RADIOIMMUNOASSAY OF THYROID RELATED HORMONES AND TSH IN PRIMARY HYPERTHYROIDISM

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Abstract

One hundred and forty seven clinically proven hyperthyroid subjects were studied using radioimmunoassay techniques for the estimation of serum thyroid hormones and pituitaryTSH concentrations. In females toxic goitre was more frequently seen in the age group 21 to 30 years and in males 31 to 40 years.
The mean levels for serum T4, T3, FT4 and FT1 were significantly elevated while the mean level for serum TSH was normal. A significant positive correlation was found between T4 and T3 and between FT4 and FT1 while correlation between T4/FT4, T3/TSH and T4/TSH were found to be insignificant. Serum T3 : T4 ratio was found to be high in hyperthyroid patients. There was no significant difference in the mean of various hormones between the two sexes. Serum T3 was found to be the most sensitive single test that can be performed for the diagnosis of hyperthyroidism. (JPMA 37: 215 , 1987).

INTRODUCTION

Overt hyperthyroidism can easily be detected clinically but the diagnosis of subclinical, mild to moderate hyperthyroidism presents certain problems. It is difficult to differentiate various levels of hyperactivity without knowing the concentration of serum thyroid hormones (total or free). So the estimation of serum T3 and T4 or Free T3 and Free T4 are used as additional evidence in support of clinical diagnosis. Serum TSH determination is not useful to distinguish euthyroid from hyperthyroid patients because of its extremely low circulating levels' and lack of sensitivity of the common TSH assay.
Data on hormonal levels in hyperthyroidism has been lacking in Pakistan. This study was undertaken to establish the levels of thyroid related hormones and pituitaryTSH in hyperthyroid patients.

MATERIAL AND METHOD

One hundred forty seven patients with clinically proven hyperthyroidism (toxic goitre) seen at the Atomic Energy Medical Centre, Jinriah Postgraduate Medical Centre, Karachi were investigated. Their age, sex and socioeconomic status were recorded and blood was drawn for the estimation of Thyroxine (T4), Triiodothyronine ('F3), Free Thyroxine (FT4), T3 uptake and Thyrotropin (TSH).
Iodine uptake was performed according to standardised technique, i.e. 2 hours, 24 hours and 48 hours after oral administration of radio iodine. Serum T3 T4 and TSH were measured by radioimmunoassay technique using RIA kits. Two types of kits were used, the simple and solid phase Amerlex RIA kits of Amersham, International, U.K. Though the principle of RIA kits was similar but the method was slightly modified in Amerlex RIA kits. The normal ranges of the two kits were slightly different. Serum T3, T4, TSH in 96 cases were measured by simple RIA kits and 51 cases by Amerlex kits. In 71 cases FT4 was measured by Amerlex RIA kit only.
The normal values of serum T3, 14, TSH, FT4 and 13 uptake used in this study were our own established normal ranges in 70 apparently healthy subjects.4 In all the cases free thyroxine index was

calculated as the product of 13 uptake and T4 which approximate the absolute free circulaating concentration of 14. The performance and accuracy of the assay was checked by using a set of quality control sera (supplied by Amersham, U.K) in duplicate with each assay. To count the radioactivity, a miniassay gamma counter type 6-20 was used. The results were further checked by multidector (computerized) gamma counter (model : 1612 Nuclear Enterprises) using four parameter non linear curve fitting method. Coefficient of correlation was calculated by Karl Pearson's formula and probability of significance was noted. Ratio of thyroxine and triiodothyronine was also calculated in each case.

RESULTS

Out of 147 patients with hyperthyroidism 41(27.89%) were males and 106 (72.10%) females, ratio was 1:2.58. The mean (±SE) age for males was 34.10 (±1.85) and females 30.54 (±1.57) years, their ages ranged between 20 to 56 and 14 to 60 years respectively.
Majority of males (8536%) and females (92.45%) belonged to Karachi, while 2 males (4.86%) were from Punjab, 3 (7.29%) from Sind and 1 (2.43%) male and 4 (3.77%) females were from India. 51.21% males and 54.7 1% females belonged to poor socioeconomic and 40.78% males, 45.28% females to middle class.
The 2 hours, 24 and 48 hours iodine uptake was elevated in both male and female patients.

