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CONCORDANCE BETWEEN FREE T4 AND T4 IN THYROID FUNCTION TESTS

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ABSTRACT

Background: Thyroid hormones regulate the metabolism of virtually all cells in the body. The frequency of thyroid dysfunction in our population compels every physician to be skilled in the diagnosis of thyroid disease. Direct measurement of serum concentration of TSH, T4 and T3 is used to establish the diagnosis of primary hypothyroidism and hyperthyroidism. The free unbound fraction of T4 (FT4) and T3 (FT3) which is less than 0.03% is the active form of the hormone. Free T4 (the biologically active T4) is less sensitive to changes in serum binding proteins and hence FT4 levels could best represent the thyroid functional status **Aim**: To evaluate the concordance between total T4, T3 levels and free T4 levels in patients with abnormal thyroid function test reports where the full panel of TFT has been ordered. **Materials & Methods**: Consecutive TFT reports of patients in whom the full panel of TFT have been ordered over a two month period were included in the analysis. The results obtained were statistically analyzed by the help of Microsoft Excel and SPSS software. **Results**: In the hypothyroid group, the median TSH was 6.89 (Mean=21.25) with a mean T4 of 6.75 and mean FT4 of 1.03. In this group, the mean T3 was 0.91 with a range 0.195-1.95. In the hyperthyroid group, the mean TSH was 0.049 with a mean T4 and FT4 of 11.01 and 2.07 respectively and the mean T3 was 1.3 with a range of 0.62 to 4.33. Statistical analysis using Karl Pearson's method showed a significant correlation (r = 0.8) between T4 and FT4 values. It was also found that T3 also had a significant positive correlation with FT4 and T4. No significant influence of age and gender on TFT was found. 14% of the T4 values in the hypothyroid group and 40 % of T4 values in the hyperthyroid group were in the normal range while Free T4 alone showed changes consistent with thyroid dysfunction. **Conclusion:** FT4 correlates highly with T4 in both primary hypothyroidism and hyperthyroidism, and in conjugation with TSH its measurement serves as a better tool than total T4 in the diagnosis of thyroid disorders.

KEYWORDS: Thyroid function test correlation; Free T4 concordance.

INTRODUCTION

Thyroid hormones regulate the metabolism of virtually all cells in the body. These hormones in their active forms interact with their receptors and increase the transcription of several genes involved in the control of metabolism.[1] Thyroid gland produces two hormones, thyroxine(T4) and tri-iodothyronine (T3). Thyroxine is converted by the target tissues and organs into tri- iodothyronine which is its active form. Around 80% of T3 in the body is derived from conversion of T4 in the tissues (a process mediated by deiodinase enzymes) while the remaining 20% come directly from the thyroid gland. The gland is under the control of thyroid stimulating hormone (TSH) originating from pituitary gland which in turn is under the control of Thyrotropin releasing hormone (TRH) originating from the hypothalamus. Dysfunction of the thyroid gland results in hyperthyroidism and hypothyroidism.[2]

The frequency of thyroid dysfunction in our population compels every physician to be skilled in the diagnosis of thyroid disease. In addition to clinical features, thyroid function tests help to distinguish hyperthyroidism and hypothyroidism from the euthyroid states and to quantify them. In view of overlapping clinical features between non-

thyroidal and thyroidal illnesses, thyroid function studies must be selected and interpreted with a specific question in mind. This requires a grasp of thyroid physiology, knowledge of the limitations of the tests in question and an understanding of the individual patient. Direct measurement of serum concentration of TSH, T4 and T3 is used to establish the diagnosis of primary hypothyroidism and hyperthyroidism. It has to be noted that most (>99%) of the thyroid hormone in the blood is bound to proteins and is not available to the target cells. The free unbound fraction of T4 (FT4) and T3 (FT3) which less than 0.03% is the active form. The concentration of T4 in the circulation exists as an equilibrium mixture of protein bound T4 (to TBG, albumin and pre albumin by 75%, 15% and 10% respectively) and unbound (free) T4.[3] This equilibrium maintains a constant level of FT4 by replenishing the stores from bound forms. Pregnancy, illnesses, drugs and dysproteinemic conditions affect the binding of T4. Estrogens, genetic defects in binding protein levels (familialhyperalbuminemia),HIV and acute liver diseases are associated with increased thyroxine binding, while androgens, steroids, chronic liver disease, stress, protein-losing enteropathy, nephrotic syndrome, salicylates and phenytoin can decrease the binding of thyroxine to these proteins. But FT4 levels are maintained so as to maintain a constant supply of FT4 to the tissues.[4] Hence Free T4 (the biologically active T4) is less sensitive to changes in serum binding proteins and hence FT4 levels could best represent the thyroid functional status.[5]

