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Familial abnormalities of thyroxine binding proteins: some problems of recognition and interpretation

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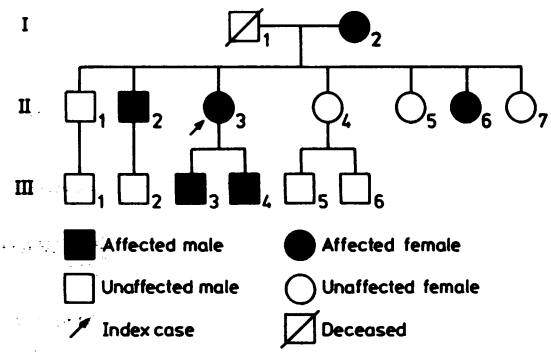
SUMMARY A three generation family study was carried out after inappropriate treatment with radioactive iodine of a 50 year old woman with a raised serum total thyroxine concentration and free thyroxine index. Subsequent investigations showed that she and five members of her family had raised thyroxine binding globulin concentrations. Free thyroxine and free triiodothyronine concentrations were normal. Problems encountered in the recognition of this thyroxine binding protein disorder are discussed. Clinicians and clinical biochemists should be aware of these pitfalls and thus avoid further incorrect treatment on the basis of biochemical findings, even though free hormone estimations are now becoming readily available.

Since 1959[1,2] there have been reports of patients with apparent thyroid disease treated inappropriately because of abnormal biochemical results. Most of these cases were related to the presence of abnormal concentrations of thyroxine binding globulin. Recently, but more rarely, some cases of hyperthyroxinaemia in euthyroid individuals have been shown to be due to excessive binding of thyroxine to either a modified albumin[3–5] or thyroxine binding prealbumin.[6,7] Many clinicians and clinical biochemists are unfamiliar with this problem and we therefore describe a family study carried out on a patient who was incorrectly treated with radioiodine on the basis of her biochemical results.

Case report

A 50 year old woman (patient II3, Figure) presented in 1977 with dizzy spells and was noted to be pale and to have mild exophthalmos. Results of investigations showed a haemoglobin concentration of 9·1 g/dl (normal range 12–14 g/dl) with an iron deficient blood film. Serum total thyroxine concentration was raised at 262 nmol/l (normal range 60–150 nmol/l). The anaemia responded to iron supplements, her symptoms resolved, but as she appeared clinically euthyroid she was referred to hospital for investigation of her thyroid. On examination, her eyes were prominent but with no lid-lag or exophthalmos; her pulse rate was 76 beats per minute; there was no tremor; and her thyroid was not palpable. Results of investigations were as follows: abnormal total thyroxine concentrations (greater than 300 nmol/l), 3,5,3′-triiodothyronine binding capacity 149% (normal range 92–117%), and free thyroxine index more than 201 (normal range 53–144), but a normal technetium-99m 20 minute uptake of 2·1% (normal range 0·7–3·0%). She remained well, but because the persistently raised thyroxine concentration and free thyroxine index suggested hyperthyroidism she was treated in



Three generation study of a family with an excess of thyroid binding globulin.

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February 1978 with 111 MBq of ^{131}I. Ten months later she was gaining weight, had developed a dry skin and slow relaxing reflexes, and investigation showed a raised thyroxine concentration of 165 nmol/l and a greatly raised thyroid stimulating hormone (TSH) value of 58 mU/l (normal less than 5·5 mU/l).

She was clinically hypothyroid and the TSH value remained raised for several weeks; so despite the raised thyroxine concentration she was treated with L-thyroxine. Six months later she was clinically euthyroid and has remained so on 0·1 mg of L-thyroxine daily. At this time total thyroxine was 210 nmol/l, 3,5,3′-triiodothyronine binding capacity 153%, free thyroxine index 137, total triiodothyronine 5·7 nmol/l (normal range 1·2–2·8 nmol/l), TSH 7·6 mU/l, and thyroid antibodies were positive. She was noted to have an antinuclear antibody positive IgG titre of 1/160 (diffuse) with an equivocal DNA binding of 22 U/ml. ^{99m}Tc 20 minute uptake was low at 0·6% and a thyrotrophin releasing hormone test gave an exaggerated TSH response of 12 mU/l at 0 minutes and 50 mU/l at 20 and 60 minutes. Subsequent assay showed the thyroxine binding globulin to be considerably raised at 51 mg/l (normal range 6–16 mg/l).

These tests confirmed the iatrogenic hypothyroidism and showed that the raised total thyroxine and triiodothyronine values were secondary to an excess serum thyroxine binding globulin (TBG) concentration. A family study was therefore undertaken.

