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DIAGNOSTIC SENSITIVITY OF TWO RADIO RECEPTOR ASSAYS (TRAK-ASSAY AND TRAK DYNO HUMAN) FOR DETECTION OF TSH RECEPTOR ANTIBODIES

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ABSTRACT

Radio receptor assays for detection of TSH receptor antibodies in serum are typically based on binding competition of TSH-R antibodies and \(^{125}\)I “labeled” TSH for membrane preparation of thyrocytes (TBII tests). Sensitivity of available tests utilizing porcine cell membranes was around 80%. New test (TRAK Dyno human, BRAHMS) utilizes human recombinant TSH receptor and human standard material that are supposed to improve performance of the test. We have compared results of these two assays. Sensitivity of TRAK Assay tested in 356 patients with untreated Graves’ disease was 85% and 97.5% for TRAK Dyno human in 111 newly diagnosed patients. Both tests were performed from the same serum specimen for 60 of all investigated patients. TRAK Assay was positive in 50 patients (83.2%) and TRAK Dyno human in 59 patients (98.3%). Specificity of the new radio receptor assay was also improved.

Key words: Radioreceptor assay, human receptor, TSH receptor antibody, Graves’ disease

INTRODUCTION

Graves’ disease is immunogenic form of hyperthyroidism which main pathogenic agent is antibody to TSH receptor (TSH-R Ab, TRAb) on thyrocytes\(^1\). The most practical way for detection of these autoantibodies is radioreceptor assay (RRA). This test is based on binding competition of TSH-R antibodies and \(^{125}\)I labeled TSH for membrane preparation of thyrocytes (TBII tests)\(^1,2,3\). The most convenient RRA utilizes membrane preparation of solubilized porcine thyrocytes and standardized preparations with thyrostimulatory activity \(^4\) as non-radioactive standards. Sensitivity of available tests was around 80% \(^5,6,7\).

New test (TRAK Dyno human, BRAHMS) utilizes human recombinant TSH receptor, human standard material and solid phase (coated tubes) separation procedure, that is supposed to improve performance of test \(^8,9,10,11,12\).

Aim of this study was to compare results of these two assays in patients with immunogenic hyperthyroidism (Graves’ disease) for the assessment of diagnostic sensitivity of the new generation RRA (TRAK Dyno human).
METHODS

Materials:

b) TRAK Dyno human (BRAHMS, Germany). Normal (negative) value under 2 U/l.

Diagnostic protocol for Graves’ disease:
Clinical: hypermetabolic state, ophtalmopathy (negative finding is not exclusive); Biochemical: elevated thyroid hormones (after 1995 “free hormones”) and maximaly suppressed TSH (ultrasensitive DELFIA);
Scintigraphic: diffuse enlarged thyroid with homogenous distribution of tracer and elevated uptake of $^{131}$I or $^{99m}$Tc pertechnetate.

Patients:


130 patients: 118 at various stages of Graves’ disease (60 non treated- subgroup I, including in group A and group B; 38 in the course of medicament treatment, 13 in stable remission after medicament Th; 7 after $^{131}$I Th) and 12 with non-immunologic hyperthyroidism. Testing period from 2000 to 2002. Tested by TRAK-assay and TRAK Dyno human (in the same serum specimen).

20 patients (including in group C) with not-expected immunological (TRAb) activity (11 in long term stable remission of Graves’ disease; 6 with non-immunogenic hyperthyroidism; 3 with pain-less thyroiditis). Tested by TRAK-assay and TRAK Dyno human (in the same serum specimen).

The groups were homogenous for age and sex structure. Age from 7 to 78 yrs (median 46). Sex: female to male ratio 7:1.

RESULTS

Clinical diagnostic sensitivity (true positive findings in untreated patients with Graves’ disease) of TRAK-assay (tested in 356 patients with untreated Graves’ disease was 85% and for TRAK Dyno human (tested in 111 corresponding patients) 97% respectively (Graph 1, section A).

For 60 patients with untreated patients with Graves’ disease (subgroup I of group C), both tests were performed from the same serum specimen with similar results: TRAK assay was positive in 50 patients (83%) and TRAK Dyno human in 59 patients (98.3%) (graph 1, section B).
Četrto poglavlje: Autoimunitet i štitasta žlezda

Graph 1  
A. Incidence of TSH-R Ab positive findings in patients with untreated Graves’ disease  
B. Incidence of TSH-R Ab positive findings in patients with untreated Graves’ disease – TRAK-assay and TRAK Dyno human from the same serum specimen

Correlation between these two tests, in 130 patients (Group C) was very good, $r=0.88$ (graph 2, section A). Similar result was also obtained for subgroup I C (untreated patients with Graves’ disease) (graph 2 section B).

Graph 2  
A. Correlation between TRAK-assay and TRAK Dyno human findings in 130 patients with hyperthyroidism (group C)  
B. Correlation between TRAK-assay and TRAK Dyno human results in 60 untreated patients with Graves’ disease.

Principal difference between two tests is significantly higher Thyrotropin Binding Inhibition effect in the TRAK Dyno human test. (graph 3).

DISCUSSION

It is generally accepted that autoantibodies to TSH-R have pathogenetic role in Graves’ disease. Conversely, only about 85% of untreated patients with this disease have positive findings of TSH-R antibodies. The principal reason for this divergence is (species) unspecific model of TRAb testing: porcine thyrocytes as source of TSH receptors preparation, bovine labeled TSH, standards (unknown origin?)with thyrostimulatory activity, versus human TSH-R antibodies as tested materials. It is possible that some technical procedures, like polyethylenglycol (PEG) separation, contribute to these unsatisfactory sensitivity. In addition, in some reports with high percent of TRAb negative findings, Graves’ disease may have not been correctly diagnosed. Some authors consider that patients with TRAb negative “diffuse toxic goiter” have disseminated thyroid autonomy.

New test (TRAK Dyno human) uses recombinant human TSH receptor (transfected on leukemia cell line K562) as receptor preparation. This receptors material is affinity immobilized by monoclonal antibody BA8 on coated tubes, which improves separation procedure and eliminates invariable PEG. “Standards” are also of human origin. Thyrotropin (¹²⁵I) binding inhibition (TBI) which is the base of this test is significantly higher in this new test than in conventional (porcine) assay (graph 3). The result of this improved TBI is better clinical diagnostic sensitivity: about 98% versus 85% in porcine assay (graph 1). Our findings support some recent reports (10,11, 14-19).

Specificity of the new test is also improved (14-19). In this study, in group D consisting of 20 patients (where positive findings are not expected) - assay was positive in 6 patients (32%) - (“false positive”?) and TRAK Dyno human was “true negative” in all. Nevertheless, testing for specificity requires further study: healthy persons, more non-immunogenic hyperthyroidism, other autoimmune thyroid diseases, non-thyroid illnesses etc.

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