The objective of our study was to evaluate the concordance between total T4, T3 levels with free T4 levels in patients with abnormal thyroid function test reports wherever the full panel of TFT has been ordered In our institute, TSH alone or in combination of FT4 is the commonly ordered thyroid tests. Less commonly a complete panel of TFT (TSH, T4, T3 and FT4) is preferred by doctors particularly when it is ordered for the first time in a patient.

MATERIAL & METHODOLOGY

Study design: This observational analytical study

Research place: time bound study was conducted at the departments of Biochemistry and Endocrinology of the Father Muller Medical college in Mangalore, Karnataka, a tertiary care health facility.

The study was conducted over period of two months from February 1st to March 31st 2015.

Inclusion criteria: : Consecutive TFT reports of patients both male and female,with age more than 1 year in whom the full panel of TFT have been ordered over a two month period were included in the analysis irrespective of the disease condition.

Exclusion criteria: Pregnant subjects and those receiving hormonal therapy for gynecological conditions were excluded from the study.

Sample size: During the study period, a total of 793 samples were received in our lab for complete thyroid function analysis. Abnormal TSH values were observed in 205 subjects. After applying the exclusion criteria, 186 TFT reports were included for the statistical analysis.

Methodology: In our lab, random venous samples collected from the cubital vein were centrifuged and analyzed for T3, T4, TSG, FT4 by Electro-chemiluminescence immunoassay using Cobas 6000 auto analyzer.[5] A two step competitive immunoassay principle is used in the immunoassay of FT4.[6,7]

Grouping : The 186 test reports with abnormal TSH was divided into hypothyroid (TSH > 4.2 mIU/ml) and hyperthyroid groups (TSH < 0.01mIU/ml). Two patients were excluded based on the criteria. Serum T4 values (in mcg/dl) less than 5.1 and more than 14.1 were considered low and high respectively. Similarly, serum FT4 values (in ng/dl) less than 0.93 and more than 1.7were considered low and high respectively. For serum T3, values (in ng/ml) less than 0.8 and more than 2.0 were considered low and high respectively. Based on the T4 and FT4 values, hypothyroid group was further divided into four subgroups. [NN: Normal T4 AND FT 4, DD: Decreased T4 AND FT4, ND: Normal T4 AND Decreased FT4, DN: Decreased T4 AND Normal FT4.]. Similarly the hyperthyroid group was also divided into four subgroups. [NN : Normal T4 AND FT4, II: Increased T4 AND FT4, NI : Normal T4 AND Increased FT4, IN: Increased T4 AND Normal FT4].

STATISTICAL ANALYSIS

The TFTs reports were analyzed statistically using Microsoft Excel and SPSS software. The mean, SD and range of various parameters were analyzed. The relation between FT4 and T4 in the different groups was analyzed using Karl Pearson's correlation and ANOVA. Age and gender wise comparison of the various parameters of the TFT were also done.

RESULTS

A total number of 186 reports were found suitable for analysis. (Table 1a). The maximum number of patients was in the 41 to 50 age group closely followed by 31 to 40 age group. (Table 1b). The reports with abnormal TSH values were further divided into two groups i.e. hypothyroid and hyperthyroid. A total of 143 TSH values were in the hypothyroid range (TSH > 4.2) and 43 were in hyperthyroid range (TSH < 0.01).