Material and methods

Total thyroxine and total triiodothyronine concentrations were determined by conventional radioimmunoassays using poly(ethylene glycol) 6000 to separate bound and free radioactivity. Serum levels of 3,3′,5′-triiodothyronine (reverse triiodothyronine) were determined by a double antibody radioimmunoassay as previously described.[8] Amerlex kits (Amersham International) were used to measure TSH, free thyroxine, and free triiodothyronine concentrations in serum.

Indices of free thyroxine concentrations were calculated either as the ratio of the concentrations of thyroxine and thyroid binding globulin (recommended by Burr *et al*[9]) or as the free thyroxine index, derived by dividing the thyroxine concentration by the relative 3,5,3′-triiodothyronine binding capacity of serum determined using the Thyopac 3 kit (Amersham International).

Concentrations of the three thyroid hormone binding proteins, TBG, albumin, and prealbumin were determined by rocket immunoelectrophoresis[10] using commercially available antisera (TBG, Seward Laboratory; prealbumin and albumin, Behringwerke). Electrophoresis was carried out at 2·5 v/cm overnight in 1% (wt/vol) agarose gels containing the appropriate antiserum in 0·03 M barbitone buffer pH 8·6. The gels were then washed in saline and water before being dried on to their carrier glass plates and stained with amidoschwarz.

Results

The five affected relatives of the index case (II3) had TBG concentrations between 31 and 58 mg/l, that is 1·9–3·6 times the upper limit of normal (Table). Their normal consanguinous relatives had TBG val-

Results of thyroid function test results of the family study

Subject	*Sex*	*TBG (mg/l)*	*T4 (nmol/l)*	*free T4 (pmol/l)*	*T3 (nmol/l)*	*free T3 (pmol/l)*	*rT3 (nmol/l)*	*T3b (%)*	*FTI*	*T4:TBG*	*TSH (mU/l)*	*Prealbumin (mg/l)*	*Albumin (g/l)*
I2*	F	31	275	20·5	3·45	6·9	0·85	132	208	8·7	2·1	236	34
II1	M	8	103	16·5	2·48	6·5	0·31	80	129	12·9	2·0	200	50
II2*	M	43	345	16·0	4·95	7·1	0·77	136	254	8·0	1·5	305	44
II3*	F	51	>300	19·5	4·0	5·5	0·61	149	>201	> 5·9		219	36
II4	F	11	97	10·0	1·90	4·0	0·29	75	109	8·8	1·0	236	30
II5	F	13	125	13·5	2·00	5·6	0·34	76	164	9·6	1·3	262	43
II6*	F	39	198	14·0	3·58	6·0	0·61	126	157	5·0	2·1	335	38
II7	F	12	113	15·0	2·15	5·6	0·37	107	106	9·6	2·3	276	42
III1	M	13	122	25·5	1·60	6·8	0·47	100	122	9·6	1·7	287	56
III2	M	12	110	15·5	2·34	7·3	0·39	88	125	9·4	2·5	372	51
III3*	M	39	236	9·5	2·15	5·0	0·44	129	183	4·0	2·6	276	30
III4*	M	58	260	15·0	5·08	6·2	0·45	133	195	4·5	0·7	252	50
III5	M	9	92	8·0	2·08	6·8	0·25	86	107	9·8	2·1	302	52
III6	M	9	68	11·5	1·95	6·4	0·30	79	86	7·5	1·4	302	52
Reference range		6–16	60–150	8–23	1·2–2·8	3–8	0·11–0·44		92–117	53–144	4·0–12·0	<5·5	150–350	35–55

*Affected subjects I, II, and III refer respectively to the first, second, and third generation of the family.
> = greater than, < = less than.
TBG = thyroxine binding globulin; T4 = thyroxine; T3 = triiodothyronine; rT3 = reverse triiodothyronine; FTI = free thyroxine index; TSH = thyroid stimulating hormone.

ues in the middle of the normal range within 8–13 mg/l. Total thyroxine, triiodothyronine, and reverse triiodothyronine concentrations were generally raised above the upper limit of normal in these affected members as would be expected from the high values of the major transport protein, but normal TSH concentrations indicated their euthyroid state. In the five untreated members of the family with high TBG values (I2, II2, II6, III3, and III4) the free thyroxine index as calculated from thyroxine and 3,5,3′-triiodothyronine binding capacity failed fully to correct for the high thyroxine due to the protein abnormality, although the thyroxine to TBG ratio was normal. Direct measurement of the free hormone concentrations using Amerlex kits also gave normal values for the affected members of the family.

Interestingly, in three of the unaffected relatives with normal TSH concentrations one of the three measures of free thyroxine was abnormal: thus in II1 the thyroxine to TBG ratio, in II5 the free thyroxine index, and in III1 the Amerlex free thyroxine values were slightly raised: free triiodothyronine values were normal in all unaffected subjects.