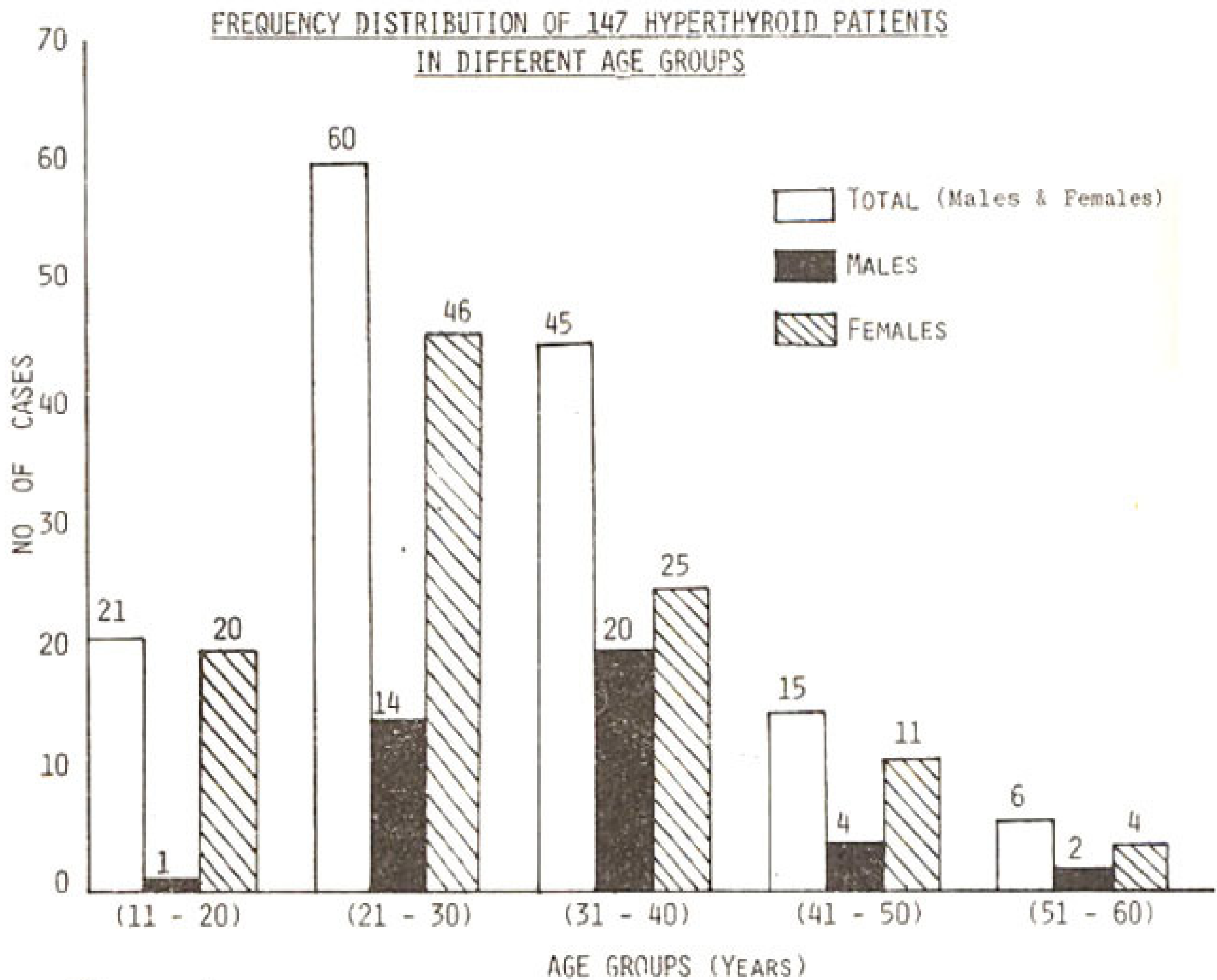

Figure 1. Frequency distribution of 147 hyperthyroid patients in different age groups.

Figure shows the distribution of 147 cases of priinaiy hyperthy roidism in different age groups. In females the peak of percentage frequency was in the age group of 21 to 30 years and in males 31 to 40 years.

