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Assay Advancements: Exploring Varied Assays for Thyroid-Stimulating Hormone Measurement in Subclinical Hypothyroidism Diagnosis in Riyadh, Saudi Arabia

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Abstract

Thyroid-stimulating hormone (TSH) assay is considered a decisive diagnostic method for thyroid disorders. The lack of different TSH immunoassay standardization often leads to misdiagnosis of subclinical hypothyroidism (SCH). This study aims to differentiate between two analytical platforms for TSH measurement, namely Abbott Laboratories and Snibe Diagnostics. This study employed 173 thyroid profile samples to detect SCH. Initially, the sample was run on Abbott and then by Snibe immunoassay. Statistical analysis showed a significant difference of 0.87 mIU/L ($P < 0.001$) between the TSH values of the two assays. The proposed study corroborates with previous literature by manifesting notable differences in the TSH measurements of two different assays. The findings revealed some insights regarding the differences between the two assays, which conclude that the diagnosis of SCH requires vigilance because of inadequate assay standardization. Thus, future research involving larger cohorts with standardization efforts is imperative to improve the reliability of TSH measurements using different analytical platforms.

Keywords

Immunoassays, Subclinical Hypothyroidism, Thyroid Stimulating Hormone

1. Introduction

Subclinical hypothyroidism (SCH) is a prevalent medical condition that faces challenges in diagnosis all across the globe [1].

The findings of Al Eidan (2018) revealed that the prevalence of subclinical hypothyroidism is around 10.3% in Riyadh, Saudi Arabia [2].

Most recent publications propound SCH as a medical condition with elevated TSH level > 4.0 mU/I and a normal range of free thyroxin (FT4) serum level [3]. In SCH, the pathophysiology behind elevated TSH levels is explained as follows: when the thyroid gland fails to produce sufficient amounts of T4 to meet the body's requirements, the pituitary gland compensates by increasing TSH levels. This rise in TSH aims to stimulate the thyroid gland to work harder and produce more T4.

There are risk factors that can increase the chance to develop SCH like age and gender. It is more common in older populations. SCH is becoming prevalent especially among women as 15% of women above the age of 60 years suffer from SCH whereas only 8% of men suffer from this condition [4]. There are several other conditions that can cause SCH, certain medications as lithium or amiodarone, can impact thyroid function and potentially lead to subclinical hypothyroidism [5].

Nutritional deficiency has an association with SCH. Thyroid hormone synthesis require iodine, iron, selenium, zinc and vitamin A. A 3-month observational study found that after changing the diet, the TSH levels normalized for the pediatric cohort [6]. Although most of SCH cases are asymptomatic [7], recent literature has revealed that untreated SCH condition is associated with numerous health issues including Atherosclerotic cardiovascular disease, hypertension, metabolic syndrome, obstetric issues and many other complications [8]. However, due to technological limitations and high screening costs, no fixed criteria have yet been established regarding the SCH diagnosis [9].

Different recent guidelines manifest varying standards of SCH diagnosis, for instance, China's guidelines on the treatment and diagnosis of SCH characterize it as a medical condition with elevated levels of TSH and normal concentration of serum total thyroxin (TT4) and serum FT4 along with no clinical symptoms [10]. Literature clearly reveals that TSH level interpretation is affected by numerous factors, like age and sex, but the effects of different laboratory assays have not yet been discovered [11]. The variations in the reference ranges of TSH and serum FT4 levels have been examined previously and are well interpreted in literature, but the assay-related differences in the diagnosis and treatment of SCH remain insubstantial [12]. Identifying the variations between different TSH assays will eventually enhance the quality of clinical management and diminish the risk of misdiagnosis in the SCH treatment.

Immunoassays, are widely used methods in clinical laboratories for diagnosing and monitoring various diseases. In spite of that, they have some limitations. One of the common limitations is low specificity secondary to cross-reactivity, where antibodies bind to unintended antigens [13]. The advances in immunoassays have decreased the interference issues, but not totally eliminated. Also endogenous interferents from some patient sample cannot be ignored. Other Immunoassays limitation is variation in the sensitivity levels and detection limits, which may not be appropriate for detecting very low concentrations of biomarkers. This can affect

the accuracy of early disease diagnosis and the monitoring of rare biomarkers.

