Concentration of Thyroid Hormones in Blood Serum by Chemilumiscence Immunoassay

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Abstract: Thyroid gland is located at the base of the neck and release two important hormones T3 and T4. The concentration of triidothyronine (T3), thyroxin (T4) and thyroid hormone varies in males and females. Different factors are involved in which concentration of TSH increases like age, hormonal changes in genders, stress, anxiety and environmental factors etc. Concentration of the thyroid hormones in blood stream can be finding out by many ways. One of the processes in the list is chemiluminiscence immunoassay in which we find the concentration of a macromolecule in solution by using antibody. The analyte for thyroid test is present in the serum. Serum in the sample cup is run in the immulite and then test unit is run. The test unit and the sample of analysis are examined by bar coder. After incubation and substrate addition reaction take place. Photomultiplier tube (PMT) counts the emitted photon. The number of emitted photons is converted into concentration with the help of computer software and the results are then displayed in the form of slope. The values of slope are then analogized with the recorded standardvalues in computer during the entry of kit. In the present study 956 patients were referred to the thyroid lab, checked for the abnormality by CLIA. The percentage of female patients was higher than the male patients.

Key words: Thyroid gland · TSH · T3 · T4

INTRODUCTION

Endocrine system and the nervous system are two main controllers of body’s processes. In endocrine system chemical messengers are involved which are released by endocrine glands and convey the message to the target or effectors cell. Hormones are endocrine messengers [1]. Various hormones are produced by specialized and which perform vital functions throughout the human body. Like growth hormone is involved in growth of cells and tissues of the body.

Thyroid stimulating hormone is responsible for the production of T3 and T4 hormones in thyroid gland.

The largest endocrine gland is the thyroid gland which is located at the base of neck on either side prior to trachea. Two lobes of thyroid gland are coupled together with the help of isthmus. Usually the weight of thyroid gland is approximately 20-40 grams; however, it is variable depending on the body physiology [2].
Goiter is the enlargement of thyroid gland. Hyperthyroidism as well as hypothyroidism may result in goiter called toxic goiter and non-toxic goiter respectively. In hypothyroidism, although the size of gland is increased but the production of hormones is decreased [1].

MATERIALS AND METHODS

The laboratory of Institute of Radiotherapy and Nuclear Medicine (IRNUM) Peshawar provided the facility to carry out this research. IRNUM facilitates the mankind by providing the diagnosis as well as treatment using advanced technologies. The concentration of thyroid hormones is determined by Chemiluminescence Assay (CLIA) in the fully equipped laboratory of IRNUM. CLIA is a type of immunological and biochemical assay that finds the concentration of macromolecules in the blood by using antigen-antibody reaction. An analyte is the macromolecule going to be detected.

**Principle:** The principle of CLIA is to localize antigens in blood by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker. During this assay, an antibody binds to the epitope of an antigen and a signal is produced.

The signal produced is measureable because of detectable labels. They either give off radiations, a color change is produced in a solution, fluoresce is produced or they can be persuade to release light [13].

The concentration of thyroid hormones is measured by using an instrument called immulite which is also used to measure other body hormones as well.

**Sample Collection:** 4-5 cc (cubic centimeter) blood samples are taken with the help of a disposable sterile syringe of five cubic centimeters. Blood is poured into the small test tube after discarding the needle of syringe. All the needed information like patient name, sex and district, type of test etc are noted on the test tube.

**Sample Preparation:** Blood clot coagulates when sample is permitted to rest for five to seven minutes. After blood clotting, centrifugation is done in a sterile test tube. For this purpose, the centrifuge machine was turned on 1500-2000 rpm and centrifugation was done for 5 minutes approximately. The serum was collected from the supernatant and pellet was discarded. All the informations regarding the patient are recorded including test to be performed. Sample cup was moved into the immulite.

