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Original paper

THE T_4/TBG RATIO IN HEALTHY SUBJECTS AND IN PATIENTS WITH DISORDERS OF THYROID FUNCTION: CORRELATION WITH FT_4 CONCENTRATIONS

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Summary: The proportion of free hormones can be estimated in the presence of protein-bound hormones by a variety of direct assay approaches or by a two test strategy to calculate a free hormone »index«. The T_4/TBG ratio, determined from measurements of total thyroxine (TT_4) and thyroxine-binding globulin (TBG) concentrations, is an appropriate index with improved diagnostic accuracy compared with T_4. In this study, we calculated the T_4/TBG ratio after determining serum TT_4 and TBG by radioimmunoassay (INEP, SCG) in euthyroid persons (n=102), in patients with hypothyrosis (n=33) and hyperthyrosis (n=66), as well as in healthy pregnant women (n=346). The reference range for each group of patients was determined. Values obtained for the T_4/TBG ratio in healthy subjects varied from 2.1 to 4.4. They were lower in hypothyrosis (0.5 –2.2) and elevated in hyperthyrosis (4.7–13.1). The reference intervals of the T_4/TBG ratio were also determined for each trimester of pregnancy. A significant correlation was obtained between the values for T_4/TBG ratio and FT_4 concentration, determined by the two-step radioimmunoassay (CIS-bio international, France), in healthy subjects and patients with thyroid disorders (r=0.93), as well as during pregnancy (0.59). In conclusion, we determined reference intervals for the T_4/TBG ratio, confirming its practical usefulness in the assessment of thyroid function.

Key words: T_4/TBG ratio, FT_4 concentration, correlation, reference intervals

Introduction

Thyroxine (T_4) in the circulation represents an equilibrium mixture of protein bound T_4 and free T_4, the physiologically active form of the hormone. Since up to 99% of circulating T_4 is reversibly complexed with certain serum proteins, predominantly with thyroxine-binding globulin (TBG), serum total T_4 (TT_4) concentration is proportional to the TBG-bound T_4 concentration irrespective of free T_4 (FT_4) concentration. Consequently, altered levels of TBG, which is known to fluctuate under the influence of hormones, drugs (1, 2), diseases (3–5) and genetic factors (6), profoundly affect TT_4 measurement leading to incorrect interpretation of the results obtained for its concentration in the serum. Considering these influences, determination of FT_4 concentration is of more significant clinical value than TT_4 measurements (7).

In order to predict the minute FT_4 concentration, various indices have been proposed. Methods that employed physical separation of free from bound hormone, like the gold standard methods – equilibrium dialysis and ultrafiltration (8), were technically demanding, time consuming and nowadays are predominantly available only in reference laboratories. Routine clinical laboratories use a variety of free hormone tests that estimate the FT_4 concentration in the presence of protein-bound hormone. These procedures employ either indirect determinations, using a two test strategy to calculate a free hormone »index« (9, 10) or a variety of direct immunoassays (11, 12). Index methods require two separate measurements.

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One test is a total hormone measurement (TT_4 or TT_3), while the other is an assessment of thyroid hormone binding protein saturation, using either T_4 or T_3 uptake tests, to calculate the free thyroxine index (FTI) or an immunoassay for measuring TBG concentration, in order to calculate the T_4/TBG ratio.

When these indirect estimations of FT_4 concentration were compared, the T_4/TBG ratio was found to be superior to the FTI in the diagnosis of thyroid dysfunction. This is particularly true for the common occurrence of TBG elevation in cases of acutely ill patients, pregnancy, estrogen treatment and hereditary TBG abnormality (13, 14). It was shown that the T_4/TBG ratio correlates well with both FT_4 concentrations and clinical thyroid status (9, 15), with the exception of congenital TBG deficiency and excess (10).

Here we report a study of the T_4/TBG ratio, using TT_4 and TBG radioimmunoassays developed in our laboratory (16), in sera obtained from euthyroid subjects, hypo- and hyperthyroid patients and those with normal pregnancy. The results were compared with FT_4 concentrations measured by a direct (two-step) radioimmunoassay (CIS-bio international, France). The reference intervals (central 95% range) for the T_4/TBG ratio were also established for each examined group.

Materials and Methods

Subjects

Serum specimens were obtained from 102 euthyroid subjects, 33 and 66 patients with hypothyrosis and hyperthyrosis respectively, and 346 women with uncomplicated pregnancy. The euthyroid subjects included 45 male and 57 female healthy blood donors from the Blood Transfusion Centre of Zemun Clinic, SCG, with no evidence of thyroid disease.

Serum from patients with clinically and biochemically proven hypothyrosis (3 males, 30 females) and hyperthyrosis (14 males, 52 females) were obtained from the Department of Nuclear Medicine, Medical Centre Zaječar, SCG. An ultrasensitive TSH immunofluorometricassay (Wallac, Finland) was used for biochemical evaluation of their thyroid status.