Since 1960s, the development of the pioneer thyroid assay is evaluating thyroid function using TSH concentration [14]. However, the ancient methods used to evaluate TSH concentration had low precision, poor sensitivity, limited high detection and the cross-reaction with other hormones like Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) or Human chorionic gonadotropin (hCG) [15]. Later in mid-1980s, the second-generation immunoassay has an upgraded system by incorporating the use of two antibodiesthat provided more specificity. Shortly afterwards, a third generation of thyroid assays was matured with more precision and better sensitivity [16]. Currently, the measurement of TSH levels is sufficient to the diagnose SCH [17]. The International Federation of Clinical Chemistry (C-STFT) has devoted over a decade to standardize thyroid function tests and minimize the variations in different testing methods [18]. After dedicated efforts, C-STFT was able to develop reference method measurement for FT4 using equilibrium dialysis isotope dilution-liquid tandem mass spectrometry [19]. Despite this furtherance, integrating the technique of mass spectrometry to evaluate FT4 level has certain drawbacks when compared to the automated immunoassays approach. These drawbacks encompass laborious manual processing, delayed results, and excessive costs. As a result, at present the immunoassays approach is preferred over mass spectrometry techniques in clinical laboratories [20]. Moreover, C-STFT preferred to integrate immunoassays statistically because the reference measurement technique for TSH evaluation remains unprocurable subsequently [21]. In UK, 75% of clinical laboratories employ Roche diagnostics and Abbott laboratories for producing TSH and FT4 immunoassays [22]. While in Saudi Arabia the most practical platforms for that include Abbott laboratories, Roche diagnostics and Snibe diagnostics. Although there is limited data on these assays' variations, Abbott laboratories and Roche diagnostics variation have been presented distinctly for SCH tests in a UK publication [23] [24], whereas the variations between Abbott laboratories and Snibe diagnostics have not been identified yet and need to be indicated to provide a clear picture on how different assays impact the diagnosis and treatment of SCH. Hence this study is aimed to compare the variations in the assays of Abbott laboratories and Snibe diagnostics specifically to aid accurate diagnosis and clinical management for subclinical hypothyroidism.

2. Methods and Material

2.1. Instrumentation and Sample Handling

This study is a prospective cross-sectional study conducted at two large medical centres located in Riyadh, Saudi Arabia over a period of 22 days in January 2023. The samples were collected from 173 patients from primary health care clinics who had laboratory order for a thyroid profile including TSH and T4. The demographic data including patient's age, sex, presence of other medical conditions was obtained from patient's medical record then the inclusion and exclusion criteria were applied. Any patient less than 18 years old or pregnant was excluded. Moreover,

patients having thyroid disorder, on levothyroxine or other medications that affect thyroid hormones like (Lithium, Metformin, Amiodarone, antiepileptics), having kidney diseases, been treated with radiotherapy, known to have nephrotic syndrome or tumours were excluded from the study. The confidentiality of patients was maintained by ensuring that the name, personal ID or medical record number of patients was not revealed during the testing period. Initially the samples were collected from one medical centre that had incorporated Abbott laboratory automation. The patient's serum then pooled on a plain tube and later deep freezed at a temperature of −80˚C. Following the WHO guidelines safety measurements, the samples were transported from the first medical centre to the second medical centre. Initially, the samples tubes were enclosed by primary and secondary second watertight, leak proof containers. Then the package placed on the transporter box that was sealed and filled with iced gel bags. The stability and storage of the samples were sustained during the transportation according to the reagents kit manufacture instruction. For instance, TSH and T4 samples remain stable up to 7 days at 2˚C - 8˚C and for longer period at −80˚C. In the second medical centre, the samples were re-run on Snibe diagnostics automation to evaluate TSH. Both of the laboratories involved in the study were CEBAHI accredited. The values were collected from the laboratory's information system. The results obtained from the assays in two laboratories were interpreted and compared using SPSS software to identify potential differences between Abbott and Snibe assays. According to the American Thyroid Association (ATA), and the American Association of Clinical Endocrinologists (AACE) guidelines for subclinical hypothyroidism, the data collection criteria set as follows: subclinical hypothyroidism definition; TSH level of >4.6 mU/L and <10 mU/L for grade 1 and TSH level of ≥ 10 mU/L for grade 2 subclinical hypothyroidism, with normal T4 which is (12 - 22 pmol/L) [25]-[27].