In the hypothyroid group, the median TSH was 6.89 microIU/ml (Mean=21.25) with a mean T4 of 6.75 and mean FT4 of 1.03. In this group, the mean T3 was 0.91 ng/ml with a range 0.195-1.95. In the hyperthyroid group, the

mean TSH was 0.049 microIU/ml a mean T4 and FT4 of 11.01 microgm/dl and 2.07 ng/ml respectively and the mean T3 was 1.3 ng/ml] a range of 0.62 to 4.33. The mean, range and the standard deviations of the TFT values are given in Table 2.

Table 1a. Showing the distribution of abnormal TFTs for the two months.

MONTH	Hypothyroid	Hyperthyroid	TOTAL
Month 1	65	18	83
Month 2	78	25	103
TOTAL	143	43	186

Table 1b. Showing the frequency of thyroid disorders in different age groups

	Frequency of thyroid	
Age	**disorder**	**Percent**
20 and below	7	3.8
21 - 30	24	12.9
31 - 40	39	21.0
41 - 50	45	24.2
51 - 60	35	18.8
61 - 70	24	12.9
Above 70	12	6.5
Total	186	100.0

Table 2. Showing mean, SD and range of T3, T4, FT4 AND TSH in both groups.

		Mean± SD	Range	NORMAL RANGE UNITS:
Hyperthyroid	T4	11.01 ±3.26	5.8 – 22.9	5.1 - 14.1 µg/dl
	FT4	2.07±1.05	1.09-6.37	0.93 - 1.7 ng/dl
	T3	1.3 ± 0.73	0.62-4.33	0.8 - 2.00 ng/ml
	TSH	0.049 ± 0.05	<0.005 - 0.0179	0.27- 4.2µIU/ml
Hypothyroid	T4	6.75 ± 2.25	3.37-12.26	5.1 - 14.1 µg/dl
	FT4	1.03 ± 0.36	0.27-1.78	0.93 - 1.7 ng/dl
	T3	0.91 ± 0.29	0.195-1.95	0.8 - 2.00 ng/ml
	TSH	21.25 ± 45.18	4.49-154.5	0.27 - 4.2µIU/ml
Median=6.89				

Table 3. Showing the distribution of cases in the various subgroups of Hypothyroidism

Hypothyroid	NN	DD	ND	DN
Month 1	41	15	8	1
Month 2	46	18	14	0
TOTAL	87	34	21	1

NN: NORMAL T4 AND FT4, DD: DECREASE D T4 AND FT4, ND: NORMAL T4 AND DECREASED FT4, DN: DECREASED T4 AND NORMAL FT4.

Table 4. Showing the distribution of cases in the various subgroups of Hyperthyroidism

Hyperthyroid	NN	II	NI	IN
Month 1	7	3	6	2
Month 2	9	4	11	0
TOTAL	16	7	17	2

NN : NORMAL T4 AND FT4 , II: INCREASE D T4 AND FT4 , NI : NORMAL T4 AND INCREASED FT4, IN: INCREASED T4 AND NORMAL FT4.

Table 5. Showing mean, SD and range of T4,FT4 and TSH in the subgroups of hypothyroidism

Hypothyroid		Mean ± SD	Range
NN	T4	8.06± 1.42	5.05 – 12.26
	FT4	1.25± 0.2	0.95-1.70
	TSH	7.43± 5.24	5.29-29.03
	Median=5.88		
DD	T4	3.61 ± 1.16	0.42-5.00
	FT4	0.55 ± 0.23	0.023-0.90
	TSH	66.6 ± 79.15	4.49 – 154.5
	Median=50.16		
ND	T4	6.53 ± 1.06	5.17-8.02
	FT4	0.89 ± 0.17	0.75-1.53
	TSH	13.17 ± 12.65	4.28-21.73
		Median=8.41	

NN: NORMAL T4 AND FT4, DD: DECREASE D T4 AND FT4, ND: NORMAL T4 AND DECREASED FT4.

Table 6. Showing mean, SD and range of T4,FT4 and TSH in the subgroups of Hyperthyroidism.

