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Comparison of the Performance of Second (Fast TSH) and Third (HYPERsensitive TSH) Generation Automated TSH Immunoassays in Healthy Euthyroid Subjects

ORIGINAL ARTICLE

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ABSTRACT

Objective: We aimed to compare the performances of HYPERsensitive and Fast thyroid-stimulating hormone (TSH) methods in euthyroid subjects.

Materials and Methods: The study included 500 euthyroid subjects. We measured the TSH levels of study subjects using HYPERsensitive and Fast TSH methods. We compared the performances of the two methods. Moreover, free triiodothyronine and thyroxine, anti-thyroglobulin, and anti-thyroperoxidase levels were determined in study subjects.

Results: Mean serum TSH levels were determined as 1.76±1.06 and 1.85±1.12 µIU/mL using Fast and HYPERsensitive TSH methods, respectively. Differences were statistically significant ($p<0.05$). We found a positive correlation between Fast and HYPERsensitive TSH methods ($r=0.960$, $p<0.001$).

Conclusion: Although the sensitivity and precision of HYPERsensitive TSH method is better than that of Fast TSH method, the Fast TSH method needs lower sample volume, and the reaction time is shorter than that in HYPERsensitive TSH method. Thus, clinical biochemistry laboratories should select an appropriate method according to their requirements.

Keywords: Fast TSH, HYPERsensitive TSH, method comparison, generation

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INTRODUCTION

Thyroid disorders are common endocrine disorders affecting almost every organ system. Early detection of thyroid disorders is difficult because the initial signs of the disorders may be nonspecific (1–3). Changes in thyroid hormone concentration lead to significant alterations in blood thyroid-stimulating hormone (TSH) concentration (4). Therefore, TSH measurement is considered to be the primary test for detecting thyroid disorders (5).

Standard methods for measuring TSH are the sensitive immunoassays that can be used as screening tests to assess thyroid function (6). Immunoassays are classified based on the lowest detection limit of serum or plasma TSH levels from the first to fourth generations. The lowest detection limits of the first, which are not used nowadays, and second generation TSH kits were 1–2 µIU/mL and 0.1–0.2 µIU/mL, respectively. The lowest detection limits of the third and fourth generation TSH kits were 0.01–0.02 µIU/mL and 0.001–0.002 µIU/mL, respectively (7–9). Both third and fourth generation (ultrasensitive) TSH kits can be used as thyroid function screening tests in routine clinical laboratories (10).

Beckman Coulter Diagnostics uses two immunoassay methods to measure human TSH (hTSH) levels. These two immunoassay methods are third generation, also called HYPERsensitive hTSH method, and second generation, also called Fast hTSH method. In the present study we aimed to evaluate the performances of HYPERsensitive and Fast hTSH methods.

MATERIALS and METHODS

Participants and Specimens

The study was prospectively conducted at the Departments of Biochemistry and Endocrinology in the Ankara Numune Training and Research Hospital, Turkey. Five hundred euthyroid subjects who had no thyroid disorder and other known chronic diseases, including diabetes mellitus, malignancy, and kidney failure, were included in the study. No subject was <18 years old. Blood samples were collected from all participants into red top tubes (Becton Dickinson, UK) after an overnight fast. Before centrifugation, serum samples were allowed to clot. Subsequently, all samples were centrifuged at 3500 rpm for 15 min. Thereafter, serum from all samples was aliquoted and immediately analyzed. Hemolytic, lipemic, and icteric samples were excluded. All the participants were informed about the study. The study was approved by the local ethics committee in accordance with the Declaration of Helsinki (1028/2015).

Biochemical Analyses

Serum TSH levels of study subjects were measured using Fast and HYPERsensitive hTSH methods based on a paramagnetic particle chemiluminescent immunoassay. The serum levels of anti-thyroid peroxidase (anti-TPO), free triiodothyronine (fT3), free thyroxine (fT4), and anti-thyroglobulin (anti-TG) concentrations were determined for all participants. Measurements of these parameters were performed using DXI 800 (Beckman Coulter Inc., Brea, CA, USA). The reference value for both hTSH methods was 0.34–5.6 µIU/mL. The differences between Fast and HYPERsensitive TSH kits are given in Table 1.

The imprecision of Fast and HYPERsensitive hTSH methods was expressed as the coefficient of variation (CV) for within-run and between-days. With this aim, two levels of hTSH control materials (Bio-Rad Lypochek) were assayed 10 times consecutively in a single day to determine within-run CV and 20 times on consecutive days to determine between-days' CV for these methods (11).