TABLE – I

Mean Values of Various Thyroid Hormones and Pituitary TSH in Hyperthyroid Patients by Simple & Amerlex RIA.

Simple RIA	T_4 (SIU) (No)	T_3 (SIU) (No)	TSH (SIU) (No)	T_3-Uptake (%) (No)	FT_4 (SIU) (No)	FTI (SIU) (No)	T_3T_4 (No)
Hyperthyroid Males	343.57 ± 33.09 (26)	10.74 ± 0.70 (26)	4.21 ± 0.38 (26)	34.29 ± 0.73 (26)	–	119.49 ± 12.54 (26)	0.03 (26)
Normal Males	119.43 ± 4.01 (12)	2.39 ± 0.09 (12)	3.32 ± 0.48 (12)	29.94 ± 0.90 (12)	–	34.78 ± 0.82 (11)	0.019 (12)
Hyperthyroid Females	384.55 ± 37.35 (69)	9.58 ± 0.46 (70)	3.58 ± 0.24 (68)	34.00 ± 0.59 (69)	–	154.55 ± 24.73 (69)	0.026 (67)
Normal Females	115.19 ± 4.1 (16)	2.38 ± 0.136 (16)	4.34 ± 0.59 (18)	28.2 ± 0.73 (16)	–	32.5 ± 1.6 (16)	0.019 (16)
Amerlex RIA							
Hyperthyroid Males	255.29 ± 20.29 (15)	7.7 ± 0.67 (15)	1.42 ± 0.33 (15)	37.26 ± 0.97 (14)	172.14±78.87 (20)	94.33 ±8.98 (14)	0.029 (15)
Normal Males	103.61 ± 3.91 (21)	1.7 ± 0.06 (20)	1.33 ± 0.14 (21)	–	20.7 ± 0.47 (20)	–	0.016 (21)
Hyperthyroid Females	313.35 ± 37.97 (36)	9.317 ± 1.107 (36)	1.01 ± 0.17 (36)	37.95 ± 0.80 (36)	158.93±28.29 (36)	123.31 ± 17.77 (36)	0.028 (36)
Normal Females	104.27 ± 3.55 (18)	1.74 ± 0.11 (19)	2.15 ± 0.28 (16)	28.71 ± 0.97 (10)	18.38 ± 1.38 (16)	28.79 ± 1.88 (19)	0.015 (19)

Results are in Mean ± S.E.

Table 1 shows the mean (±SE) of thyroid hormones and TSH in male and female patients by simple and Amerlex RIA. There was no significant difference in the mean of various hormones between the two sexes.

The mean levels for T4, 13 and Free T4 were significantly high (~< 0.001) in both male and female patients as compared with controls. The mean difference of FTI between patients and controls was highly significant (P< 0.001).

TABLE II
Range of Thyroid Hormones in Hyperthyroid Patients.

SIMPLE RIA	T_4 (SIU)	T_3 (SIU)	TSH (SIU)	T_3-UPTAKE(%)	FT_4 (SIU)	FTI (SIU)
Hyperthyroid Males	168.60–880.08	6.31–18.69	0.2–5.7	25.66–40.32	–	50.45–316.91
Normal Males	95.88–144.14	1.84– 2.92	0.60–6.6	25.08–34.47	–	29.85–38.45
Hyperthyroid Females	176.31–2467.19	3.31–30.76	0.0–6.7	25.8–42.32	–	52.50–1478.00
Normal Females	90.09–146.72	1.31–3.23	1.52–6.9	20.00–33.14	–	23.55–45.93
Amerlex RIA						
Hyperthyroid Males	179.54–436.67	4.31–13.55	0.0–2.19	33.53–42.58	44.91–200.25	55.85–164.34
Normal Males	77.86–136.42	1.17–2.09	0.0–2.75	–	18.02–23.94	–
Hyperthyroid Females	160.87–1364.46	3.57–16.17	0.0–2.9	27.21–45.04	16.73–1199.35	50.96–393.45
Normal Females	83.6–134.23	1.09–2.46	0.39–4.2	22.36–33.40	10.29–28.70	19.27–36.70

