Serological diagnosis of thyroid disorders in patients using minividas TsH, T3, T4 Enzyme linked fluorescent immunoassay (ELFA)

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Abstract

Serum samples 363, 243 and 234 for measuring the level of TsH, T3 and T4 respectively were taken from patients of both sexes clinically suspected with the thyroid disorders using enzyme linked fluorescent immunoassay (ELFA) for measuring TSH, T3 and T4. Results were showed that out of 363 serum samples only 36 (9.88%) were positive for the hyperthyroidism, the level of TSH were < 0.15 miu/ml accompanied by raised level of T3 > 2.33 nmol/L in 38 (15.6%) and raised level of T4 > 120 nmol/L in 32 (13.6%). And out of 363 serum samples only 31 (8.5%) were positive for primary hypothyroidism, the level of TsH were > 7 miu/ml accompanied by low levels of T3 <0.92 nmol/L in 18 (7.4%) and low level of T4< 60 nmol/L in 27 (11.5%). Three (0.82%) serum samples were positive for secondary hypothyroidism accompanied by low level of TsH together with subnormal level of T3 and T4. Euthyroidism cases were positive in 293 out of 363 (80.8% serum samples examined with normal level of TsH 0.15-7 miu/ml and normal level of T3 and T4 (0.92-2.33 and 60-120 nmol/L) respectively. Conclusion:

1. Minividas TsH, T3 and T4 enzyme linked fluorescent immunoassay (ELFA) are of value to confirm clinical diagnosis of thyroid disorders.
2. Hyperthyroidism disorder were present in 9.88%, hypothyroidism (primary and secondary) were present in 8.5%, 0.82% respectively, where as Euthyroidism were present in 80.8% of serum samples examined.

التشخيص المصلي لآفات الغدة الدرقية باستعمال تقنية الأنزيم المرتبط الفلورسيني للـ T4، T3 و TsH بجهاز الـMinividas

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الخلاصة

تم قياس مستوى هرمونات الغدة الدرقية (TsH, T3, T4) في نماذج أمصال مرضى الغدة الدرقية المشخصين سريرياً وباستعمال جهاز Minividas وبتقنية الأنزيم المرتبط الفلوريسيني (ELFA)، حيث تبين من خلال فحص 363 مصل فقط 36 (9.88%) موجب لحالة فرط الغدة الدرقية مصحوباً بقلة مستوى هرمون TsH تحت الطبيعي (< 0.15 min/ml) وزيادة في مستوى هرمون T3 (> 2.33 nmol/L) في 38 (15.6%) مع زيادة في مستوى هرمون T4 (>120 nmol/L) في 32 (13.6%). ومن خلال فحص 363 مصل فقط 31 (8.5%) موجب لحالة ضمور الغدة الدرقية البدائي مصحوباً بزيادة مستوى هرمون TsH فوق الطبيعي (>7miu/ml) مع قلة مستوى هرمون T3 تحت الطبيعي (<0.92 nmol/L) في 18 (7.4%) مع قلة مستوى هرمون T4 (<60 nmol/L) في 27 (11.5%) سجلت 3 نماذج (0.82%) من المصول حالة ضمور الغدة الدرقية الثانوي مصحوباً بقلة في مستوى هرمون TsH مع قلة مستوى هرمون T4,T3 تحت الطبيعي. حالات الغدة الدرقية الحسنة Euthtroidism كانت موجبة في 293 (80.8%) من المصول المفحوصة 363 وبمستوى

طبيعي لهرمون TsH (0.15−7 miu/ml) وبمستوى طبيعي لهرمون T3 and T4 (0.92-2.33 and 60-120 nmol/L) على التوالي. الاستنتاجات:

1. فحص الإنزيم المرتبط الفلوريسيني (ELFA) بجهاز Minividas له أهمية وكفاءة في تشخيص مستوى هرمونات TsH, T3 and T4 لمرض الغدة الدرقية المشخصين سريرياً.
2. حالة فرط نمو الغدة الدرقية كانت موجبة في (9.88%) اما حالة ضمور الغدة الدرقية البدائي والثانوي كانت (8.5، 0.82)% على التوالي بينما حالة الغدة الدرقية الحسنة كانت (80.8%) في نماذج المصول المفحوصة للمرضى.