2.1.1. Abbott Total Automation

The first laboratory integrated the use of Abbott total automation assay using Alinity i analyzer for evaluating TSH. Alinity i TSH assay is basically a two-step immunoassay to determine the presence of TSH in the sample using Chemiluminescent Microparticle Immunoassay technology with flexible assay protocols, referred to as Chemiflex.

2.1.2 Snib Diagnostic

The second assay that was used for re-testing the TSH sample was Snibe using MAGLUMI 800 analyzer. It is solid phase, two-site immunometric assay, performed using two different monoclonal anti-TSH antibodies. The assay calibrators are traceable to the WHO third International Reference Preparation 81/565. During the study period on the 2 analysers, the quality control was within acceptable level and external quality assurance performance was also satisfactory.

2.1.3. Power Analysis and Sample Size

The estimation of the sample size was determined using PASS® software version 11.0.10 [1983-2011.NCSS, LLC]. By considering the prevalence rate of 10.3% of SCH

in primary healthcare visitors (2), a sample size of 173 achieves 80% power to detect a difference (P_1 - P_0) of 0.007 using a two-sided binomial test. The target significance level is 0.05. The actual significance level achieved by this test is 0.032. These results assume that the population proportion under the null hypothesis is 0.103.

2.1.4. Statistical Analysis Procedure

All categorical variables such as gender, chronic disease, and SCH classification are presented as frequency and percentage values. Continuous variables such as age, TSH, and FT4 are expressed as median [IQR]. The Kolmogorov-Smirnov test was used to confirm the assumption of normal distribution. If the data was biased, a nonparametric test was used. Pearson chi-square/Fisher's exact test was used to determine significant associations between categorical variables, depending on whether the cell was expected to have an expected frequency of less than 5. Wilcoxon-Signed Rank test was employed to determine the median significant differences between TSH Abbott and Snibe assays. A two-sided p-value of less than 0.05 was considered statistically significant. All data was entered and analysed using the SPSS 25 Statistics Package (SPSS Inc., Chicago, Illinois, USA).

3. Result

A total of 173 patients were recruited in the study among which 49 were male (28.3%) and 124 were female (71.1%), with a mean age 50.12 years. All of these patients were under gone thyroid profile laboratory test and submitted their samples along with history of chronic disease like diabetes mellitus, dyslipidemia or hypertension. By using Abbott assays 167 patients (96.5%) were classified with grade 1 subclinical hypothyroidism and 6 patients (3.5%) had subclinical hypothyroidism grade 2 based on the definition of subclinical hypothyroidism *i.e.* TSH level of >4.6 mU/L and <10 for grade 1 and TSH level of ≥10 mU/L for grade 2 subclinical hypothyroidism, with normal T4 (12 - 22 pmol/L). Similarly, as per Snibe assays 22 patients (12.7%) were classified as normal, 138 patients (79.8%) had grade 1 subclinical hypothyroidism and 13 patients (7.5%) were classified with grade 2 subclinical hypothyroidism respectively. The average (median) value of TSH Abbott assays was 5.99 mIU/L, FT4 average (median) value was 10.91 pmol/L whereas the average (median) value of TSH using Snibe assays was 6.86 mIU/L and FT4 average (median) value 13.25 pmol/L respectively. All the information is depicted in Table 1.

Table 1. Demographic and clinical characteristics of patients (n = 173).