**Assay Procedure:**
- Kit was entered in computer by means of scanner or by entering the barcodes of kit in computer (manually).
Kit was adjusted via two vials i.e. low and high adjustors of lyophilized free T4 in processed human serum, with preservative. The concentration of adjustors is known.

Each vial is reconstituted by adding 2.0 ml distilled or deionized water.

The lyophilized material was converted into the working form by gradual mixing.

Four test units were run after the low adjustors in the immulite.

Four test units were run after the high adjustor in the immulite.

Kit was adjusted and we get a slope.

Slope was in acceptable range according to master curve.

Sample was taken with the help of pipette in a sample cup and it should be more than the mandatory volume.

Sample cup containing sample was run in immulite and depending on the type of test test units are run after sample cup.

Bar coder read the sample and test unit of test to be held.

With the help of pipette reagent and serum was taken and added them in test unit vial which already contained beads coated by antibody.

Its incubation period was 30 minutes.

Substrate was added electronically.

Reaction took placed.

The powdered form of adjustors was dissolved by continuously shaking until the powdered form of adjustors is completely dissolved.

Slope was obtained having an acceptable range as per master curve.

By means of pipette sample was taken in sample cup.

Principle Procedure for TSH:

TSH is a solid-phase chemiluminiscent immunoassay and incubation period is 90 minutes. For the test of TSH, the mandatory concentration of serum is 75 micro liters and sample cup should contain 10 micro liters.

Assay Procedure:

For the manual entry of kit, barcode of the kit was entered.

Adjusters of both types were made for TSH. In the 4.0 ml of distilled water, the adjusters of known concentrations were reconstituted.

The powdered form of adjustors was dissolved by continuously shaking.

High and low adjusters were moved in the immulite and after adjustors four test units are run respectively.

Slope was obtained having an acceptable range as per master curve.
• The test units were run after the sample cup.
• The sample cup and test units were interpreted by barcoder and saved.
• 75ul sample was taken from the sample cup and reagent from carousel with the help of pipette.
• Sample and reagents were put in the test unit containing labeled beads.
• It is left for 1 hour and 30 minutes (incubation period).
• A substrate was added so that a particular reaction can occur.
• The emitted photons were counted by PMT and then converted into slope by software already installed in the computer.
• At end, the slope was compared with standard slopes.

RESULTS AND DISCUSSION

When a doctor or a medical consultant is not able to diagnose a disease of a patient from the apparent symptoms, he will advise them some laboratory tests. These tests are basically confirmatory tests with the help of which we exactly come to know about the nature of disease. These confirmatory tests help to know about the type of disease and also to treat it.

Thyroid lab of Institute of Radiotherapy and Nuclear Medicine (IRNUM) is fully validated and operational enough to hold the tests of thyroid hormones and others in the series. An immense number of patients suffering thyroid problems are referred by their doctors for thyroid tests at IRNUM. Consultants become more confident over their treatments after the results.

The thyroid patients referred to the lab were from different age groups and from different localities of Khyber Pakhtoonkhwa. Then the results are examined after getting the relationship of thyroid with the gender using different statistical tools. The prescription criteria for hormonal tests by doctor, the rates of recurrence of pathological conditions and the area of the patient from which he belongs were also determined from the data.

Relationship Between Thyroid and Gender: From these observations it is concluded that, thyroid hormones has gender-specific response. The value of TSH concentration is lower in males as compared to females.

With the increase of age, TSH is also increased but the concentration of T3 and T4 is not changed with age. In females, the value of T3 concentration increases between the age of 35-44 and 55-64 years. The value of T4 decreases between the age of 15-24 and 25-34 but then increases with age after 25-34, few exceptions are present. From testing, it is observed that TSH concentration is changed in females only with age but does not change in males. The concentration of T3 decreases in age between 25-64 age groups and again decreases in males of age between 55-64 years. The concentration of T4 decreases in males between the age group of 15-24 and 25-34 while values remain constant over 25-34 age group males [14].

