Prevalence of thyroid antibodies in Shiraz, Iran, an area with iodine deficiency

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Summary

The prevalence of thyroid antibodies was 2% among 53 healthy Shirazi volunteers, 3% among 30 consecutive patients with goitre, 41% among 17 patients with thyrotoxicosis and 67% among 18 consecutive patients with myxoedema. A surprising finding was that in a sub-group of 9 of the latter, a high incidence of diabetes (55%) was found and when both diseases co-existed, the prevalence of thyroid antibodies was 80% compared with 67% with myxoedema alone.

Serum cholesterol was marginally lower in the goitre than the control group (P , 0.05-0.025) but when females alone were compared there was no significant difference. It was also lower in the thyrotoxic group (P , 0.0025) and higher in the myxoedema group (P , 0.0025). Serum triiodothyronine was lower in the goitre group than the controls (P , 0.0005) but again, when females alone were considered, this difference lessened (P , 0.025-0.0125). Serum thyroxine and thyroid stimulating hormone levels showed no significant differences in the goitre and control groups.

Introduction

Iodine deficiency in Fars province in southern Iran shows variable degrees of severity. In Ardekan, a small town receiving its water from one main spring, the low iodine content and the high prevalence of goitre is known, although cretinism and deaf mutism are rare, suggesting in relative terms that iodine deficiency is only moderate (Delange *et al.*, 1972b).

Caughey stated that Shiraz was an area endemic for iodine deficiency and that the iodine deficiency was mild (Caughey, 1971). In his study of 1440 teenage girls, 85% had a goitre, although in most it was small. Water with an iodine content below 2.5 $\mu\text{g/l}$ almost always results in goitre (Roche and Lissitzky, 1960), and values above 25 $\mu\text{g/l}$ usually protect against it (Malamos *et al.*, 1971). Caughey also found that in 33 patients with goitre the mean urinary iodide was 25 $\mu\text{g/g}$ creatinine. Olwen-Williams and Hammick (1974) quote Caughey as

showing that the drinking water in Shiraz contained 12 $\mu\text{g/l}$ of iodine. Both these values correspond to those found in areas of mild to moderate iodine deficiency (Ibbertson, 1979). As areas of mild iodine deficiency are more likely to reveal other factors, such as autoimmune thyroiditis in the pathogenesis of goitre, Shiraz was chosen as the venue for the study. Furthermore, if autoimmune thyroiditis is prevalent, one might expect a high incidence of positive thyroid antibodies in newly found patients with myxoedema and thyrotoxicosis, as in the United Kingdom and elsewhere (Doniach, Hudson and Roitt, 1960; Roitt and Doniach, 1960). It was thus decided to estimate the incidence of thyroid antibodies in health, goitre, myxoedema and thyrotoxicosis in Shiraz.

Patients and methods

A group of 53 healthy volunteers, comprising 28 males and 25 females living and working in the university town of Shiraz acted as controls. None of these had a palpable or visible goitre. Thirty consecutive patients with diffuse goitre, 28 of which were female and 2 male and all of whom were clinically euthyroid comprised the goitre group. The term goitre here denotes both a visible and palpable swelling of the thyroid gland moving freely upwards on swallowing. Twelve of these had thyroid scans showing diffuse enlargement and one patient previously shown to have a cold nodule and a further one a multinodular goitre were excluded. Eighteen consecutive patients with primary hypothyroidism and 17 with thyrotoxicosis due to diffuse thyromegaly, in 11 confirmed by thyroid scans, acted as the other 2 groups. The term primary hypothyroidism is defined as underactivity of the thyroid gland resulting in low circulating thyroid hormones and a raised serum TSH level. The term is used interchangeably with myxoedema in the text except that when the latter is used describing the Shirazi patients the presence of non-pitting oedema is implied. Thyrotoxicosis is defined as the clinical state resulting from excessive thyroid hormone activity and associated with raised

circulating levels of thyroxine (T_4) and/or triiodothyronine (T_3). Where thyroid scans were not done (e.g. 18 of the patients with goitre, 6 with thyrotoxicosis) this was because radioiodine was unfortunately unavailable owing to the political upheaval occurring in Iran.