Variables	Description	Number (n%)
Gender	Male	49 (28.3%)
	Female	124 (71.7%)
Age	Mean+/−Standard Deviation	50.12 ± 18.12
Chronic diseases	Anxiety disorder	1 (0.6%)
	Atrial fibrillation	2 (1.2%)

Continued

Chronic diseases	Coronary artery diseases	4 (2.3%)
	Dilated cardiomyopathy	2 (1.2%)
	Dyslipidemia	27 (15.6%)
	Diabetes mellitus	31 (17.9%)
	Heart failure	1 (0.6%)
	Hypertension	22 (12.7%)
	Lumber neuritis	1 (0.6%)
	Major depression	2 (1.2%)
	Neuropathy	1 (0.6%)
	Osteoarthritis	2 (1.2%)
	Polycystic ovarian syndrome	1 (0.6%)
	Tetralogy of Fallot	1 (0.6%)
	Vitamin D deficiency	1 (0.6%)
Subclinical hypothyroidism Abbott Assays	Grade 1 (TSH 4.5 - <10 mlU/L)	167 (96.5)
	Grade 2 (TSH > 10 mlU/L)	6 (3.5%)
Subclinical hypothyroidism Snibe Assays	Grade 1 (TSH < 4.5 mlU/L)	22 (12.7%)
	Grade 1 (TSH 4.5 - <10 mlU/L)	138 (79.8%)
	Grade 2 (TSH > 10 mlU/L)	13 (7.5%)
TSH mlIU/L (Abbott)	Median [IQR]	5.99 [6.8 - 5.41]
FT4 pmol/L (Abbott)	Median [IQR]	10.91 [11.71 - 10.21]
TSH mlIU/L (Snibe)	Median [IQR]	6.86 [8.23 - 5.56]
FT4 pmol/L (Snibe)	Median [IQR] 22 (12.7%)	13.25 [14.14 - 12.25]

A paired analysis was performed to identify the differences between two assays platforms Abbott and Snibe pertaining to TSH value. Therefore, Wilcoxon Signed Rank test was used to detect the 0.87 mIU/L unit statistically significant difference ($P < 0.001$) between two assays as shown in Table 2. Similarly, cumulative frequency distribution was generated to observe the variations between two assays. In general, the assays TSH Abbott and Snibe are closely similar to each other. However, as per the observation, the minimum value of TSH Abbott assay was observed 5.00 mIU/L whereas TSH Snibe assay had a value of 2.56 mIU/L. Moreover, the maximum value of TSH Abbott assay was observed 13.00 mIU/L while TSH Snibe assay had a maximum of 18 mIU/L. Besides this, the mean value of TSH Abbott assay was observed to be 6.36 mIU/L while TSH Snibe assay's mean was 7.20 mIU/L respectively. Figure 1 illustrates the cumulative frequency distribution of the two assays under consideration.

Table 2. Paired comparison between TSH Abbott and TSH Snibe.

Description	TSH Abbott	TSH Snibe	Abs [M. D]	P-value
Median [IQR]	5.99 [6.8 - 5.41]	6.86 [8.23 - 5.56]	0.87	*<0.001

Note: Continuous data expressed as Median [IQR: Interquartile Range]; M. D: median absolute difference* shows that the P-value is significant at $P < 0.05$.

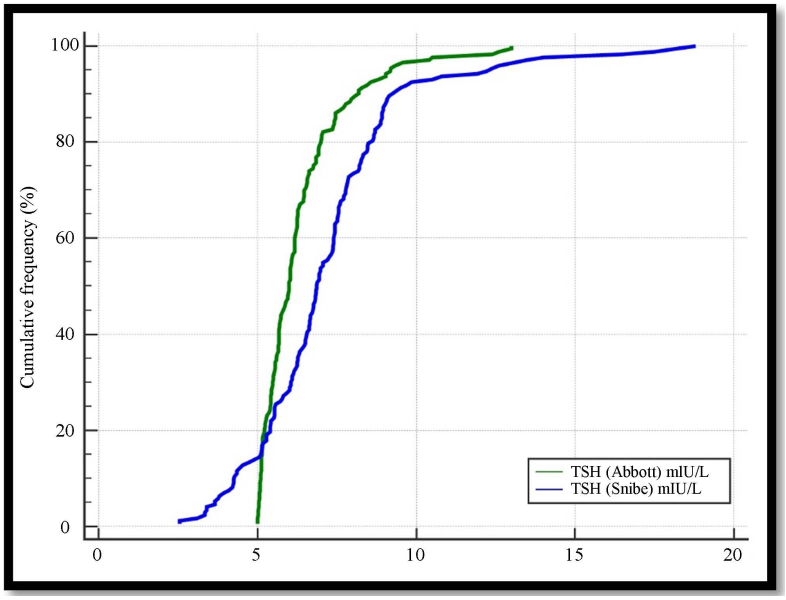

Figure 1. Cumulative frequency distribution of TSH Abbott & Snibe.

