The Diagnostic Utility of Third Generation TSH Electro-Chemiluminescence Immunoassay in Detecting the Incidence of Thyroid Dysfunctions in Saudi Arabia

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Abstract

Objective: To demonstrate how to get use of the highly sensitive third generation electrochemiluminescence immunoassay (ECLIA) in determining the incidence of various thyroid dysfunctions and making proper differentiation for these different thyroid disorders in KSA.

Setting: The Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Taibah University, Outpatient Clinics and the Laboratory Section in Ohud Hospital at Almadinah Almunawarah, KSA.

Subjects: Two groups were involved: Group I included 117 subjects of young volunteers (20-30 years) from Taibah University (TUV) as an apparently healthy group with no specific thyroid symptoms and group II represented by all attendants to outpatient clinics in Ohud Hospital at the same collection period (569 Subjects).

Methods: All subjects were subjected to measurement of serum TSH and free thyroid hormones by ECLIA. The main 2 groups were subdivided on biochemical basis according to their TSH and Thyroid Hormones (TH) levels.

Results: By the use of this immunoassay a percentage of 5.1% for subclinical hypothyroidism among TUV (group I) was reported. A higher percentage of 21.1% for subclinical hypothyroidism in group II was reported (group IIc). The difference between these two percentages was highly significant (p<0.0001). Percentages of 4% for overt hypothyroidism (group IId) and 7.2% for those with high TSH and border line thyroid hormones (group IIb) were also recorded among group II. The sum of percentages of cases with high TSH in group II (32.3%) was highly significantly increased when compared with TUV group (p<0.0001). Percentages of 2% for subclinical hyperthyroidism and 0.7% for overt hyperthyroidism in group II were reported, but no cases of hyperthyroidism (subclinical- or overt) were recorded in TUV group. Also, hypothyroidism was found to be the main thyroid dysfunction when compared to hyperthyroidism in group II (32.3% versus 2.7%) and also in group I (5.1% versus 0%). The mean values for TSH in all subgroups included in group II showed highly significant differences when compared with the normal control group (group Ia). The mean values for thyroid hormones (FT4 & FT3) showed significant differences in some but not all subgroups in group II when compared with normal control.

Conclusion: The third generation TSH-ECLIA immunoassay is the single sensitive first-line test for accurate screening and early diagnosis of thyroid dysfunctions in clinical laboratory. From the 2 studied Saudian population samples, the test proves that increasing the age increases the susceptibility of having a thyroid diseases especially hypothyroidism. The age period of 20 to 30 years could be the period that regular screening for subclinical hypothyroidism should be done in order to face a warning silent pandemic of hypothyroidism and prevent its major complications.

Key Words: TSH – Electrochemiluminescence Immunoassay – Third generation immunoassay – Subclinical thyroid diseases.

Introduction

The whole system of thyroid pituitary hypothalamic axis is like a thermostat. When the body senses that there is no enough thyroid hormone, the hypothalamus releases Thyrotropin Releasing Hormone that causes the pituitary to secrete Thyroid Stimulating Hormone (TSH) that stimulate the thyroid gland to make and release thyroid hormones. When there is too much thyroid hormone the releasing hormones are turned off and the thyroid stops producing thyroid hormones [1]. The major function of thyroid hormones is their control of the basal metabolic rate and calorigenesis. Thyroid hormones are known to (a) Stimulate neural development and normal growth, (b) Promote sexual maturation, (c) Stimulate adrenergic activity with increased heart rate and myocardial contractility, (d) Stimulate protein synthesis and carbohydrate metabolism, (e) Increase the synthesis and degradation of cholesterol and triglycerides, (f)
Increase the requirement for vitamins, (g) Increase the calcium and phosphorus metabolism, and (h) Enhance the sensitivity of adrenergic receptors to catecholamines. These effects are typically magnified in patients with either an overactive thyroid gland, such as in hyperthyroidism or reduced in patients with a sluggish thyroid function such as in hypothyroidism [2,3].

Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein having a molecular weight of approx. 30000 Daltons and consisting of two subunits. The β-subunit carries the TSH-specific immunological and biological information, whereas α-chain carries species-specific information and has an identical amino acid sequence to α-chains of LH, FSH and hCG. TSH is formed in specific basophile cells of the anterior pituitary. The hypothalamic release of TSH is the central regulating mechanism for the biological action of thyroid hormones. TSH has a stimulating action in all stages of thyroid hormone formation and secretion; it also has a proliferative effect [4]. The thyroid hormone thyroxin (T4) is physiologically a part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The major fraction of the total thyroxin is bound to transport proteins (TBG, prealbumin, and albumin). The free thyroxin (FT4) is the physiologically active thyroxin component [4-6].