In addition, sera from healthy pregnant women without any thyroid disorder and with known gestation stage, were obtained from the Gynaecological Department of Zemun Health Centre, SCG.

All samples were stored at –20 °C until analysis.

Laboratory methods

Radioimmunoassays were used to measure TBG, TT_4 and FT_4 concentrations in all sera. Thus, serum TBG was determined by RIA TBG (PEG)-INEP (SCG), TT_4 by RIA T_4 (PEG)-INEP (SCG) and FT_4 by RIA CT FT_4 (CIS-bio international, France). All assays were performed according to the manufacturer's instructions.

The T_4/TBG ratio was calculated for each sample from the equation:

T_4/TBG ratio = T_4 (nmol/L) : 1.287/ TBG (mg/L).

The reference interval for each parameter was determined as the central 95% range between the 2.5th and the 97.5th percentiles as proposed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (17).

All statistical calculations were done using the STATGRAPHICS programme, version 4.2. Medians were analysed by the nonparametric Mann-Whitney U test. A value of Z higher than 1.96 (p=0.05) was considered statistically significant.

Results

The values of the T_4/TBG ratio determined for all the studied groups are shown in *Table I*. Statistical analysis revealed significant differences between the T_4/TBG ratios in euthyroid subjects and hypothyroid or hyperthyroid patients (medians: 3.2, 1.4, 7.5 respectively). Determination of the 95% central range as reference intervals showed that the T_4/TBG ratios

Table I Reference intervals for the T4/TBG ratio in various subjects are given as the central 95% range (2.5th, the median, 97.5th percentiles).

Subjects		T4/TBG ratio		
		2.5th	Me	97.5th
euthyroid (n=102)		2.1	3.2	4.4
hypothyroid (n=33)		0.5	1.4*	2.2
hyperthyroid (n=66)		4.7	7.5*	13.1
non-pregnant women (n=57)		1.9	3.1	4.2
pregnant women (n=346)	1st trimester (n=102)	2.1	3.4*	5.5
	2nd trimester (n=107)	1.7	2.9*	4.6
	3rd trimester (n=137)	1.5	2.5*	4.5

*Statistically significant difference (Z>1.96; p=0.05) when comparing hypothyroid and hyperthyroid with euthyroid, pregnant women of the 1st trimester with non-pregnant women (control group), 2nd trimester with 1st trimester and 3rd trimester with 2nd trimester.

for the hyperthyroid patients (4.7–13.1) were well differentiated from the euthyroid range (2.1 – 4.4), but those for the hypothyroid patients (0.5–2.2) overlapped a little. *Figure 1* clearly reveals the distribution of T_4/TBG ratios in euthyroid subjects and patients with thyroid disorders.

The median T_4/TBG ratio in pregnant women during the 1st trimester of gestation (median 3.4) was significantly higher than that for the non-pregnant women used as a control group (median 3.1) (*Table I*). Statistically significant decreases were observed during the 2nd and 3rd trimesters (medians: 2.9 and 2.5 respectively). Thus, there is a need to modify the normal T_4/TBG ratio range for pregnant women in each trimester as follows: 2.1–5.5, 1.7–4.6, 1.5–4.5, for the 1st, 2nd and 3rd trimesters respectively. Figure 2 shows the distribution of T_4/TBG ratios in pregnant women sera and the corresponding control group. During pregnancy, the ratios exhibited a trend towards hypothyroid values, i.e. 2%, 9% and 23% of values in the 1st, 2nd and 3rd trimester respectively were in the hypothyroid range.

The relation between the T_4/TBG ratio and FT_4 in the euthyroid, hypothyroid and hyperthyroid subjects studied is given in *Figure 3*. Although it was not linear ($y = 1.7858 \times^{1.6414}$), a significant correlation was found ($r = 0.93$).

The correlation coefficient between the T_4/TBG ratios and FT_4 concentrations in 72 sera from pregnant women was 0.59. The equation for the line is $y = 4.8659 \times^{0.9029}$ (*Figure 4*).