Introduction

Thyrotroponine or thyroid stimulating hormone (TsH) is a glycoprotein with a molecular weight of 28.000-30.000 Daltons. TsH is composed of two non-covalently bound (α, 92 aminoacid and β, 112 aminoacid) peptide subunits bound to glycan residues and determine the biological and immunological properties of the hormone (1). This TsH produced by thyrotrophic cell in anterior pituitary gland, secreted into blood stream and stimulate thyroid gland to secrete triiodothyronine (T3) and thyroxin (T4) (1,2). T3 and T4 are a hormones secreted by thyroid glands T3 are produced 80% by deiodination mechanism which converts T4 to T3 and 20% T3 secreted by thyroid gland. T3 is physiologically more active than T4 it plays important role in maintaining euthyroidism (3). Both T3, T4 are bound predominantly into carrier protein 99.9% (thyroxine binding globulin). The fraction that remain free of each hormone is considered as the active part of the hormone (3, 4). During the importance of the thyroid disorders and their recurrence, this study aimed at:

1. Measuring the levels of TsH, T3 and T4 using minividas enzyme linked fluorescent immunoassay (ELFA) in patients sera.
2. Confirm clinical diagnosis of hyperthyroidism, hypothyroidism, and Euthyroidism among patients.

Materials and Methods

Serum samples from thyroid patients of both sexes were taken in Al-Razi private clinical laboratory during the period between 8.1.2011 to 23.1.2013. All serum samples 363 were tested for TsH, 243 for T3 and 234 for T4 confirm clinical diagnosis of thyroid disorders using TsH, T3, T4 Enzyme in minividas instrument.

Principle:

1. In case of TsH, the assay combines a one-step enzyme immunoassay sandwich method, in case of T3, T4 the assay combines an enzyme competition method, with final fluorescent detection (ELFA).
2. The solid phase receptacle (SPR) serve as the solid phase as well as the pipetting devices for the assay, all reagents for the assay are ready to use predispended in the strips and all the assay steps are performed automatically by the minividas instruments.
3. In case of TsH, the samples is transferred into the well containing anti TsH antibody labeled with alkaline phosphatase (conjugate). The sample/ conjugate mixture is cycled in and out the SPR, the Ag bind to Abs coated on the SPR and to the conjugate forming sandwich.
4. In case of T3, T4 competition occurs between the Ag present in the sample and labeled Ag for specific anti T3 or anti T4 antibodies (sheep or mouse) coated on the interior of the SPR, unbound components are eliminated during washing.
5. During the final detection step. The substrate (4- methy/ umbelliferyl phosphate) is cycled in and out the SPR the conjugate enzyme catalyzes the hydrolysis of the

substrate in a fluorescent product measured at 450 nm. The intensity of the fluorescent product is proportional to the concentration of sample Ag (hormone). Results are automatically calculated by instrument.

Contents of the kits:

1. Strips for TsH, T3, T4.
2. SPR (solid phase Receptacle) sensitized with monoclonal anti TsH immunoglobulin as (mouse) or coated with anti T3 monoclonal Abs (sheep) or anti T4 monoclonal Abs (mouse).
3. C1 control for TsH, T3, T4.
4. S1 calibrator for TsH, T3, T4.
5. MLE card (master lot Entry) required to calibrate the test.

Procedure:

1. All the required reagent must be allow them to come to room temperature.
2. Use one strip and one SPR for TsH, T3, T4 for each sample, control or calibrator to be tested.
3. Type or select TsH or T3 or T4 on the instrument to enter the test code. The calibrator must be identified by S1 and tested in duplicate and for control is to be identified by C1 for each TsH or T3 or T4.
4. Mix the calibrator, control and sample using vortex type mixture.
5. Pipette the 200 µl of calibrators, control or samples into sample well for TsH or T4 and 100 µl of calibrators, control or samples into sample well for T3 assay.
6. Insert the SPR and strips for TsH, T3 or T4 assay into the instrument.
7. Initiate the assay as directed in the operator's manual, All the Assay steps are preformed automatically by the instrument and the assay will be completed within 40 minutes.
8. After the assay is completed remove SPR and strips from the instrument and dispose it.