Diseases of the thyroid gland are among the most abundant disorders worldwide second to diabetes. About 95% of the time hypothyroidism is the result of malfunction of the thyroid gland itself (primary hypothyroidism). Causes of primary hypothyroidism can be either, congenital or acquired. The most common occurs in iodine deficient countries, a daily iodine intake below 25 μg particularly in preterm infants, is a more frequent cause of hypothyroidism accounting for many cases in Europe, Asia and Africa. Hypothyroidism is usually due to autoimmune thyroiditis and thyroid auto-antibodies are classified into three main categories, antibodies against microsomal components including; anti-microsomal antibodies (the thyroid peroxidase TPO), related to the production of thyroid hormones and other group of antibodies against TSH receptors. They included TSH stimulating antibodies and TSH binding inhibitory antibodies. The third group includes antithyroglobulin antibodies [7]. There are several causes of hyperthyroidism. The most common include immunologic conditions (Graves’ disease and thyrotoxicosis), toxic thyroid nodules (adenomas), and toxic multinodular goiter [1].

The term subclinical hypothyroidism is used for patients who have mildly increased levels of serum TSH but normal thyroid hormones levels [8]. The term Subclinical hyperthyroidism is defined as a persistently suppressed serum TSH with normal thyroxin and triiodothyronine in patients who do not have symptoms. Subclinical hyperthyroidism can be caused by the same thyroid disorders that result in clinical hyperthyroidism [9]. While signs and symptoms of overt hyper and hypothyroidism are well known, sub clinical thyroid conditions have subtle clinical manifestations and may mimic other diseases. Hence it is important to develop rational laboratory strategies to differentiate the various conditions to guide the physician towards correct diagnosis and treatment [10].

The determination of TSH serves as the initial test in thyroid diagnostics. Even very slight changes in the concentrations of the free thyroid hormones bring about much greater opposite changes in the TSH level. Accordingly, TSH is a very sensitive and specific parameter for assessing thyroid function and is particularly suitable for early detection or exclusion of disorders in the central regulating circuit between the hypothalamus, pituitary and thyroid [11-14]. The determination of free thyroxin is an important element in clinical routine diagnostics. Free T4 is measured together with TSH when thyroid function disorders are suspected. The determination of FT4 is also suitable for monitoring thyro-suppressive therapy. The determination of FT4 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins. Thus, additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. A variety of methods are available for estimating the free thyroid hormone levels. The direct measurement of FT4 and FT3 via equilibrium dialysis or ultra filtration is mainly used as a reference method for standardizing the immunological procedures generally used for routine diagnostic purposes, but those methods are tedious and could not be used as routine tests [4-6].

Determination of TSH by sensitive electrochemiluminescence immunoassay "ECLIA" is currently judged as the most sensitive and also most cost-effective first-line approach to thyroid function testing. Measuring TSH concentrations by third generation assay turned out to be advantageous in the follow-up of many clinical situations, (a) In patients with mildly suppressed but well detectable TSH concentrations due to functional thyroid autonomy (0.03-0.3mU/l), also, overt hyperthyroidism can be excluded by third generation TSH
measurement alone without the need of additional thyroid hormone measurements; (b) In patients receiving long term suppressive T4 treatment after thyroidectomy for differentiated thyroid cancer, measurement of basal TSH by third generation assays allows accurate monitoring of hormone therapy without the need for TRH testing; (c) In most patients with severe non-thyroidal illnesses and decreased TSH levels, TSH concentrations measured by third generation assays are only moderately suppressed and could be clearly discriminated from undetectable levels in overt hypothyroidism. Thus, the use of third generation TSH immunoassays is recommended in specialized clinical laboratories frequently analyzing samples taken in one of those clinical situations [15].