Statistical Analyses

The conformity of continuous variables to normal distribution was tested using the Kolmogorov–Smirnov test, and normal distribution of the groups was observed. The paired t-test was used to determine whether there was a statistically significant difference between the groups with normal distribution. Descriptive statistics of continuous variables were expressed as mean±SD. Pearson correlation test was used to determine whether there was a correlation between the normally distributed groups. Bias and 95% limit values were obtained using the Bland–Altman analysis. The two hTSH methods were compared using the Passing–Bablok regression analysis. Systematic and proportional errors were considered based on the confidence intervals (CIs) of the estimated regression coefficients. A systematic error was considered to be present if the CI of the constant excluded 0, whereas a proportional error was considered to be present if the CI of the slope excluded 1. Analyses were conducted using the R 3.3.0 (www.r-project.org) software. A p-value <0.05 was considered statistically significant.

RESULTS

The mean serum TSH levels of 500 euthyroid subjects were determined as 1.76±1.06 (90% CI, 1.67–1.85) and 1.85±1.12 (90% CI, 1.75–1.95) µIU/mL using Fast and HYPERsensitive hTSH methods, respectively. There was a significant difference between the two methods ($p<0.05$). As a result of linear regression analysis, the r^2 value of hTSH was found to be 0.923, and the relation equation between the two methods was calculated as y=1.0183x – 0.0573 (y: HYPERsensitive hTSH and x: Fast hTSH) (Figure 1). In the Passing–Bablok regression analysis for comparing HYPERsensitive and Fast hTSH methods, the equation was calculated as hTSH (Fast)=0.026+0.943x hTSH (HYPERsensitive) (Figure 2). The constant value for the analysis was 0.943 (95% CI, 0.924–0.960), whereas the slope was 0.026 (95% CI, 0.001–0.053). In the Passing–Bablok regression analysis, β0 prediction CI did not include 0, and β1 prediction CI did not include 1. Therefore, we observed both constant and relative systematic errors. A strong positive correlation was found between the results of the two methods (r=0.960, p<0.001). According to the Bland–Altman graph, bias was found to be 0.1 (95% limit of agreement between –0.5 and 0.7) (Figure 3).

Table 1. Comparison of analytical characteristics of hTSH methods using DXI 800 autoanalyzer

Analytical parameter	Fast hTSH	HYPERsensitive hTSH
Standard	WHO 2nd IRP 80/558	
Method	Paramagnetic particle chemiluminescence sandwich immunoassay	
Generation	2nd	3rd
Sample volume	55	110
Result time, min	20	45
AMR, µIU/mL	0.030–100	0.015–100

AMR: Analytical measurement range; WHO: World Health Organization; (ref. 33820)

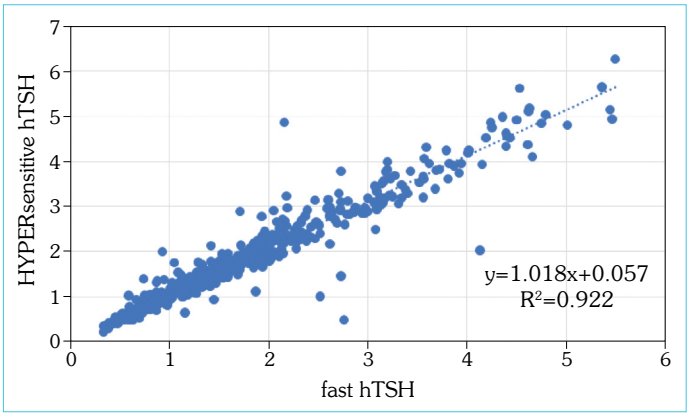

Figure 1. Linear regression analysis results for Fast and HYPERsensitive hTSH methods

Regression equation: y=1.0183x+0.0573 r^2=0.93 (y: HYPERsensitive hTSH and x: Fast hTSH)

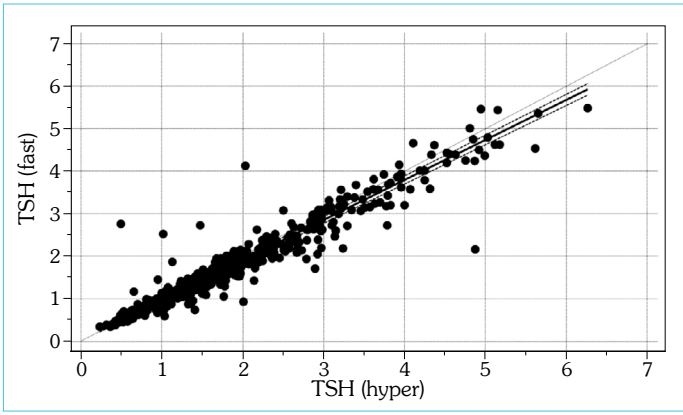

Figure 2. The Passing–Bablok analysis results for Fast and HYPERsensitive hTSH methods

The Passing–Bablok regression equation: (y=0.026 [95% CI, 0.001–0.053] + 0.943 [95% CI, 0.924–0.960]x) (y: Fast hTSH and x: HYPERsensitive hTSH)

Data obtained from control levels 1 and 2 were evaluated, in which we found that CVs for within-run and between-days for HYPERsensitive hTSH method were lower than those for Fast hTSH method (Table 2). Demographic characteristics and thyroid function test (fT3, fT4, anti-TPO, and anti-TG) values are shown in Table 3.