All subjects gave verbal and, where possible, written consent. Each subject was assessed regarding thyroid status, size of goitre and where possible, aetiology. Associated clinical conditions were noted. Blood was taken from an antecubital vein and the serum divided into 2 portions. The first was stored at 4°C until its despatch to Charing Cross Hospital, London, where thyroid autoantibodies were estimated within 10 days. An indirect immunofluorescent technique, using Coon's reagent diluted to 1 to 10 and multiblocks including human thyroid gland, was used. This was conjugated with antihuman IgG and examined for microsomal antibodies using a fluorescent microscope. The result was graded as negative or +, ++ or +++ positive. No actual titres were performed except in 2 representative patients with weakly positive (+) results. These included one patient with myxoedema (titre 1 in 80²), and one with thyrotoxicosis (titre 1 in 40²). All weak positive results are indicated in Table 1 but in general most positives were strongly so at ++ or more and these correspond in the authors' laboratory to titres of 1 in 80² or more.

The remaining portions were stored at -20°C until estimations of serum T_4 , T_3 , TSH and cholesterol were done. The former 3 were estimated using a radioimmunoassay procedure with either Amersham (Radiochemical Centre, Amersham) or Phadebas Kits either in Shiraz or London. The sera were randomly distributed in roughly equal numbers between the 2 centres. Serum cholesterol was measured using a cholesterol oxidase/phenol/4-aminophenazone method as supplied by B.C.L. Ltd on an AA II autoanalyser.

A sub-group of 9 myxoedematous patients had blood glucose measurements using a glucose oxidase method. Either single fasting readings were used when these were clearly abnormal (5 patients) or a standard oral 50-g glucose tolerance test performed in the rest. The term diabetes here is defined either as a fasting blood sugar above 7.2 mmol/l, or when the 50-g glucose tolerance test is used, as a peak level exceeding 8.9 mmol or a level at 2 hr exceeding 6.7 mmol.

Radioactive iodine uptakes using ¹³¹I at 2 and 24 hr were performed on 11 patients with goitre, and 11 with thyrotoxicosis had thyroid scans done.

Results

These are summarized in Table 1. There were no significant differences between the goitre and control

groups in the mean serum T_4 , TSH, cholesterol or incidence of thyroid antibodies. Only one patient in each of these groups had weakly positive antibodies and unfortunately no titres were possible. Antibodies were present in 67% of the myxoedema and 41% of the thyrotoxic group. Both these groups included one patient each with weakly positive antibodies in addition to the 2 previously cited, the remainder showing ++ or +++ readings. The mean serum T_3 was significantly lower in the goitre group than in the controls. Serum cholesterol was significantly lower in the thyrotoxic group and higher in the myxoedema group, but the mean value of 6.6 mmol/l would have been considered at the upper limit of normal for a British population.

The ranges are also tabulated and 2 patients had raised TSH level in the goitre group despite appearing clinically euthyroid and having normal T_4 and T_3 levels. However, TSH levels have been found to be high in endemic areas despite the patient appearing clinically euthyroid (Chopra, Herschmann and Hornabrook, 1975; Patel *et al.*, 1973; Delange, Herschmann and Ermans, 1971). Here the raised TSH levels may reflect a compensatory mechanism by the body to iodine deficiency rather than implying a failing thyroid as it does in autoimmune thyroiditis (AIT) (Ibbertson, 1979). Thus these 2 patients are included in the goitre group. One patient with goitre had a low T_4 level and a low T_3 level but her serum TSH was at the upper limit of normal and her cholesterol was only 3.9 and she also had weakly positive antibodies. This patient might have had mild hypothyroidism but clinically she appeared euthyroid and there was no change over 10 months. Low thyroglobulin levels might have explained her low serum T_4 and T_3 but neither a free T_4 nor a TRH test to exclude hypothyroidism could be done.