Electrochemiluminescence is one of the best of these immunoassays and it differs from chemiluminescence and bio-luminescence in that the reactive species that produce the chemiluminescent reaction are electrochemically generated from stable precursors that are at the surface of an electrode. Electrochemiluminescence process has been demonstrated for many different molecules by several different mechanisms, including an oxidation-reduction reaction with tris ruthenium and tripropylamine. The chemiluminescence precursors are stable and relatively small and can be used to label haptns or large molecules like TSH. Multiple labels can be coupled to proteins or oligonucleotides. Their advantages include improved regent stability, simple regent preparation, and enhanced sensitivity. With its use, detection limits of 200 f mol/L could be achieved. It could be used as either in competitive or sandwich immunoassays. With the ruthenium label, various assays have been developed in a flow cell, with magnetic beads as the solid phase. Beads are captured at the electrode surface, and unbound label is wasted from the cell by a wash buffer. Label bound to the bead undergoes electrochemiluminescent reaction, and the light emission is measured by an adjacent photo-multiplier tube [16].

The aim of this work was to demonstrate how to get use of this highly sensitive third generation electrochemiluminescence immunoassay in determining the incidence of various thyroid dysfunctions and making early proper differentiation for these different thyroid disorders in Almadinah Almunawarah, KSA.

**Subjects and Methods**

The current study included two groups of Saudi subjects, group (I) Included 117 volunteer female subjects from Taibah University at Almadinah Almunawarah, KSA, they were having no specific symptoms of any thyroid disorders (their ages ranged from 20 to 30 years {an important inclusion criteria}), and group (II) Included 569 untreated subjects from all attendants at the outpatient clinics of Ohud hospital at Almadinah Almunawarah, KSA in the same period of collection (month 3 and 4 -1432), their ages ranged from 18 to 80 years and females represented 83% of this group (males were 17%). The two groups were subjected to laboratory tests that included highly sensitive serum TSH, FT4 and FT3 measurements.

Five ml of venous blood was extracted from every subject and blood samples were allowed to clot at room temperature in vacutainer tubes with serum separator. The tubes were centrifuged and serum was separated and freezed at -20°C till assayed. The TSH and thyroid hormones were estimated using a highly sensitive electrochemiluminescence immunoassay method (ECLIA) on ELECSYS 2010 immunoassay analyzer from Roche. The reference ranges for the three hormones were primarily established as in the literature. These are 0.27-4.2 g IU/ml (m IU/L), 12-22 p mol/L and 3.1-6.8 p mol/L for TSH, FT4 and FT3 respectively [16].

The two groups were subdivided biochemically according to TSH and thyroid hormones (TH) levels into subgroups. Group I was divided into group Ia that included 111 subjects with completely normal TSH and TH, this group was considered as the normal control for the whole studied groups and group Ib that included 6 subjects with elevated serum TSH level and normal TH. Group II was also subdivided biochemically into 6 subgroups, group IIa included those with normal TSH and normal TH, group IIb with high TSH and border line TH, group IIc with elevated serum TSH level and normal TH, group II d with elevated serum TSH level and low TH, group IIe with low serum TSH level and normal TH and group II f with low TSH level and high TH.

**Test principle and steps of serum TSH automated electrochemiluminescence immunoassay method:**

This electrochemiluminescence TSH immunoassay employs monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium complex consist of a chimeric construct from human and mouse-specific components. As a result, interfering effect due to human anti-mouse antibodies are largely eliminated. It is an immunoassay with sandwich principle. The total
duration of assay is 18 minutes. In the first incubation; 50 µl sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex. In the second incubation; after addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substance is then removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode [16].

Test principle and steps of serum FT4 & FT3 automated electrochemiluminescence immunoassay method:

In this electrochemiluminescence assay, the determination of free hormone is made with the aid of a specific anti-T4 (or T3) antibody labeled with a ruthenium complex. The quantity of antibody used is so small that the equilibrium between bound and free T4 (or T3) remains virtually unaffected. It is an immunoassay with competition principle. Total duration of assay is 18 minutes. In the first incubation, 15 µl sample and T4 (or T3) specific antibody labeled with a ruthenium complex are mixed. In the second incubation, after addition of biotinylated T4 (or T3) and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode [16].

Statistical methods:

The SPSS ® (Statistical Package for Social Sciences version 11) was used for data management and analysis and the Microsoft Excel for charts construction. Quantitative data were presented as mean±SD. For comparison of the means, the Student’s t-test was used. The Chi-square test was used for comparison of percentages. All tests were two tailed and considered significant when p<0.01 and highly significant when p<0.0001.