Hyperthyroid		Mean ± SD	Range
NN	T44	8.55± 1.37	**5.8-9.9**
	FT4	1.44± 0.22	**1.09-1.79**
	TSH	0.05± 0.04	**<0.005-0.149**
II	T4	16.23± 3.71	**12.97-22.98**
	FT4	3.79 ± 1.46	**2.5-6.37**
	TSH	0.006 ± 0.0018	**<0.005-0.008**
NI	T4	10.78± 1.2	**9.33-12.9**
	FT4	1.95 ± 0.29	**1.47-2.65**
	TSH	0.05± 0.07	**<0.005-0.22**

NN : NORMAL T4 AND FT4 , II: INCREASE D T4 AND FT4 , NI : NORMAL T4 AND INCREASED FT4.

The numbers of subjects in each subgroup of hypothyroid and hyperthyroid patients are given in Table 3 and Table 4 respectively. The mean, SD and range of three subgroups of hypothyroid patients (NN, DD and ND) is given in table 5. Among the hypothyroid group, 87 (61%) had normal levels of both T4 and FT4 [NN], 34 (24 %) showed a decrease in both T4 and FT4 [DD]. However 21 subjects (14.8%) had normal T4 along with decreased FT4 levels (ND subgroup). The mean T4 was 6.53 while the mean FT4 was 0.89 in these 21 subjects of the hypothyroid group. Similarly in the hyperthyroid group,

53 % of the values were concordant (NN and II of table 4). But 40% (17/43) of the hyperthyroid subjects i.e. NI subgroup had increased FT4 alone with a normal T4. In these 17 hyperthyroid patients, the mean T4 was 10.78 while the mean FT4 was 1.95. Statistical analysis using Karl Pearson's method showed a significant correlation (r = 0.8) between T4 and FT4 values. It was also found that T3 also had a significant positive correlation with FT4 and T4. No significant influence of age and gender on TFT was found.

DISCUSSION

The clinical features of thyroid dysfunction can be non-specific or sub-clinical. Thyroid function tests confirm or rule out thyroid dysfunction. Serum levels of TSH are used to screen for both hypothyroidism and hyperthyroidism and also to evaluate adequacy of thyroxine replacement. Total T4 is the standard hormone tested to confirm and quantify the extent of thyroid dysfunction once the initial TSH screening has pointed towards the possibility of an existing thyroid gland dysfunction. After TSH, Total T4/ free T4 are more sensitive indicators of evolving hypothyroidism than T3 or Free T3, and are therefore preferred for confirming hypothyroidism. Routine testing of total T3 is not helpful in many situations including hypothyroid states (late to decrease) and non thyroidal illness/ Sick Euthyroid state (early to decrease).[7]

During certain situations, the reliability of total T4 (and total T3) is questionable due to problems with the levels of binding proteins or problems with the binding of hormones per se to the binding proteins. Free T4 is theoretically a better test than T4 or T3 as it is not affected by the problems in binding of hormones to transport proteins. However the concentration of free hormones in normal serum is extremely small and hence its measurement is associated with technical difficulties. The most reliable way is to separate free hormone fractions by equilibrium dialysis or ultrafiltration, and subsequently measure by immunoassay or mass spectrometry. But these steps are cumbersome and are best suited for research and reference laboratories. Before the advent of automated methods for measuring free T4, methods to approximate free hormone by measuring the total T4 concentration and using indirect methods like resin uptake and Free Thyroid Index (FT4I) to assess protein-binding capacity were used. These methods are no longer useful. The widely used are the direct methods i.e. Free T4 in one-step or two-step immunoassays. However they are not fool-proof in some situations as the serum proteins that bind the hormones deviate significantly from normal concentrations and alter the concentration. But in a majority of patients, these assays provide reliable estimates of FT4 concentration.[3]

This study made an attempt to look for correlation between T4 and FT4 in thyroid disorders. In our study, over a period of two months approximately one-fourth (23%) of all the thyroid tests done as a part of screening or treatment follow-up had shown abnormality in the thyroid functions. The female:male ratio of 2.3:1 for hypothyroid subjects and 3:1 for hyperthyroid subjects in our study is in accordance with the previously known fact that thyroid disorders are more common in women.[3,8] Maximum number of patients were in the 41 to 50 years (24.2%) and 31 to 40 years age group (21%).[9,10]