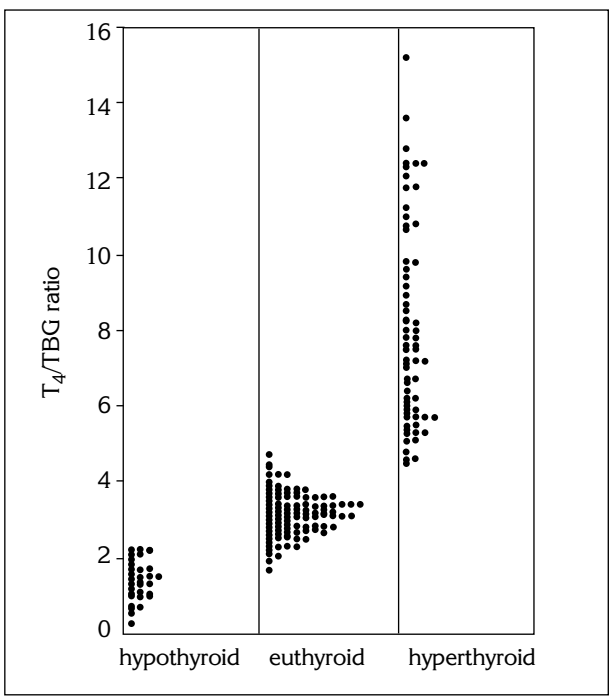

Figure 1 T4/TBG ratio in euthyroid subjects (n=102) and in patients with hypothyrosis (n=33) and hyperthyrosis (n=66).

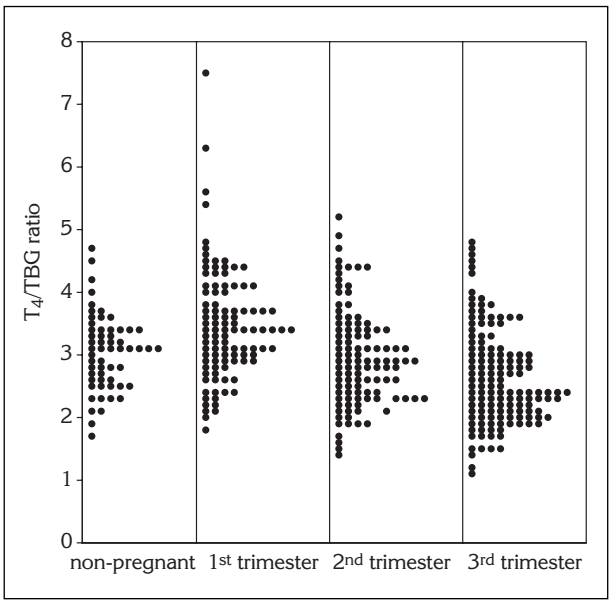

Figure 2 Values for the T_4/TBG ratio determined in pregnant women (n=346; 1st =102; 2nd =107; 3rd =137) and the control group (57 non-pregnant women)

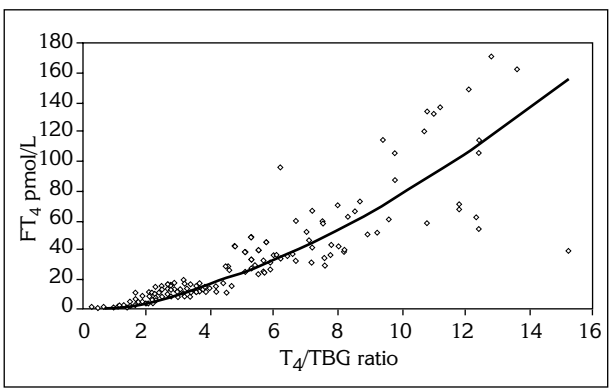

Figure 3 Correlation between the T_4/TBG ratio and FT_4 concentrations in 102 euthyroid subjects, 33 patients with hypothyrosis and 66 hyperthyroid patients ($y=1.7858 \times^{1.6414}$, $R^2=0.8659$, $r=0.93$)

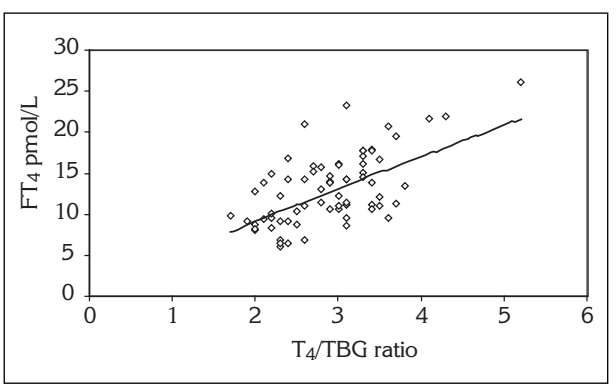

Figure 4 Correlation between the T_4/TBG ratio and FT_4 concentrations in 72 pregnant women ($y=4.8659 \times^{0.9029}$, $R^2=0.3533$, $r=0.59$)

Discussion

The determination of total thyroxine concentration is not always the most appropriate laboratory diagnostic analysis for the assessment of functional thyroid disorders. The main reason is that its concentration varies with total binding protein concentration, principally TBG. Thus, estimation of free T_4 concentration is of much greater clinical interest for determining thyrometabolic status.