Results

All the results of the current study are shown in the following figures and tables. The pie charts (Figs. 1-3) represent the percentage of various thyroid disorders diagnosed on biochemical basis in both groups; Fig. (1) for group I (TUV) and fig. (2) for percentages of different TSH levels (normal, high & low) in group II which represents hospital outpatient clinics attendants. Fig. (3) for all subgroups in group II. Table (1) shows the comparison of mean values for TSH and thyroid hormones in subclinical hypothyroid TUV versus normal TUV subjects. Table (2) shows the comparison of mean values for TSH and thyroid hormones in attendants to hospital laboratory in relation to normal TUV group. Table (3) shows the comparison of percentages of cases of subclinical hypothyroidism in TUV group with other subgroups in group II that also has either the same subclinical hypothyroid disorder, all hypothyroidism (subclinical- or overt) or all subjects with increased TSH level regardless the level of thyroid hormones. Table (4) shows comparison of percentages of cases with high TSH versus cases with low TSH in the main 2 groups. Figs. (4&5) show the comparison of mean values of serum TSH, FT4 and FT3 in all studied groups in relation to normal control respectively.
Table (1): Comparison of mean values for TSH and thyroid hormones in subclinical hypothyroid TUV & normal TUV

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal TUV (N = 111)</th>
<th>TUV with Subclinical Hypothyroidism (N = 6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH X±SD (m IU/L)</td>
<td>1.53±0.77</td>
<td>5.59±1.51**</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FT4 X±SD (p mol/L)</td>
<td>14.18±1.44</td>
<td>13.51±2.24</td>
<td>0.67</td>
</tr>
<tr>
<td>FT3 X±SD (p mol/L)</td>
<td>5.3±0.43</td>
<td>4.95±1.38</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table (2): Comparison of Mean Values for TSH and Thyroid Hormones in Attendants to Hospital Laboratory in Relation to Normal TUV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ia</th>
<th>Ib</th>
<th>Ic</th>
<th>Id</th>
<th>Ie</th>
<th>If</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal TSH+ Normal TH</td>
<td>High TSH+ Normal TH</td>
<td>High TSH+ Normal TH (Subclinical Hypothyroidism)</td>
<td>High TSH+ Low TH (Hyperthyroidism)</td>
<td>Low TSH+ Normal TH (Subclinical Hypothyroidism)</td>
<td>Low TSH+ High TH (Hyperthyroidism)</td>
</tr>
<tr>
<td>N</td>
<td>370 (65%)</td>
<td>41 (7.2%)</td>
<td>120 (21.1%)</td>
<td>23 (4%)</td>
<td>11 (2%)</td>
<td>4 (0.7%)</td>
</tr>
<tr>
<td>TSH X±SD (m IU/L)</td>
<td>2.34±1.06**</td>
<td>8.09±6.53**</td>
<td>8.67±6.67**</td>
<td>44.43±30.02**</td>
<td>0.062±0.046**</td>
<td>0.06±0.04**</td>
</tr>
<tr>
<td>FT4 X±SD (p mol/L)</td>
<td>14.1±2.36</td>
<td>10.92±0.62**</td>
<td>14.11±2.02</td>
<td>8.76±3.36**</td>
<td>17.2±2.7**</td>
<td>24.4±1.33**</td>
</tr>
<tr>
<td>FT3 X±SD (p mol/L)</td>
<td>4.81±0.85**</td>
<td>4.39±0.64**</td>
<td>4.77±0.86**</td>
<td>3.90±1.45**</td>
<td>4.84±0.95**</td>
<td>5.2±0.92</td>
</tr>
</tbody>
</table>

Table (3): Comparison of Percentages of Cases of Subclinical Hypothyroidism in TUV group with other related groups in group II.

<table>
<thead>
<tr>
<th>% Of cases in TUV (group Ib)</th>
<th>% Of cases in TUV (group Ib)</th>
<th>% Of cases in Groups Ic &amp; Id</th>
<th>% Of cases in groups</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclinical- Hypothyroidism</td>
<td>Subclinical- Hypothyroidism</td>
<td>Total Hypothyroidism (subclinical &amp; overt)</td>
<td>Total cases with high TSH within group II</td>
<td></td>
</tr>
<tr>
<td>within group I</td>
<td>within group I</td>
<td>within group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1%</td>
<td>21.1%</td>
<td>25.1%**</td>
<td>32.3%**</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table (4): Comparison of percentages of cases with high TSH with cases with low TSH in the main 2 groups.