Radioiodine studies showed a diffuse goitre in all the subjects in the goitre and thyrotoxic groups that were studied and all the patients with thyrotoxicosis had high uptakes. Evidence for iodine deficiency was present in the majority of patients with goitre who had uptake studies and 8 out of 11 had raised uptakes at 24 hr, 5 had raised levels at 2 hr and the remaining 6 high normal readings (14–20%).

The commonest cause for primary hypothyroidism found was autoimmune thyroiditis and 11 patients had positive antibodies alone (61%), one had had surgery and antibodies, one surgery and radioactive iodine, and 2 had had radioactive iodine alone. In the remaining 3, one had previously been treated for Graves' disease with drugs alone and one was a cretin.

A sub-group of 9 myxoedematous patients (50%) had blood glucose estimations. In 5, fasting blood glucose was raised at 8.8, 14, 17, 11 and 7.6 mmol/l using venous blood. Some of these patients were

TABLE 1. Results of thyroid investigations on controls and patients with goitre, myxoedema, and thyrotoxicosis

	Controls				Goitre				Myxoedema				Thyrotoxicosis				Units
	M	F	All		M	F	All		M	F	All		M	F	All		
Mean age (range in brackets)	33	30	32 (19-54)		32	37.5	28 (16-60)		47	44	45 (23-62)		34	26	30 (16-43)		Years
Number of serum T ₄	27	24	51		2	28	30		5	13	18		7	10	17		-
Mean serum T ₄ (range)	106	109	107 (61-175)		78	103	† (46-165)		16	26	24 (>10-51)		236	238	237 (171-360)		nmol/l
Number of serum T ₃	28	25	53		2	27	29		3	12	15		2	5	7		-
Mean serum T ₃ (range)	2.3	2	2.2 (1.2-2.9)		1.2	1.7	* (<0.5-2.8)		1.2	0.8	0.9 (<0.5-2.2)		7.4	6.4	* (2.8-10)		nmol/l
Number of serum TSH	28	23	51		2	26	28		3	10	13		3	4	7		-
Mean serum TSH (range)	3	5	4(<1-8)		2	4	† (2-16)		44	80	71(12-185)		2	2	† (2-2-3)		i.u./l
Number of serum cholesterol	28	24	52		2	19	21		4	10	14		3	4	7		-
Mean serum cholesterol (range)	5.1	4.5	4.8 (2.9-9)		4.9	4.2	0 (2-8.9)		7.1	6.4	6.6 (3.1-15)		3.6	3.5	θ (2.8-4.5)		nmol/l
Antibodies (on all subjects)	0	Δ	2%		0%	Δ	3%		60%	Δ	67%		Δ	30%	41%		%
Mean size goitre (range)					65	48	50(35-100)			69%			57%	55	55(40-100)		-
Number of radioisotope studies					1	10	11						5	6	11		-
High normal 2-hr uptake (14-20%)					1	6	6						-	-	-		%
Above normal 2-hr uptake (>20%)					1	4	5						4	6	10		%
Above normal 24-hr uptake (>50%)					1	7	8						5	6	11		%
Diffuse thyromegaly on scan						12	12						5	6	11		-

* $P < 0.0005$; † = not significant; θ = includes one patient with reading, and no titre done (other positives 2 + or more).

Normal values: T₄: 106-196 nmol/l; T₃: 1.2-2.9 nmol/l; TSH: 0.5-2.8 i.u./l.

symptomatic but none was insulin dependent. Of these, 3 were known diabetics, and in 2 the diabetes had preceded the myxoedema. One other patient had a borderline glucose tolerance test (fasting, 30-min, 60-min, and 120-min readings being 5.25, 7.0, 6.5, 6.5 mmol/l). The remaining 3 patients had normal glucose tolerance tests. The incidence of diabetes was 55%. The prevalence of thyroid antibodies in the diabetic myxoedematous group was 80% and, if the borderline patient was included, 83%, whereas the prevalence of antibodies in the myxoedema alone was 66.6%.

