 assays are usually unaffected or only slightly affected by Arg218His and were helpful in monitoring treatment for Graves' disease in our patient.[10] Other rarer forms of FDH may present with higher FT3 on routine immunoassays. When TFTs are discordant and unexpected, liaison with the laboratory is recommended to investigate potential interferences and avoid unnecessary investigations for thyroid hormone resistance or TSH-oma, or misattribution to hyperthyroidism.

COMPETING INTERESTS
Nil.

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