<table>
<thead>
<tr>
<th>% of cases with high TSH in group I</th>
<th>% of cases with low TSH in group I</th>
<th>p value</th>
<th>% of cases with high TSH in group II</th>
<th>% of cases with low TSH in group II</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1%*</td>
<td>0%</td>
<td>*&lt;0.05</td>
<td>32.3%**</td>
<td>2.7%</td>
<td>**&lt;0.0001</td>
</tr>
</tbody>
</table>
The Diagnostic Utility of Third Generation TSH Electro-Chemiluminescence Immunoassay

Discussion

It is well known that the most useful test for assessing thyroid function is the measurement of serum TSH. Measuring TSH with sufficient sensitivity to distinguish low levels from normal levels has become the preferred first-line test for accurate diagnosis of thyroid dysfunction. Immunoassay is the method of choice for the measurement of serum TSH in the clinical laboratory. Highly sensitive assays for TSH have become available that employ various detection signals, including chemiluminescence and assays with low end detection limits in the 0.01 to 0.05 mIU/L range [17]. Clinically, these assays are capable of measuring TSH at concentration required to accurately differentiate the low concentration of serum TSH found in patient with hyperthyroidism as represented in our study by 2% with subclinical hyperthyroidism; group IIe in group II (11 out of 569) in addition to 0.7% with overt hyperthyroidism in group IIIf (4 out of 569) from other causes of low TSH. This means that low concentrations of TSH due to hyperthyroidism could be easily differentiated from the suppressed concentration found in patient with non-thyroidal illnesses that can develop because of other conditions not related to primary thyroid disease like hospitalization, some acute illnesses,... etc. Group I in our study as we mentioned before is a group of volunteer people from Taibah University with no history of any thyroid disorders or non thyroidal illnesses and with no symptoms or signs of any of them. Their ages were ranging from 20 to 30 years which means with low susceptibility of developing any thyroid dysfunction especially hyperthyroid states which usually manifest in older ages. In spite of this young age and small sample volume (117), six cases with subclinical hypothyroidism were discovered (5.1%). We also reported percentage of 21.1% of subclinical hypothyroidism among group II subjects.

The prevalence of subclinical hyperthyroidism varied amongst other reports. Prevalence of subclinical hyperthyroidism was reported to be 10% in Whickham Survey [9] and 12% in the Framingham study [18]. The colorado thyroid disease prevalence study involving 25,862 subjects showed a prevalence of 2.1% [19]. This is in agreement of our results for group IIe that showed percentage of 2%. Other investigators studied a representative population of 17,353 people aged 12 and above and found that the prevalence of subclinical hyperthyroidism was only 0.7% [7]. This means that increasing the age, increases the susceptibility of having thyroid diseases.

The prevalence of overt hyperthyroidism in women is between 0.5 and 2%, and is ten times more common in women than in men in other reports. In the Whickham survey, the prevalence of undiagnosed hyperthyroidism was 4.7 per 1000 women (0.47%) [20]. The prevalence data in elderly persons show a wide range between 0.4% to 2.0%
Our results showed a close percentage (0.7%) in group II. A cross-sectional study of 2799 healthy adults aged 70 to 79 years in the US found evidence of hyperthyroidism (defined biochemically as a serum TSH concentration less than 0.1 mU/L, and a serum-free T4 concentration greater than 23 p mol/L) in only five subjects. In Leiden study, only 2 of 558 subjects aged between 85 and 89 years had newly diagnosed overt hyperthyroidism [22,23]. The prevalence of a further 1% of adults was having a history of toxic nodular goiter [24]. In a population sample of 2656 from Copenhagen, newly diagnosed thyrotoxicosis was found in 1.2% of women and no men, and the prevalence of known thyrotoxicosis was 1.4% [25].