Crosstab analysis shows that 22 patients (100%) diagnosed with TSH Snibe normal range were upgraded in Abbot assay with grade 1 TSH, and 138 patients (100%) with TSH grade 1 in Snibe assays were diagnosed as same TSH grade 1 in Abbott assays too. Furthermore, 7 patients (53.8%) of TSH grade 2 in Snibe assay were downgraded in Abbot assay with TSH grade 1. Lastly, 6 patients (46.2%) with TSH grade 2 in Snibe assay had similar result with Abbot assay *i.e.*, TSH grade 2 respectively. These variations in the two assays diagnosis are presented in Table 3.

Table 3. Comparison of upgraded and downgraded ranges between TSH Snibe Assay and Abbott Assays.

Variable	Description	TSH Snibe			P-Value
		Normal TSH (<4.6)	TSH Grade 1 (>4.6 - <10)	TSH Grade 2 (≥10)	
TSH Abbott	TSH Grade1 (>4.6 - <10)	22 (100%)	138 (100%)	7 (53.8%)	<0.001
	TSH Grade 2 (≥10)	0	0	6 (46.2%)	

Note: Categorical data presented as frequency (%); *shows that the P-value is significant at $P < 0.05$.

4. Discussion

The results obtained in the study analysis meticulously compared the outputs of TSH Abbot and TSH Snibe diagnostics. The findings of the study revealed some pivotal insights regarding the performance and differences between the two assays. The study conducted Wilcoxon Signed-Rank test that identified the significant variations between the TSH values obtained from Abbot and Snibe analytical platforms. A statistically significant difference of 0.87 mIU/L ($P < 0.001$) was observed between the two assays; Abbot and Snibe which indicated that there is a measurable difference in the TSH value from the two respective assay platforms. The cumulative frequency distribution of the two assays showed some analogy, but also identified significant differences in the minimum and maximum values of TSH. For instance, the minimum TSH value for Abbot was 5.00 mIU/L but the minimum TSH for Snibe was 2.56 mIU/L. Similarly, the maximum TSH value for Abbott assay was 13.00 mIU/L whereas Snibe has a maximum value of 18 mIU/L. These variations in the TSH levels clearly indicate that the results of thyroid profile from two different assays may not provide homogeneous results for the same patients when their samples are tested in two different laboratories. Using the crosstab analysis, it was easier to identify how patients can be classified in terms of TSH grade, grade 1 or grade 2, for both the Abbott and Snibe assays. The results of the analysis revealed that patients classified as normal in Snibe assay were upgraded to TSH grade 1 using Abbott assay. Contrarily, patients classified with TSH grade 1 in Abbott assay remained congruous in Snibe assay too. In some cases, patients with TSH grade 2 in Snibe assay were downgraded to TSH grade 1 in Abbott assays which manifests discrepancy between the two assay profiles. The findings of this study are consistent with literature that has previously delineated differences in TSH measurements when employing different assay platforms. Moreover, discrepancies in the reported cut-off values of TSH serum in different studies have further sparked the controversies regarding the clinical significance and diagnosis of SCH [28]. Bearing in mind the differences in the measurements of TSH and thyroxin levels while testing subclinical hypothyroidism, it would be fair to say that SCH is a laboratory-based disorder. A publication of the Royal Wolverhampton NHS Trust declared that the diagnosis and clinical management of SCH is phenomenally different when comparing TSH assays from Abbott or Roche analytical platform. The authors also proclaimed that the variations between assays from different manufacturers and the variability in the reference ranges have a significant impact on the diagnosis and treatment of subclinical hypothyroidism [29]. Another thyroid relating publication on Endocrinology Advisors revealed that the diagnostic results for SCH using Roche TSH assay were considerably higher than the results from Abbott assays [23]. The study also proposed that laboratory technicians and clinicians must be aware that variations in reference ranges and the differences between assays directly impact the clinical management of subclinical hypothyroidism. The paramount publication in the field of thyroid assays and their notable differences is the one conducted in UK, comparing