Table II shows the range of various hormones in patients and controls.
Significant difference was noted between the patients and controls in various hormonal ranges. Ranges for T4, T3, FT4 and Fl! were markedly elevated in hyperthyroid patients. The minimum values for T4, T3, in both male and female patients were higher than the maximum values in controls. Serum TSH concentration in patients was within the normal range.
A significant positive correlation was seen between 13 and 14 in both male ($r=0.76$, $P< 0.05$) and female ($r=0.89$, $P <0.05$) patients. The correlation between T4 and TSH, FT4 and TSH, T3 and TSH were found to be insignificant. There was a good positive correlation between FTI and FT4 in hyperthyroid males (r=().68, $P <0.05$) and females ($r=0.79$,$P <0.05$).
As there was no significant difference in two sexes the data was further analysed after combining the results of males and females in both simple and Amerlex RIA methods.

TABLE III

T_3, T_4 and T_3/T_4 Ratio in Total (Male & Female) Hyperthyroid Patients.

Simple RIA`	2.38 ± 0.08 (28)	116.75 ± 2.92 (28)	0.019 (28)
Hyperthyroid Patients	10.16 ± 0.58 (96)	364.06 ± 35.22 (95)	0.028 (95)
Amerlex RIA			
Normal	1.73 ± 0.06 (39)	104.71 ± 2.39 (39)	0.015 (39)
Hyperthyroid Patients	8.5 ± 0.88 (51)	284.32 ± 29.13 (51)	0.029 (51)

Results are in Mean ± S.E.

Table III shows the mean (±SE) of T4 and T3 in all subjects by simple and Amerlex RIA. The mean 13/14 ratio was found to be high in hyperthyroid patients.

DISCUSSION

The diagnosis of hyperthyroidism presents few problems in the majority of patients and may be confirmed by measuring the serum T4 and T3 concentration. There is, in general a good correlation between 13 and T4 levels over a wide range of concentration. [1] However, the T3 values can be disproportionately high with respect to the 14 levels (T3-toxicosis)[2] and the 14 values may be equivocal in some patients with mild disease.

An increase in both the serum 14 and T3 concentration is the usual pattern of change seen in patients with hyperthyroidism.[3] In present study both 14 and T3 levels were elevated in all patients as compared with the normal levels.[4] Usually, the increase in 13 concentration Is pro portiónately greater than the increase in serum T4, so that the T3/T4 ratio in serum is almost always elevated3 Similar Increase in T3/T4 ratio Is seen In present study. This indicates that in hyperthyroidism the serum T3 reflects not only peripheral generation from T4 but also hypersecretion from the gland. In the majority of patients diagnosis is established by serum total T4 and 13 and there is accumulating evidence that the T3 level may be the most sensitive test for hyperthyroidism.[5] However, in order to exclude the possibility that the increase in serum total 14 and 13 concentration is the result of an increase in hormone binding in the blood, measurement of free honnone concentration is needed.

Normally, the concentration of free hormones (FT4 and FT3) are effectively independent of binding protein concentration.[6] In hyperthyroidism both the proportions and absolute concentrations of free 14 and free 13 are increased and it has also been reported[7] that free hormone levels (FT4 and Fl3) showed a greater rise than total 14 and T3. In all patients of this study free 14 levels were elevated with a similar elevation in total 14' and no subject was found to have normal total 14 with an elevated free T4 because all patients studied were clinically proven cases of primary hyperthyroidism.

It is also reported[8,9,10] that in hyperthyroidism free T4 and free 14 index (FTI) showed equal elevations in more than 90% cases. Similar elevations and a good correlation between free 14 and FTI was seen in this study. In hyperthyroidism the hyperactivity of thyroid gland results in elevation of serum T4 and T3 and reduction of serum TSH levels to almost zero. In present series the levels for serum TSH were found to be normal rather than zero. This could be due to the lack of sensitivity of TSH assay. The most recently developed test is an immunoradiometric assay (IRMA) for TSH which is sensitive enough to discriminate on a basal serum sample the undetectable levels of overt and subclinical hyperthyroidism from those found in euthyroid patients.[7] It appears that measure- ment of serum T3 concentration together with some indicator of hormone binding (FT3 or FT4) will establish or exclude the diagnosis of hyperthyroidism in an even greater proportion of patients than will values of the serum T4 concentration and might, therefbre be regarded as the best initial approach.

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