FREQUENCY OF THYROID PROBLEM: Thyroid hormones as well as thyroid stimulating hormones T3 T4 and TSH respectively are actually responsible for thyroid abnormalities. The concentration of thyroid hormones and thyroid thought-provoking hormones are the cause of diseases called thyrotoxicosis (hyperthyroidism) and hypothyroidism. In hyperthyroidism the levels of T3 and T4 are more and the level of TSH is less than the normal range. While in hypothyroidism the levels of T3 and T4 are less than the normal and the level of TSH is higher than the normal range. Total 956 patients with thyroid problems were referred to the thyroid lab at IRNUM for diagnostic tests, 58.78% patients were normal from total patients. The results we obtained showed that for most of the time the values for thyroid hormones are in standard range. A doctor should have known how about the diagnosis of thyroid problems before referring the patient to thyroid laboratory. Due to which the assets of hospitals will be saved and also cure will become available to most of the victims. The highest number of normal female patients of T4 was 218, for TSH was 166 and for was T3 73. The highest number of normal male patients of T4 were 45, for TSH was 45 and for T3 was 15. The percentage of normal male patients of T3 was 68.1%, for T4 70.3% and for TSH was 57.69%. The percentage for the normal female patients for T3 was 63.47%, for T4 was 67.7% and for TSH was 46.76%.

Table 1: Normal concentration of thyroid hormone in blood stream

<table>
<thead>
<tr>
<th>Test Required</th>
<th>Units</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>pg/ml</td>
<td>1.5—4.1</td>
</tr>
<tr>
<td>T4</td>
<td>ng/dl</td>
<td>0.89—1.76</td>
</tr>
<tr>
<td>TSH</td>
<td>µIU/ml</td>
<td>0.4—4.0</td>
</tr>
</tbody>
</table>
Table 2: Data collected from IRNUM showing the number of normal and abnormal patients in both male and females

<table>
<thead>
<tr>
<th>Gender</th>
<th>T3 Normal</th>
<th>T3 Abnormal</th>
<th>T4 Normal</th>
<th>T4 Abnormal</th>
<th>TSH Normal</th>
<th>TSH Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>15</td>
<td>7</td>
<td>45</td>
<td>19</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td>Female</td>
<td>73</td>
<td>42</td>
<td>218</td>
<td>104</td>
<td>166</td>
<td>189</td>
</tr>
</tbody>
</table>

Percentage of normal patient of thyroid hormone

<table>
<thead>
<tr>
<th>Patients</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>68.1%</td>
<td>70.3%</td>
<td>57.69%</td>
</tr>
<tr>
<td>Females</td>
<td>63.7%</td>
<td>67.7%</td>
<td>46.76%</td>
</tr>
</tbody>
</table>

Percentage of abnormal patient of thyroid hormone

<table>
<thead>
<tr>
<th>Patients</th>
<th>T3</th>
<th>T4</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>31.8%</td>
<td>29.68%</td>
<td>42.3%</td>
</tr>
<tr>
<td>Females</td>
<td>36.52%</td>
<td>32.29%</td>
<td>53.23%</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The values of T3, T4 and TSH obtained showed that the concentration of thyroid hormones is more in females rather than the males. The values in the table show that the highest percentage of abnormality for TSH in males was 42.3% while it was 53.23% in females. Abnormal values of T4 for females are 32.29% and for males it was 29.68%. The abnormal percentage of T3 for females is 63.47% and in males it was 31.8%. From these results we can conclude that the irregularity in thyroid hormones is more commonly found in females than males. Depending upon symptoms of disease, age and gender of patient, different tests for thyroid hormones are advised by the doctor, most commonly T4 and TSH. The people from Peshawar and neighboring areas who come to hospital show that they are cultured and take care of their health. They seek advice from their doctor and doctor may recommend diagnostic tests in case of any abnormality.

**REFERENCES**