Roche diagnostics and Abbott laboratories, and it is a major reference for all other relevant literature findings [23]. The findings of Kalaria *et al.* reflected that during the analysis, 53 patients were diagnosed with subclinical hypothyroidism on Roche assay but when analysed with Abbott assay, 40 of these patients (75.5%) were characterized with normal thyroid function and only 13 patients (24.5%) had SCH [22]. This clearly depicts how the diagnosis of SCH is different when using varied assays; in this case Abbott laboratories and Roche diagnostics. According to literature, SCH is a laboratory-based disorder and its prevalence worldwide is in the range 1% - 10% [30]. The ubiquity of SCH in Saudi Arabia alone is 10.3% which calls for the attention of researchers and medical specialists to identify different assays and standardize reference values in order to improve SCH patient management in the region [2].

The most common assays used in Saudi Arabia for thyroid profile tests are Roche diagnostics, Snibe diagnostics and Abbott laboratories. Roche and Abbott assays have been critically examined in the past studies and they are considered efficient methods when comparing their performance [23]. However, the evaluation of Snibe assay and its comparison to other assays remains obscure and it is high time for scientists to figure out the discrepancies in the TSH assays and how they impact the diagnosis and treatment of SCH in Saudi Arabia. Thus, the clinical assessment of Snibe compared to other platforms is mandatory [31]. Additionally, the findings of this study suggested that Snibe diagnostic platform shows a potential performance in terms of diagnostic accuracy and the identification of subclinical hypothyroidism cases and non-cases. The difference in reference cut-off values resulting from multiple assays might lead to either higher or lower readings of TSH and FT4 serum levels triggering the overestimation or underestimation of measurements and eventually leading to misdiagnosis of SCH. The overestimation of SCH provokes the patient's anxiety, results in an unnecessary expense on the test requests and costs of regular follow-up visits, and involves undue treatment procedure [32]. On the contrary, the underestimation of the SCH condition would delay the identification and hamper the treatment of symptomatic individuals, ultimately escalating the risks of other severe medical conditions associated with SCH like cardiovascular diseases, or some psychiatric disorders as depression [33] [34]. However, it is crucial for clinicians to observe differences between different assays while measuring TSH value, select suitable platform depending on the clinical settings, and interpret results accordingly. The study also highlights that medical specialists, laboratory technicians and clinicians must be very careful when interpreting TSH results as the patient's management and diagnosis decisions rely on the measurement of TSH values. Hence there is an exigency to standardize TSH assays in all laboratories and clinics in order to minimize potential disparities and ensure consistent patient care.

5. Limitations and Future Recommendations

It is worth acknowledging that this study has certain limitations. Firs, the sample

size in the study was relatively small. The study must also be conducted over a longer duration to enhance the reliability of the gathered data. Second, the study design didn't investigate the impact of assay calibration and lot variability on the discrepancies between Abbot and Snibe assays. Controlling these factors in future research studies would further ameliorate the accuracy of the comparison between Abbott and Snibe assays.

6. Conclusion

The proposed study provides significant corroboration of the differences between TSH outputs of Abbot and Snibe assays. The statistically significant differences evaluated using numerous statistical tests identified the variations in the TSH measurements of patients and the high risk of patients being misdiagnosed. This study thus emphasizes on the importance of using standards and calibration when using various assays for thyroid tests. Therefore, clinicians and medical specialists must remain vigilant regarding the potential differences between assays and must diagnose thyroid disorders accordingly to ensure consistency. Future researchers must conduct validation studies in order to generalize these research findings employing a diverse population and using different clinical settings.

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There is no personal interest of the researcher in this study objective. Collected data from laboratory reports was anonymously analysed and reported solely in aggregate form. Thus, confidentiality and anonymity were maintained throughout the study as the participant's identity was not revealed in any stage of the research.

Conflicts of Interest

The authors declare no conflicts of interest involving this publication.

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