Thus our current highly sensitive TSH assay appeared to be very useful in differentiating mild, subclinical hyperthyroidism from overt Graves’ disease, and because of this reason, it could also be used in monitoring thyroid cancer patients on thyroxin, and in monitoring the adequacy of thyroid hormone replacement in hypothyroid patients. Also, if the basal serum TSH levels could be detected by ultrasensitive immunoassay as the electrochemiluminescense immunoassay used in our study, then no diagnostic benefit is gained by performing a TRH stimulation test that is a dynamic test that requires the availability of TRH (which is not commercially available in a lot of countries) and the acceptance of patient to do this dynamic tedious test. This means that the use of this current sensitive TSH immunoassay will save a lot of time and money and also efforts needed to reach the proper and correct diagnosis and only by very cheap easy fully automated accurate and precise immunoassay. Also, by this sensitive immunoassay we could detect an incidence of subclinical hyperthyroidism (group Ib) that was 5.1% in group I subjects belonging to the apparently normal subjects that were taken from Taibah University volunteers. In addition to greater percentages that were shown among group II that included all attendants to hospital outpatient clinics, those subjects included mainly people with some non specific symptoms or signs of thyroid disorder. Those with high serum TSH and low FT4, group IId, (hypothyroid patients) represented 4% of group II in this study and those with high serum TSH and normal FT4, group IIc, (subclinical-hypothyroid patients) represented 21.1% of group II. Also we have an additional group, group Ib that contained those subjects with high serum TSH and border line FT4 in which we recorded a percentage of 7.2%. This means that the total percentage of subjects with high serum TSH levels represented 32.3% in group II which is significantly higher than that recorded in group I (5.1% in TUV in our study), (see Table 3). The greater percentages of those with altered TSH levels among group II in the current study could be attributed to the difference in ages in the two included groups (age range for group I is 20-30 years & for group II is 18-80 years). This means that the reported percentages for subclinical hypothyroidism ranging from 5.1% to 2.1% that varies according to age, sex and clinical presentation. The comparison between these two percentages showed highly significant difference ($p<0.0001$). This means that the incidence of subclinical hyperthyroidism increases with increasing age and if this condition could be detected early in life, we can minimize the development of overt hypothyroidism with its complications. Also, all subjects with high TSH levels (32.3%) i.e. those with hyperfunction represented majority in thyroid dysfunctions found in group II when compared with those with low TSH levels (hyperfunction) and we recorded a percentage of 2.7% for them and the difference was highly significant ($p<0.0001$).

Other investigators reported close figures. The percentage of subjects with a high serum TSH concentration was higher for women than men in each decade of age, and ranged from 4 to 21% in women and 3 to 16% in men. An increase in serum TSH concentrations was also found in men in the NHANES III study. In the same study serum TSH concentrations increased with age in both men and women and were higher in whites than blacks, independent of serum antithyroid antibody concentrations [7]. Whickham survey showed 8% of women (10% of women over 55 years of age) and 3% of men had subclinical hypothyroidism [20]. In the Colorado USA study, 9.4% of the subjects had a high serum TSH concentration, of which 9.0% had subclinical hypothyroidism [19]. Others reported that approximately 10% of subjects over 60 years were having serum TSH values above the normal range [26,27].

Little number of researches were done in KSA, but a percentage of subclinical hypothyroidism (35%) was reported by authors in Jeddah with 61% of them having thyroid antibodies [28]. In Makkah, other investigator reported 50.1% of cases with hypothyroidism and 49.9% of cases with hyperthyroidism out of all studied cases having various thyroid diseases but not among general population [29]. Overt and subclinical hypothyroidism were reported in 9.3% and 14.9%, respectively, for pregnant women with significantly higher maternal age and with increased risk of pregnancy-induced hypertension, gestational diabetes mellitus and
delivery by cesarean section in a research that was
done at Almadinah Almounawarah [30].

The clinical significance of identifying those
with subclinical hypothyroidism and adjusting
thresholds for treatment remain some-what unclear
and are a continued topic of investigation. The
great value of early discovery of those cases is for
its potential benefits and risks of therapy for those
with subclinical hypothyroidism that have been
debated for two decades. The possible advantages
of treating subclinical hypothyroidism generally
include: Firstly, preventing the progression to overt
hypothyroidism. Secondly, thyroxin therapy may
improve the serum lipid profile and thereby poten-
tially decrease the risk of death from cardiovascular
causes. Finally, treatment may reverse the symp-
toms of mild hypothyroidism, including psychiatric
and cognitive abnormalities [31].

