Normal Reference Range for Serum TSH, Free T4, Total T4, and Total T3 on Roche® Platforms in Basrah, Iraq

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Background: Thyroid function tests are mandatory in clinical practice because symptoms and signs are not reliable to discriminate between various types of thyroid disease.

Aim: The aim of this study was to determine assay-specific reference range for serum free T4, total T4, total T3 and TSH among healthy non-pregnant adult cohort for Roche® platforms in Basrah (Southern Iraq) from single laboratory in a tertiary center using indirect approach of the available data.

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Methods: A Cross sectional study for non-pregnant adults 19 years and above. Sera were analyzed by using cobs e411 for thyroid functions tests.

Results: Total enrolled persons were 10,078. The 95% reference intervals for TSH were 0.20-6.50 μIU/mL, which increased with age though not linear, for free T4 were 0.8-1.70 ng/dL, for total T4 were 3.78-15.33 μg/dL, and for total T3 were 0.80-2.50 ng/mL.

Conclusion: Cobs e411 (Roche® analytical platform) analyzer reference range for thyroid function cannot be applied for Iraqi population.

Keywords: Thyroid hormones; reference intervals; Roche® analytical platform.

1. INTRODUCTION

Symptoms and signs are not reliable to discriminate various thyroid disease and we have to rely on thyroid function test.

Factors that affect thyroid hormones level are mainly age, smoking, genetic factors, iodine status but not body mass index (BMI) [1-9].

Unfortunately, there are wide range in reference range for thyroid hormone using different analytical platforms, despite they are using the same automated chemiluminescent immunoassays principles [10].

Standardization and harmonization of these assays is ongoing, but still beyond the level to reduce interlaboratory variations.

Some advocates measuring TSH and free T4 to assess thyroid function in healthy population and total T4 and TSH to assess thyroid function in those with hypothyroidism or hyperthyroidism [11].

Establishing country and analytical platforms specific reference range seems important to ensure local own normal values for thyroid hormones [12]. TSH reference interval is needed to avoid over diagnosis of subclinical hypothyroidism in elderly.

Reference interval can be obtained directly from healthy people with a rigid exclusion criterion which is commonly used by kits manufacturers, but unfortunately, they are not local country population which can affect patients’ management especially if we are talking on TSH and Free T4 in the diagnosis of subclinical and follow up of patients with clinical thyroid disease [3].

More easily, is to use the indirect method of obtaining database of large central laboratory to establish normal reference ranges for thyroid function, which is more simple and cheaper.

Trimester specific reference interval for thyroid function in pregnancy are already done in Basrah in 2016 [13].

The objectives of this study were to determine reference range, assay specific for serum free T4, total T4, total T3 and TSH among adult’s healthy non pregnant cohort for Roche® platforms in Basrah (Southern Iraq) from single laboratory in a tertiary center using indirect approach of the available data.

2. METHODS

2.1 Design

A Cross sectional study to assess normal thyroid hormone reference interval in non-pregnant adults 19 years and above. They were apparently healthy and attain the center for routine check-up.

2.2 Setting

Faiha Specialized Diabetes, Endocrine and Metabolism Center (FDEMC), laboratories in Basrah (Southern Iraq). This is tertiary care center.

2.3 Participants

We analyzed all thyroid function tests done since August 2008 up to May 2020 [Fig.1]. Smoking status assessment includes never and ever smoker (former or current). BMI were divided into obese and non-obese.

From each patient 5 ml of venous blood taken for the time 9 am to 1 pm, regardless the meal time. Analyses were done in the same day of blood sampling.
2.4 Exclusion Criteria

The following patients were excluded: Pregnant women, women on oral contraceptive pills, postpartum or abortion within 12 months, a history of hypothyroidism or using L-thyroxin, history of hyperthyroidism or using carbimazole, TSH ≥10 μIU/mL or <0.01 μIU/mL, those with positive any of the thyroid antibodies, patients with diabetes mellitus, history of thyroid surgery, hospitalized patients within previous one month (minimum one day in hospital), patients with pituitary disease or on amiodarone, anticonvulsants, and oral contraceptive pills within three months of use.

2.5 Laboratory Measurements

Sera were analyzed by using cobs e411 (Roche® analytical platform) analyzer automated chemiluminescent immunoassays (Roche Diagnostics, Mannheim, Germany).