Discussion

Both the control and goitre groups showed a low incidence of thyroid antibodies. In the former group a 2% prevalence is much lower than a 7% prevalence in a northern English community where there is no iodine deficiency, but in which microsomal antibodies were also measured (Tunbridge *et al.*, 1977). In the latter survey and in others (Couchman, Wigley and Prior, 1970) the prevalence of antibodies rose with age in women and there was a 4-fold increase in women at all ages. As the control group had a low mean age and a slight male preponderance, the lower incidence is partially explained. Nevertheless, despite the small numbers (partly because the study could not be extended owing to the political upheaval in Iran) this study failed to show that AIT plays a significant role in the pathogenesis of goitre. This is despite the fact that both the myxoedema and thyrotoxicosis groups reflected a high prevalence rate of AIT. The myxoedema group was on average 12.5 years older than the control age group, and was composed mainly of women. One might expect therefore a slightly increased incidence of antibodies. However, the differences are more than can be explained by this. Furthermore, if all the patients aged 55 years or more are excluded from the myxoedema group, the mean age out of 12 patients becomes 38 years, but the incidence of antibodies actually rises to 83%. Iodine deficiency alone might be the explanation for goitre, and the radioactive uptake studies done supported its presence as did the high female to male ratio (14:1) in the goitre group, reflecting mild deficiency. Alternatively, women would be expected to complain of goitre more frequently than would men, for cosmetic reasons. Other factors, such as unrecognized goitrogens were not tested in this study.

An unexpected finding was the high incidence of diabetes (55%) in the myxoedema group. Type I or insulin-dependent diabetes has been known to be associated with other organ specific autoimmune diseases for some time, especially thyroid disease and pernicious anaemia (Cudworth, 1978; Editorial,

1976). More recently, type I diabetes has been classified into IA and IB sub-groups (Bottazzo and Doniach, 1976) according to the temporary or more permanent persistence of pancreatic islet cell antibodies which have recently been demonstrated (Bottazzo, Florin-Christensen and Doniach, 1974; MacCuish *et al.*, 1974). Also, type I but not type II (insulin independent) diabetes has been shown to occur more frequently in HLA haplotypes B8 and BW1 and when both exist the risk is additive (Nerup *et al.*, 1974). More recently still, type I diabetes occurring in association with myxoedema has been shown to have a higher association with the haplotype HLA-B8 than type I diabetes alone (Bottazzo *et al.*, 1978). However, the present patients were type I diabetics and there is barely any increase found in these 2 diseases occurring together except for that they are both common diseases and are more likely to be present as age advances. There was a slightly higher mean age group when both were present (53 years) compared to the myxoedema group excluding the diabetics (41 years) but this would never explain the very high incidence. Furthermore, the diabetic myxoedematous sub-group had a higher incidence of antibodies against the thyroid gland, although age differences might have partly accounted for this. Nevertheless some genetic influence appears to be operating in the Shirazi diabetic myxoedematous patients and, when more pressing problems have been overcome, a study of their HLA haplotypes might be worth-while.

Finally, the mean serum T_3 was significantly lower in the goitre than in the control group. However, there was a marked (14:1) female preponderance in the goitre group and when only the females in both groups were compared the difference in the mean serum T_3 lessened considerably (P , 0.025–0.0125). One might in fact have expected serum T_3 levels to be higher in the goitre group, as in other endemic areas when the T_4 is low the patient remains euthyroid because serum T_3 levels are normal or even elevated (Delange, Camus and Ermans, 1972a). When patients with a high TSH in these areas have been given T_3 therapy, the high TSH levels have not always come down as expected, even though serum levels of T_3 have been elevated (Saber and Utiger, 1974). The explanation for the present patients' low T_3 , however, is not obvious.

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