In group II in our study we reported 23% of
subjects with overt hypothyroidism with high TSH
and low thyroid hormones (group IIId), but we did
not find any case among the small sampled group
I (TUV). This may be attributed to the young age
of this group and also the small size of the sample.
This incidence reported by our work (23% in group
II) is higher than those reported by other investi-
gators that we will discuss later and further studies
for larger population size should be done to confirm
this figures. Also, other following studies that
include tests determining the etiology of this ob-
ervation like iodine levels and thyroid auto anti-
bodies should urgently be done in different areas
in KSA. In iodine-replete communities, the preva-
ience of spontaneous hypothyroidism is between
1% and 2%, and it is more common in older women
and ten times more common in women than in
men. In the Whickham survey, the prevalence
of newly diagnosed overt hypothyroidism was 3 per
1000 women [20,21]. The prevalence of previously
diagnosed and treated hypothyroidism was 14 per
1000 women, increasing to 19 per 1000 women
when follow up was possible. The overall preva-
ience in men was less than 1 case per 1000. One
third had been previously treated by surgery or
radioiodine causes of thyrotoxicosis. Excluding iatrogenic
causes, the prevalence of hypothyroidism was 10 per
1000 women, increasing to 15 per 1000. The
mean age at diagnosis was 57 years. Other studies
in Northern Europe, Japan and the USA have found
the prevalence to range between 0.6 and 12 per
1000 women and between 1.3 and 4.0 per 1000 in
men investigated [21]. In the Colorado and
NHANES III studies, the prevalence of newly
diagnosed hypothyroidism was 4 per 1000 and 3
per 1000 respectively [7,19]. In Pescopagano, Italy,
an area of mild iodine deficiency, the prevalence
of newly diagnosed overt hypothyroidism was
0.3% of 573 women (autoimmune thyroiditis con-
irmed as etiology). There were no cases among
419 men, and no subject had been diagnosed and
treated for hypothyroidism [24]. In borderline io-
dine-deficient Copenhagen, Denmark, 6 per 1000
of the women and 2 per 1000 men had overt but
undiagnosed hypothyroidism, and 1% of all subjects
were taking thyroxin [25]. The prevalence was
higher in surveys of the elderly in the community
in other researches [21]. The overall prevalence
of hypothyroidism, including those already taking
T4, in Birmingham, UK, of 1210 subjects aged 60
and over was 4% of women and 0.8% of men aged
over 60 years. In subjects aged 60 years or more
in Framingham, 4% had serum TSH concentration
greater than 10 m U/L, of whom one-third had low
serum T4 concentrations [32]. Overt hypothyroidism
was found in 7% of 558 subjects aged between 85
and 89 years in Leiden, Netherlands [23].

From the current results and results reported
by other authors, we can say that there is a silent
pandemic of hypothyroidism that needs certain
great attention for early diagnosis of subclinical
hypothyroidy individuals. Follow up of levels of
TSH followed by thyroxin supplements if needed
could prevent or at least minimize the appearance
of this pandemic.

In addition, to the previously mentioned great
sensitivity of the current TSH immunoassay, the
calibration of the TSH by ECLIA (Elecsys 2010)
is usually done once per reagent lot and also this
is a great advantage as it reduces analysis time and
also the analyzer automatically calculates results
in m IU/L. The assay is also of good specificity as
it is not affected by jaundiced, hemolysed or lipemic
samples. The measuring range of the current assay
is 0.005-100.0 g IU/ml (m IU/L); the lower detection
limit is 0.005 g IU/ml which is the lowest
TSH level that can be distinguished from zero.
The functional sensitivity of this assay is 0.014 g

Three generations of TSH immunoassays have
been developed over the previous years. These
assays are capable of diagnosing primary hypothyr-
roidism with low thyroid hormones production and
with elevated levels of TSH. Second – generation
TSH immuno assays, with detection limits of 0.1m
U/L, can effectively screen for hyperthyroidism
(better than first generation with detection limit
of 1m U/L). However, the third generation TSH
chemiluminometric assays, with detection limits
of 0.01m U/L, are less likely to give false-negative
results and can more accurately distinguish between euthyroidism and hyperthyroidism. The assay used in our study detected very low TSH levels in group II down to 0.005 and 0.0073 m IU/L (represented by 0.18% for each), this is in addition to values like 0.01 m IU/L (3 samples out of 569 samples represented by 0.53%), 0.033 m IU/L (1 sample represented by 0.18%) and others such as 0.059, 0.076, 0.087, 0.088 and 0.09 m IU/L. Thus, the third generation TSH assays should be routinely used to monitor and adjust thyroid hormone replacement therapy as well as screen for both hyperthyroidism and hypothyroidism [33].

The sensitivity of the third generation TSH assays has lead to the ability to detect subclinical thyroid disease - or a mild degree of thyroid dysfunction due to the large reciprocal change in TSH levels seen for even small change in free T4 [34]. In the group II which represents subjects coming from outpatient clinics with potential probability of having thyroid disease but not discovered yet, and they are supposed to have some specific or important symptoms or signs of thyroid disease, we have found great percentages of most of thyroid disorders. We recorded 120 cases with subclinical hypothyroidism out of 569 subjects (21.1%), 23 cases with overt hypothyroidism (4%), 11 cases with subclinical hyperthyroidism (2%), and 4 cases with overt hyperthyroidism (0.7%).