Burr *et al*[11] reported a tendency for high TBG values to be associated with reduced prealbumin concentrations. This was not apparent in our study; the mean prealbumin concentration was 271 ± 44 mg/l in the affected group, and 292 ± 40 mg/l in the remainder (normal range 150–350 mg/l). This failure to show a difference may be due to the smaller numbers in our study. Albumin concentrations were normal in all subjects and unrelated to TBG or prealbumin values.

Discussion

In 1959 Tanaka and Starr[2] described a 38 year old man who, in an industrial survey, had a very low protein bound iodine despite being clinically euthyroid. Further investigations showed an absence of TBG but normal binding of thyroxine to albumin and prealbumin. In the same year Beierwalters and Robbins[1] reported that a 48 year old man with a raised protein bound iodine value found on routine testing had a raised TBG concentration. Both his sons had normal values but the daughter's TBG concentration was raised, which suggests a familial condition. In early family studies, abnormal TBG values were thought to be transmitted as an autosomal dominant condition.[12]

Nikolai and Seal[13] studied three generations of a large family with low TBG concentrations. In 10 males TBG was absent and in eight females intermediate values were found. The authors concluded that there was an X-linked dominant transmission, that the females were hemizygous for the abnormality, and that the variability in TBG values in the females could be explained on the Lyon hypothesis.[14] This suggests that the variable expression of X-linked traits is the result of inactivation of one randomly chosen X-chromosome during development. The results of our family study concur with this mode of transmission, although interestingly, the index case had an exceptionally high TBG value for a woman. This inheritance pattern has now been confirmed many times and has been reviewed by Burr *et al.*[11] Nikolai and Seal[13] showed absent TBG in the cord blood of a newborn boy whose mother had a low value. This suggests that TBG does not cross the placenta and that gestation can occur normally without TBG in the fetus even when some is present in the mother. A successful pregnancy may also occur in the absence of TBG in the mother.[15]

Shane *et al*[16] described a four generation family in which 13 of 15 members with raised TBG concentrations had goitres and 16 of 17 members with normal TBG values had no goitre. In general though, an association with thyroid disease appears to be relatively uncommon. Associations with growth retardation[17,18] and mental subnormality[19] have also been reported. The prevalence of familial increased TBG concentration is not known, although the prevalence of familial decreased TBG has been estimated at 0·06%[20] or 0·006%.[21] We have detected five families (14 subjects) with increased TBG values and two individuals with decreased TBG values among patients attending a clinic for thyroid diseases.

The present family study was initiated after the misdiagnosis of hyperthyroidism in a euthyroid patient. The biochemical investigations suggested hyperthyroidism and led the clinician to treat the patient with radioactive iodine. In this study all members of the family were clinically euthyroid although the index case had positive thyroid and antinuclear antibodies. Interestingly, her mother had been thought to be hypothyroid on two occasions but a high serum thyroxine concentration was found and, fortunately for her, was not pursued.

Our results confirm that reliance on measurements of total thyroxine and total triiodothyronine concentrations for diagnosis is inadequate and that correction by means of free thyroxine index is misleading in cases of raised TBG values. Measurements of reverse triiodothyronine concentrations are also of no value.

The thyroxine to TBG ratio and the Amerlex free thyroxine and free triiodothyronine methods gave normal values for the sera with high TBG values

(but the first two each failed for one euthyroid person with a normal TBG concentration). Thus re-examination of sera with total thyroxine and total triiodothyronine concentrations apparently indicating hyperthyroidism in euthyroid subjects is best made by measuring TBG, free thyroxine or free triiodothyronine concentrations. Of these, free triiodothyronine may be preferable for general use since it also records normal values for euthyroid sera containing variant albumins[22] or prealbumins[7] with enhanced affinities for thyroxine; the Amerlex free thyroxine method gives artefactually raised values due to an increased interaction of the analogue tracer with the variant albumin[3]; the thyroxine to TBG ratio is, of course, raised in these subjects since TBG values are normal. The latter result will also obtain in euthyroid subjects with a prealbumin variant.

As free triiodothyronine and free thyroxine measurements become more readily available, the majority of discrepancies between biochemical results and thyroid state will tend to disappear. Those laboratories still using total thyroxine measurements and calculating a free thyroxine index will have to take the thyroxine binding proteins into account in order to avoid misdiagnosis. The TSH value may sometimes provide a clue to the fact that an abnormal thyroxine result may be due to an abnormality of binding protein. It will help in cases of apparent hypothyroidism due to a low TBG and, with the development of more sensitive TSH assays, may do so in those with a high thyroxine due to high TBG concentrations.

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