Expected values:

TsH (Euthyroied 0.15-7 miu/ml)
(Hyperthyroid < 0.15 miu/ml)
(Hypothyroid > 7 miu/ml)
T3 (Normal range 0.92-2.33 nmol/L)
T4 (Normal range 60-120 nmol/L)

Results and Discussion

This study is revealed that out of 363 serum samples taken from patients clinically affected with thyroid disorders only 36 (9.88%) serum samples are positive for hyperthyroidism, the level of TsH are subnormal (< 0.15 miu/ml) accompanied by high level of T3 (> 2.33 nmol/L) in 38 (15.6%) out of 243 serum samples and high level of T4 (> 120 nmol/L) in 32 (13.6%) out of 234 serum samples. The results are also showed that out of 363 serum samples only 31 (8.5%) are positive for primary hypothyroidism the levels of TsH are increased (> 7 miu/ml, accompanied by low subnormal levels of T3 (< 0.92 nmol/ L in 18 (7.4%) out of 243 serum samples and low subnormal levels of T4 (< 60 miu/ ml) in 27 (11.5%) out of 234 serum samples. Also serum samples 3 (0.82%) out of 363 are showed secondary hypothyroidism accompanied with subnormal levels of TsH, T3 and T4. Euthyroidism are presented in 293 (80.8%) out of 363 serum samples accompanied with normal levels of TsH (0.15-7 miu/ml), T3 (0.92-2.33 nmol/L) and T4 (60-12 nmol/L). Although treatment of thyroid disease may be prolonged and incase of hypothyroidism, life long and it is essential to confirm the clinical diagnosis by laboratory test (5,6) which is done in this study and the excess or deficiency of circulating thyroid hormones produce a characteristic clinical changes, Although in certain cases diseases of thyroid gland may however be present without

hyper or hypofunction (7). Hyperthyroidism or thyrotoxicosis produced by excess of thyroid hormones are easily recognized but may be remain unsuspected for a long time and it is associated with speeding metabolism and clinical feature including tachycardia, tremor, weight loss, tiredness, sweating, diarrhea and anaxiety (5). In most patient with hyper thyroidism the level of T3, T4 in plasma are raised, much T3 is secreted directly from thyroid tissue and the plasma level are increased above normal and are usually evident earlier than those of T4 which is evident in this study. In certain cases only T3 level are elevated, the high levels of T3 and T4 suppress TsH secretion through negative feedback effect on pituitary gland, also TsH in these cases not even stimulated by hypothalamic thyrotrophin releasing hormone (TRH) (8). In very rare forms of hyperthyroidism, the TsH is not reduced, since the negative feedback control of the thyroid hormones has not effect (7). Hypothyroidism is due to subnormal level of plasma T3, T4, this disease condition develops into slowdown metabolism with tiredness and mental dullness, weigh gain, menstrual disturbances, dry skin, buffy face, myxedema hair falls and hoarse voice (5,6). In primary hypothyroidism, plasma level of T4 are usually low, although in very early stage the level of T4 may be within normal range, but the level of TsH is increased to abnormal high level (5). T3 level may be still with normal level in these cases of hypothyroidism. The assay for T3 may not help in making the diagnosis, so low level T4 and high level TsH confirm the diagnosis in these cases (5). Secondary hypothyroidism is due to impaired TsH secretion, caused by pituitary insufficiency in which thyrotrophin releasing hormone fails to increase TsH secretin or by failure of TRH secretion from hypothalamus. Secondary hypothyroidism due to pituitary or hypothalamus deficiency is less common than primary which is observed in this study and in the long standing secondary hypothyroidism, the thyroid gland may atrophy irreversibly, so the essential difference in finding between primary and secondary hypothyroidism is the level of TsH which is high level in primary and low level in secondary hypothyroidism (8) which is observed in this study. Euthyroid goiter represents compensated thyroid disease in which thyroid function test may be normal. In these thyroid diseases thyroxin synthesis may be impaired by iodine deficiency, drugs and minor degree enzyme deficiency, the tendency for plasma T4 to fall, increase TsH by feedback, this stimulate synthesis T4, T3 but maintain adequate normal plasma level of these hormones (TsH, T3, T4). The thyroid therefore enlarged (goiter) but hypothyroidism is avoided (9) which is observed in most of thyroid patients in this study. A similar findings are seen in low iodine areas (endemic goiter), and in thyroiditis a temporary aberrations in thyroid hormones levels but it is not common (10).

Results of thyroid hormones assays

	Hyperthyroidism	**Hypothyroidism**	
		Primary	**Secondary**
TsH	↓	↑	↓
T4	↑	↓	↓
T3	↑	↓	↓

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