The reference range quoted by manufacturer for TSH is 0.27-4.2 μIU/mL, for freeT4 is 0.93-1.7 ng/dL, total T4 is 5.1-14.1 μg/dL, and for total T3 is 0.8-2.0 ng/mL. The intra-assay imprecision of TSH is 0.1–4 μU/mL ( 5% CV) , for FT4 is 0.71-1.5 ng/dL ( 3% CV), for total T4 is 4.6-12.4 μg/dL (3% CV) and for total T3 is 1.6–6.5 ng/mL ( 3% CV).

2.6 Statistical Analysis

All Statistical analysis was done using SPSS version 23 (IBM Inc., Armonk, NY, USA). Regarding TSH, the persons were divided according to age group: 19-30, 31-40,41-50,51-60,61-70,71-80,81-90 and those older than 90 years.

The reference ranges were defined by the 2.5% and 97.5% percentiles of the modeled distribution for each group. Median also calculated. The frequency distribution curves of TSH concentration were calculated.

3. RESULTS

Total enrolled persons were 10,078 (Table-1). Age range 19-100 years. They were 7780
(77.2%) women and 2298 men. Of them 47.8% were obese and 702 (7.0%) smokers. Those above the age of 70 years were only 142 (1.4%) persons.

Table-2 shows TSH reference values in μIU/mL (2.5th percentile, median, and 97.5th percentile) according to age group and gender. Higher TSH was seen among women in this study. In men the TSH range was 0.28-6.14 and in women it was 0.18 -6.60 (p<0.001). There is an increased in upper limit of TSH reference values with age in both genders, though not linear. The 95% reference intervals for TSH were 0.26-6.50 μIU/mL while for kit manufacturer it was 0.27-4.2 μIU/mL. There is increased upper limit of TSH reference values with age in both genders, though not linear.

Free T4 reference values in ng/dL (2.5th percentile, median, and 97.5th percentile) according to age group and gender were presented in Table-3. Higher level of free T4 in men than women. For men the range was 0.80-1.72 and form women 0.80-1.70 (p<0.001). There was no increase in free T4 with age. The 95% reference interval for free T4 was 0.8-1.70 ng/dL, while for kit manufacturer it was 0.93-1.70 ng/dL.

The total T4 reference values in μg/dL (2.5th percentile, median, and 97.5th percentile) according to age group and gender are present in Table -4. Higher level of total T4 was seen in women. For men it was 1.46-13.22 and for women it was 4.13-15.90 (p=0.001). The Median total T4 did not increase, though the upper limit was significantly decreasing with age, which was more prominent in female gender. The 95% reference intervals for total T4 were 3.78-15.33 μg/dL which is widely ranged because of increase upper limit, while for kit manufacturer it was 5.10-14.10 μg/dL.

Total T3 reference values in ng/mL (2.5th percentile, median, and 97.5th percentile) according to age group and gender. Higher level of total T3 seen in women (Table -5). For men it ranged 0.70-2.06 and for women 0.80-2.54 (p=0.002). Both the median and upper limits of T3 decreased with age significantly. The 95% reference intervals for total T3 were 0.80-2.50 ng/mL while for kit manufacturer it was 0.80-2.00 ng/mL.

Normal distribution curve for women and men are present in Fig. 2 (a and b).

97.5th percentile distribution of TSH reference values is present in Fig. 3.

4. DISCUSSION

There are a lot of problems with automated immunoassay platforms of serum free T4, total T4, and total T3 especially with a low level [14]. For that reason, some suggested LC-MSMS thyroid hormone measurement for those hypothyroidism patients with target TSH, free T4, total T4, and total T3 and continued symptoms of hypothyroidism. In this situation using LC-MSMS may show low level of free T4, total T4, and total T3. However, thyroid hormone estimation by LC-MS/MS is expensive, needs a lot of expertise and cumbersome.

This study represented a large sample for age and gender specific reference intervals for thyroid hormones in apparently health population from Basrah (Southern Iraq) with no previous thyroid disease or thyroid autoimmunity.

Table 1. Baseline characteristics

| Variables          | No. (%)       |
|--------------------|---------------|
| Gender             |               |
| Women              | 7780(77.2)    |
| Men                | 2298(22.8)    |
| Age median(range) in years | 36(19-100)    |
| BMI*               |               |
| Mean ±SD           | 30.38±7.54    |
| N=9755             |               |
| Normal             | 2396(24.6)    |
| Over weight        | 2692(27.6)    |
| Obese              | 4667(47.8)    |
| Smoking state      |               |
| Ever smokers (former or current) | 702(7.0)    |
| Non-smokers        | 9376(93)      |
| Total              | 10,078        |