Indirect estimations have long been used to obtain information regarding FT_4 concentration before any direct FT_4 assays were developed. The FT_4 index (FTI), which is the product of total T_4 and T_3 uptake, although proved to be valuable in clinical practice, showed inaccurate when TBG concentrations were high. This led to euthyroid patients with increased TBG concentrations being misclassified as hyperthyroid. The utility of measuring TBG directly instead of through T_3 uptake was shown by Burr et al (13), who revealed that the relationship between T_4 and TBG in the healthy euthyroid population remained nearly linear over a wide range of TBG concentrations. This formed the basis for suggesting the use of the T_4/TBG ratio in thyroid diagnosis in order to predict FT_4 concentration. The T_4/TBG ratio was found to be a better diagnostic index than FTI, particularly when high TBG concentrations occurred: in acutely ill patients or during pregnancy. The advantages of employing the T_4/TBG ratio in the evaluation of thyroid status were confirmed by others (18, 19).

Our results for the T_4/TBG ratio in euthyroid, hypothyroid and hyperthyroid patients showed that it is an efficient thyroid function discriminator for hyperthyrosis (4.7–13.1), when compared to the 2.1–4.4 range for euthyroid subjects. Concerning hypothyroid patients, the T_4/TBG ratio range (0.5–2.2) overlapped with euthyroids (2.1–4.4), similarly as in the study of Attwood et al (18).

We observed that the serum T_4/TBG ratios of pregnant women rose significantly during the first trimester (median – 3.4 compared to 3.1 in non-pregnant women). This increase of free hormone is probably due to the intrinsic TSH-like activity of human chorionic gonadotropin. High levels in the first trimester provoke a weak, transient thyroid stimulation (20). In contrast, the T_4/TBG ratios showed a significant fall during the second and third trimester. This can be explained by greater elevation of TBG than TT_4 concentrations during pregnancy (21, 22). Thus, there is a need for trimester-specific reference intervals for the T_4/TBG ratio in order to avoid pregnant women being misclassified as hypothyroid, as was established by previous studies (23–26).

We found an excellent correlation between the T_4/TBG ratio and FT_4 concentration measured directly by two-step radioimmunoassay for the whole group of euthyroid, hypothyroid and hyperthyroid subjects ($r=0.93$). In addition, a respectable correlation ($r=0.59$) was obtained between T_4/TBG ratios and FT_4 concentrations in sera from pregnant women. These results further confirmed the value of the T_4/TBG ratio as a reliable determinant of free T_4 concentration.

In conclusion, reference ranges for the T_4/TBG ratio were established for all studied groups. Moreover, the results presented here demonstrate that the T_4/TBG ratio, determined by our TBG and TT_4 radioimmunoassays, is a useful diagnostic parameter for the determination of the proportion of FT_4 in patients with thyroid disorders, as well as for the assessment of the thyroid status of healthy adults and during pregnancy.

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VREDNOSTI T_4/TBG INDEKSA U SERUMU ZDRAVIH I OSOBA SA POREMEĆAJIMA FUNKCIJE ŠTITASTE ŽLEZDE: KORELACIJA SA KONCENTRACIJOM FT_4

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Kratak sadržaj: Za evaluaciju funkcionalnog stanja štitaste žlezde danas se, pored određivanja koncentracija ukupnih tireoidnih hormona (T_4 i T_3), određuje i slobodna, fiziološki aktivna frakcija. Pored direktnog određivanja koncentracije slobodnih hormona (FT_4 i FT_3), za procenu slobodne frakcije koristi se i T_4/TBG indeks, koji se izračunava iz koncentracija ukupnog T_4 i TBG-a. U ovoj studiji određen je T_4/TBG indeks kod zdravih, eutireoidnih osoba (n=102), kod pacijenata s hipotireozom (n=33) i hipertireozom (n=66) kao i kod zdravih trudnica (n=346). Određen je opseg referentnih vrednosti za svaku ispitivanu grupu pacijenata i utvrđen stepen korelacije sa koncentracijom FT_4. Dobijene vrednosti T_4/TBG indeksa kod zdravih su u opsegu od 2,1 do 4,4. Vrednosti T_4/TBG indeksa su snižene u hipotireozi (0,5–2,2), a povišene u hipertireozi (4,7–13,1). Referentne vrednosti T_4/TBG indeksa u trudnoći su 2,1–5,5, 1,7–4,6, 1,5–4,5 za I, II i III trimestar. Poređenjem vrednosti T_4/TBG indeksa i koncentracije FT_4 kod zdravih i pacijenata s poremećajima funkcije štitaste žlezde utvrđen je značajan stepen korelacije (r=0,93), dok je kod trudnica dobijen r=0,59. Praktična provera primenljivosti T_4/TBG indeksa u ispitivanju funkcije štitaste žlezde pokazana je korelacijom sa FT_4.

Ključne reči: T_4/TBG indeks, koncentracija FT_4, korelacija, referentne vrednosti

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