Table 2. Within-run and between-days' variation between hTSH measurements using DXI 800 autoanalyzer

TSH	Within-run	Between-days
Method	**% CV**	**% CV**
Control 1		
Fast hTSH	3.90	3.13
HYPERsensitive hTSH	2.95	3.03
Control 2		
Fast hTSH	3.51	4.08
HYPERsensitive hTSH	2.47	3.12

CV: Coefficient of variation

Table 3. Demographic characteristics and laboratory test values

Test	Mean±SD (90% CI)
Age, years	46.45±14.34
fT3, pg/mL	3.09±0.36
fT4, ng/mL	0.83±0.12
Anti-TPO, IU/mL	1.54±2.01
Anti-TG, IU/mL	1.12±0.97

SD: Standard deviation; CI: Confidence interval

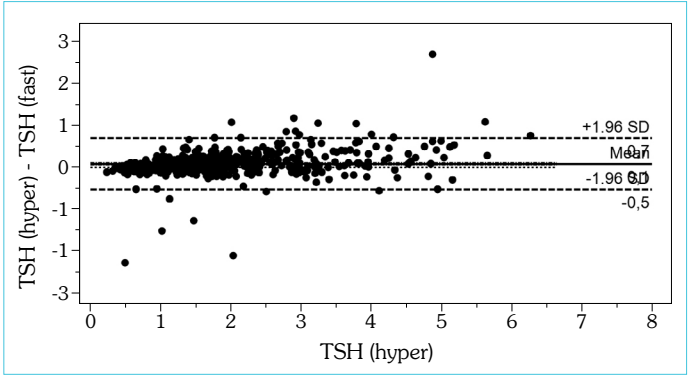

Figure 3. The Bland–Altman analysis results for Fast and HYPERsensitive hTSH methods

Bias=0.1 (95% limit of agreement between –0.5 and 0.7)

DISCUSSION

Various tests, such as fT3, fT4, and TSH, are used to evaluate thyroid functions in laboratories; among these, the most frequently used test is TSH measurement (4, 12).

TSH acts on the thyroid gland and affects T3 and T4 syntheses from the thyroglobulin. TSH secreted from the pituitary gland is regulated by negative feedback. There is a reverse log–linear relationship between serum thyroid hormones and TSH. Small changes in serum thyroid hormones lead to large changes in TSH. Therefore, TSH is important for the evaluation and follow-up of thyroid functions (4). Particularly, recent guidelines recommend that TSH should be used as the first-line test for detecting thyroid hormone-related disorders in patients who have stable thyroid status and intact hypothalamic–pituitary function (2, 3, 13). The analytical performance of TSH immunoassay tests has been gradually developed in recent years, particularly with regard to analytical sensitivity and reproducibility (14).

In our study, when the results of Fast and HYPERsensitive hTSH methods were evaluated, it was observed that HYPERsensitive hTSH method was better in within-run and between-days' reproducibility than Fast hTSH method. Our results were similar to the results of the study by Witherspoon (15). For each of the two methods, % CV values provided by the manufacturer were found to be in accordance with our results. In the study by Gabriella et al., third generation TSH was found to have % CVs close to the manufacturer's data, which was similar to our results (16). Regression analysis results showed a good correlation between Fast and HYPERsensitive hTSH methods. Different measurement methods can be used for different antibodies to measure hTSH; therefore, different results can be obtained depending on the method used. Although our study had two hTSH measurement methods working in the same manner, there was a significant difference between the two methods, and a bias of 0.1 was observed. It is believed that despite constant and proportional systematic errors, the reason for narrow CI and excluded coefficients could be related with large sample size.

The TSH level of 500 euthyroid subjects measured using Fast hTSH method was within the reference range; however, when it was measured for the same patients using HYPERsensitive hTSH method, it was observed that the results of 4 subjects were exceeded the reference range. The results showed that the use of TSH in the diagnosis of subclinical hypo- and hyperthyroidism may lead to different diagnoses of patients according to the method used. Franklyn et al. identified hTSHs in the third generation, which could not be detected in the second generation, similar to our results (17). It is important to take this situation into consideration when evaluating the results of related kits for patients' safety.

In conclusion, sensitivity and reproducibility were better in the HYPERsensitive hTSH method than that in the Fast hTSH method. However, the time required for obtaining results was shorter in Fast hTSH method than that in HYPERsensitive hTSH method. Moreover, the required sample volume was lower in Fast hTSH method than that in HYPERsensitive hTSH method. Therefore, clinical biochemistry laboratories should choose a method considering time, cost, laboratory conditions, number of patients per day, and the characteristics of the methods.

Ethics Committee Approval: Approved by the local ethics committee (1028/2015).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: EÇ. Performed the experiments or case: FS, DB. Analyzed the data: EÇ, HOD. Wrote the paper: EÇ, HOD, TT. All authors have read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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