*323 persons were without BMI measurement due to individual causes like wheelchair.
Table 2. TSH reference values in μIU/mL (2.5 percentile, median, and 97.5 percentile) according to age group and gender

| Age Years | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile |
|-----------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|
| 19-30     | 664       | 0.50          | 2.00   | 6.12           | 2832      | 0.40          | 1.94   | 6.70           | 3496      | 0.40          | 1.96   | 6.56           |
| 31-40     | 640       | 0.20          | 1.50   | 5.50           | 2064      | 0.13          | 1.80   | 6.30           | 2704      | 0.17          | 1.70   | 6.16           |
| 41-50     | 544       | 0.21          | 1.50   | 6.34           | 1584      | 0.10          | 1.70   | 6.74           | 2128      | 0.10          | 1.67   | 6.60           |
| 51-60     | 259       | 0.20          | 1.44   | 6.53           | 843       | 0.10          | 1.70   | 6.90           | 1102      | 0.13          | 1.60   | 6.70           |
| 61-70     | 133       | 0.11          | 1.70   | 6.56           | 373       | 0.08          | 1.60   | 7.17           | 506       | 0.09          | 1.60   | 7.03           |
| 71-80     | 48        | 0.50          | 1.69   | 8.13           | 64        | 0.01          | 1.25   | 7.21           | 112       | 0.01          | 1.46   | 7.95           |
| >80       | 10        | 0.05          | 2.62   | 6.8            | 20        | 0.20          | 1.57   | 6.0            | 30        | 0.05          | 2.03   | 6.80           |
| Total No. | 2298      | 0.28          | 1.60   | 6.14           | 7780      | 0.18          | 1.80   | 6.60           | 10078     | 0.20          | 1.80   | 6.50           |

Table 3. Free T4 reference values in ng/dL (2.5 percentile, median, and 97.5 percentile) according to age group and gender

| Age Years | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile |
|-----------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|
| 19-30     | 252       | 0.90          | 1.30   | 1.77           | 1138      | 0.80          | 1.20   | 1.76           | 1390      | 0.80          | 1.20   | 1.74           |
| 31-40     | 255       | 0.80          | 1.22   | 1.89           | 884       | 0.80          | 1.13   | 1.70           | 1139      | 0.80          | 1.20   | 1.70           |
| 41-50     | 200       | 0.80          | 1.20   | 1.80           | 662       | 0.71          | 1.10   | 1.71           | 862       | 0.75          | 1.13   | 1.72           |
| 51-60     | 103       | 0.58          | 1.13   | 1.70           | 381       | 0.71          | 1.10   | 1.68           | 484       | 0.70          | 1.10   | 1.70           |
| 61-70     | 49        | 0.79          | 1.10   | 1.77           | 145       | 0.75          | 1.20   | 1.85           | 194       | 0.78          | 1.16   | 1.80           |
| 71-80     | 27        | 0.70          | 1.20   | 2.00           | 27        | 0.71          | 1.20   | 1.70           | 54        | 0.70          | 1.20   | 1.89           |
| >80       | 6         | 1.10          | 1.35   | 1.70           | 4         | 0.98          | 1.22   | 1.40           | 10        | 0.98          | 1.30   | 1.70           |
| Total No. | 892       | 0.80          | 1.20   | 1.72           | 3241      | 0.80          | 1.15   | 1.70           | 4133      | 0.80          | 1.20   | 1.70           |
### Table 4. Total T4 reference values in μg/dL (2.5 percentile, median, and 97.5 percentile) according to age group and gender

| Age Years | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile |
|-----------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|
| 19-30     | 52        | 0.93          | 8.10   | 12.80          | 250       | 1.42          | 8.90   | 17.45          | 302       | 1.34          | 8.70   | 17.07          |
| 31-40     | 41        | 1.62          | 7.50   | 15.11          | 198       | 4.69          | 8.40   | 15.93          | 239       | 4.20          | 8.40   | 15.31          |
| 41-60     | 52        | 2.48          | 8.20   | 13.94          | 145       | 3.96          | 8.20   | 14.39          | 197       | 4.08          | 8.20   | 14.21          |
| 51-60     | 14        | 1.20          | 9.35   | 10.80          | 64        | 3.59          | 8.36   | 14.68          | 78        | 2.37          | 8.48   | 12.71          |
| 61-70     | 14        | 1.40          | 7.35   | 11.90          | 27        | 5.20          | 7.60   | 13.40          | 41        | 1.59          | 7.60   | 13.33          |
| 71-80     | 9         | 6.00          | 8.35   | 9.67           | 5         | 6.90          | 8.90   | 11.40          | 11        | 6.00          | 8.40   | 11.40          |
| >80       | 2         | 10.85         | 11.28  | 11.70          | 0         | NA            | NA     | NA             | 2         | 10.85         | 11.28  | 11.70          |
| Total No. | 181       | 1.46          | 8.10   | 13.22          | 689       | 4.13          | 8.50   | 15.90          | 870       | 3.78          | 8.40   | 15.33          |