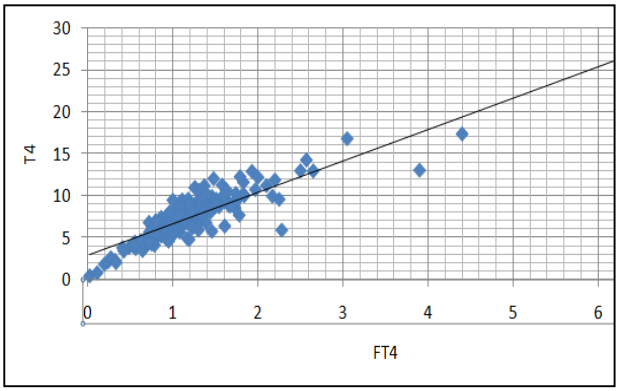
Of the 143 test values in the hypothyroid group, T4 and FT4 values were concordant in 83% (121 subjects) and significant correlation was observed between FT4 values and T4 values (r=0.8). But in 14% (21 subjects) of these hypothyroid test values, the FT4 values alone were low while the T4 values remained normal .The TSH values were highest (median 50.16) when both T4 and FT4 were decreased (i.e. DD subgroup).The lowest levels of FT4 (0.023) and T4 (0.42) were also observed in this subgroup. This indicates that higher values of TSH are associated with a greater decrease in hormone levels and the likelihood of concordance between T4 and FT4. Also in the hypothyroid group,11 subjects had normal values of T3 reiterating the point that T3 levels can remain normal in early stages of hypothyroidism and FT4 alone consistently shows a decrease.[3] These cases would be missed when T4 and T3 values alone are taken into consideration.

Similarly in the hyperthyroid group, significant correlation was observed between FT4 and T4 (r=0.9). But only 50 % of the subjects had concordance between T4 and FT4. About 40% (17 subjects) had isolated increased FT4 with normal T4 levels. Similar to the hypothyroid subjects, the suppressed TSH levels were lowest in patients who had increase in both FT4 and T4 levels (i.e. II subgroup).Thus extreme values of TSH were associated with more severe disease. In both hypothyroid and hyperthyroid situations, earlier changes were seen in FT4 values than in total T4 values. Only in frank disease states both the total T4 and FT4 were consistently concordant. Therefore FT4 levels appears to be more consistently changing in tandem with TSH while total T4 may be normal in-spite of TSH changes, at-least in the early stages of thyroid dysfunction.

Total T4 concentration when taken alone provides limited clinical information, because it reflects mostly inactive protein bound hormone. Hence isolated measurements of total T4 is inadequate in the assessment of thyroid diseases. When analysed in conjunction with free hormone measurements, total T4 levels may reveal protein-binding abnormalities that influence the ratio of bound to free hormone. In the absence of protein-binding abnormalities

total T4 should inversely correlate with TSH activity. In patients with normal serum thyroxine-binding capacity (normal albumin, TBG, and transthyretin), total T4 is proportional to the active free hormone concentration.[2] T4 can be expressed in relation to FT4 as FT4 explains 76% of variation in T4 with highly significant association (R square value of 0.76, p<0.001). An attempt to express this as a mathematical equation gives us the formula T4=2.903+3.951* FT4. (Figure 1).

Figure 1. Scatter plot showing the relationship between T4 and FT4



Hence the relation T4 = 2.903+3.751*FT4

CONCLUSION

We conclude that FT4 correlates highly with T4 in both primary hypothyroidism and hyperthyroidism, and in conjugation with TSH its measurement serves as a better tool than total T4 in the diagnosis of thyroid disorders.

Limitations of study: We have assessed biochemical values alone without taking the complete clinical situations into consideration. The influence of co-existing morbid illnesses, drug therapies etc have not been included. We have not differentiated subclinical thyroid disease and patients on drug therapy for thyroidal illness. We believe that the linear relation between T4 and FT4 as seen in our study would not have been affected significantly with these limitations

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