In subclinical hypothyroidism, the mean TSH (m IU/L) was highly significantly increased in group Ib (5.59±1.51) when compared to normal TUV, group Ia (1.53±0.77), p value was <0.0001. Also there was no significant difference between the group of subclinical hypothyroidism in TUV (group Ib) and normal TUV group (Ia) as regards the levels of FT4 & FT3 (p mol/L). The mean values were 14.18±1.44 & 13.5±2.24 for FT4 in groups Ia & Ib respectively. The mean values were 5.3±0.43 & 4.95±1.38 for FT3 in groups Ia & Ib respectively. When we compared mean values for TSH in TUV normal group (1.53±0.77) and subgroups of hospital attendants (groups Ia, Ib, Ic, IIa, Ib, Ile, II, III), there were highly significant differences between the normal TUV group and hospital groups (values were; 2.34±1.06, 8.09±6.53, 8.67±6.67, 44.43±30.02, 0.062±0.046, and 0.06±0.04 respectively) (p<0.0001). In the same way when we compared mean values for FT4 in TUV normal group (14.18±1.44) and groups including hospital attendants, there were highly significant differences between the normal TUV group and hospital groups except IIa and IIc (values were; 14.1±2.36, 10.92±0.62, 14.11±2.02, 8.76±3.36, 17.20±2.7 & 24.44±1.33 respectively, p<0.0001).

Also, when we compared mean values for FT3 in TUV normal group (5.3±0.43) and groups including hospital attendants, there were highly significant differences between the normal TUV group and all hospital subgroups except II (values were; 4.81±0.85, 4.39±0.64, 4.77±0.86, 3.90±1.45, 4.84±0.95 & 5.2±0.92 respectively p<0.0001 for all groups & =0.008 for group Ic).

In conclusion, the use of highly sensitive third generation immunoassay like ECLIA was greatly useful in reporting a percentage of 5.1% for subclinical hypothyroidism among TUV which is a homogenous group of young female volunteers without any specific thyroid symptoms (group Ib). Also a percentage of 21.1% for subclinical hyperthyroidism in larger heterogeneous group (group Ic in group II). The difference between these two percentages in the 2 groups was highly significant (p<0.0001). We also reported percentages of 4% for overt hypothyroidism (group IIb) and 7.2% for those with high TSH and border line thyroid hormones (group IIb). A total percentage of hypothyroidism (subclinical and overt) was 25.1% which was also highly significant different from cases reported in TUV. Also, the sum of percentages of cases with high TSH in group II (32.3%) was highly significant from cases with high TSH in TUV group. Thus, we can suggest that the age period of 20 to 30 years could be the period that we should screen people regularly for subclinical hypothyroidism or at least at ending the stage of secondary school education in order to face this silent pandemic and prevent its major complications. We also reported percentage of 2% for subclinical hyperthyroidism and 0.7% for overt hyperthyroidism in group II, but no cases of hyperthyroidism (subclinical- or overt) in TUV group. Also, hypothyroidism was found to be the main thyroid dysfunction when compared to hyperthyroidism in group II (32.3% versus 2.7%) and also in group I (5.1% versus 0%). The mean values for TSH in all subgroups included in group II showed highly significant differences when compared with the normal control group (group Ia). The mean values for thyroid hormones (FT4 & FT3) showed significant differences in some but not all subgroups in group II when compared with normal control. Thus, this TSH assay is the single sensitive first-line test for accurate early diagnosis and differentiation of thyroid dysfunctions in clinical laboratory. In addition to its high sensitivity, it is also of good specificity and wide measuring rang. It is quit rapid, simple, fully automated method and needs no frequent calibration.
Recommendations:

We recommend performing further studies screening larger population samples in KSA that can reveal differences in incidence of thyroid dysfunction among different age groups and also measuring serum thyroid antibody concentrations in those different groups. Also, the influence of dietary iodine intake on the epidemiology of thyroid dysfunction should be studied in more detailed further studies in order to prevent this silent pandemic by treatment that potentially could decrease the risk of deaths from cardiovascular diseases in severe cases or at least reverse the mild abnormalities in mild cases. Also, we highly recommend a large study for establishing own reference ranges for thyroid hormones in Saudian population.

References


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