### Table 5. Total T3 reference values in ng/mL (2.5 percentile, median, and 97.5 percentile) according to age group and gender

| Age Years | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile | Total No. | 2.5th centile | Median | 97.5th centile |
|-----------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|-----------|---------------|--------|----------------|
| 19-30     | 64        | 0.51          | 1.30   | 1.81           | 309       | 0.87          | 1.40   | 2.73           | 373       | 0.8           | 1.40   | 2.60           |
| 31-40     | 79        | 0.70          | 1.30   | 2.20           | 255       | 0.80          | 1.31   | 2.64           | 334       | 0.80          | 1.30   | 2.45           |
| 41-50     | 66        | 0.60          | 1.24   | 3.92           | 187       | 0.80          | 1.30   | 2.33           | 253       | 0.80          | 1.30   | 2.46           |
| 51-60     | 30        | 0.70          | 1.28   | 1.90           | 89        | 0.80          | 1.20   | 2.25           | 119       | 0.73          | 1.21   | 2.10           |
| 61-70     | 19        | 0.70          | 1.20   | 1.60           | 49        | 0.64          | 1.20   | 5.33           | 68        | 0.68          | 1.20   | 3.11           |
| 71-80     | 8         | 0.80          | 1.22   | 2.84           | 10        | 0.90          | 1.04   | 1.43           | 18        | 0.80          | 1.09   | 2.84           |
| >80       | 3         | 1.10          | 1.15   | 1.30           | 2         | 0.80          | 1.09   | 1.38           | 5         | 0.80          | 1.15   | 1.38           |
| Total No. | 269       | 0.70          | 1.29   | 2.06           | 901       | 0.80          | 1.30   | 2.54           | 1170      | 0.80          | 1.30   | 2.50           |
The upper limit of TSH increased with age, though not linear, while free T4 and total T4 do not increase with age in our study; on the other hand, the upper limits for both T3 and T4 together with the median level of T3 decreased significantly with age. Furthermore, we found higher TSH, total T4 and total T3 among women than men, while men have higher level of free T4.

As reported earlier by others, we found that median and upper limit of TSH were higher in women [15, 16]. Free T4 remained stable with age; total T4 does not increase with age and total T3 decrease with age. Previous data showed that total T4 remain stable or increase whereas total T3 decrease with age [9]. Except for free T4, all thyroid hormones were higher in women in this study.

Our findings are similar to a Sudanese study [17], where women had significantly higher level of TSH and free T4 but no difference in total T3.TSH was also higher in women in large recent study from France [16].

In one study from UAE published in 2006 [18], among 959 ambulatory persons, reference...
interval not affected by age or gender with TSH 0.30-4.32 mU/L and FreeT4 0.76-1.44 ng/dL, which differs from other countries and manufacturer’s reference intervals using the same platform (Abbott Architect i2000 immunoassay analyzer).

The reference interval for TSH was 0.18-6.60 μIU/mL, which is totally different from kit manufacturer values of 0.27-4.2 μIU/mL. In one smaller study on 337 persons from Saudi Arabia, TSH reference range was similar to kit manufacturer[19].

Study limitation: The majority of our study population were aged 70 year and below. Smaller sample for age above 70 years (1.4%) makes the interpretation of thyroid hormones in elderly in this study not clear and may explain nonlinear increase in upper limit of TSH with age.

Iodine status is unknow in Iraq, which may affect thyroid function and hormone estimation.

5. CONCLUSION

We established new reference range for thyroid hormones in adults. The 95% reference intervals for TSH, FT4, total T4, and total T3 were 0.20-6.50 μIU/mL, 0.80-1.70 ng/dL, 3.78-15.33 μg/dL, and 0.80-2.50 ng/mL respectively in Basrah, Iraq. Thyroid Roche Diagnostics kits normal values are not applicable to Iraqi population. Interpretation of thyroid hormone results should be made in the context of analytical platform type beside other variables, which affect those values like age and gender. Using these new reference values will reclassify a lot of blood samples used for thyroid function tests.

CONSENT

As per international standard or university standard, patient’s written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study protocol approved by ethical committee from FDEMC.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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