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A Study of the Correlation Coefficient for Some Thyroid Hormones and Ferritin Variables in Overweight and Obese People

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Abstract

Subjective This study was conducted for the correlation coefficient for some thyroid Hormones and Ferritin variables in overweight and obese people. ***Objective*** Blood samples were collected from the target persons in the study at the rate of 5 ml from each person. In the period between September 25, 2021 and until November 2, 2021, the number of samples was (50) samples divided into 3 groups if the first group, which was considered a control group, included (10) people who had a body mass index. Ranges between (18.5-24.9), the second group included people with overweight, who had a body mass index between (25.0-29.9), and the third group included people with obesity and had a body mass index of 30.0 or more. ***Results*** Our study results showmen significant decreased $p \leq 0.05$ in the level T3 thyroid hormone in over weight group when compared to control group whereas; no significant differences between obese and overweight groups of T3, T4 levels. Also there was no significant differences between obese group in T4 thyroid hormone level when compared to control. Ferritin level showed significant decreased in both group obese and overweight when compared to control group and there were no significant differences between obese and overweight group. Also our results indicated that were a positive correlation between Ferritin and T3, TSH, and very weak correlation with T4. ***Conclusion*** The study concluded that is Ferritin levels might have association with T3 and TSH levels in obese population. If confirmed by further studies, ferritin and thyroid hormones levels could be used in therapeutic monitoring in obese population

Keywords: obesity, correlation, correlation coefficient, Ferritin, Thyroid hormones

Introduction

Obesity is defined by the World Health Organization (WHO) as an abnormal or excessive fat accumulation that may harm health. (WHO, 2016) The term Obesity is also used to refer to excess body weight than normal weight, which is indicated by body mass index. (Body Mass Index (BMI), which is calculated by weight in kilograms divided by the square of height in meters). (Itani, et al 2020). The formula is BMI = kg/m^2

$$\text{BMI} = \text{kg/m2}$$

Where kg is a person's weight in kilograms and m^2 is their height in meters squared. (Canada, 2022) Obesity can also be defined as a state of imbalance between the energy gained and the energy consumed, in other words, the amount of calories that enter the body is higher

than the amount of calories that the body needs to carry out vital activities. Fat accumulated under the skin, abdomen, and large muscles, they surround vital internal body organs such as the intestines, heart, and arteries. (Al-Muhanna, 2017).According to the US Food and Drug Administration (FDA), the normal measure of ideal weight has a BMI = 24, but in the case of excess of the ideal weight, the body mass index is BMI = 26, but if the body mass index (BMI) = 28, this is an indicator on having obesity. From a measurement of BMI = 30 and above, it is called hyper-obesity (FDA, 2020). Being overweight is associated with many conditions, including cardiovascular disease, type 2 diabetes, musculoskeletal disorders, and impaired respiratory function. (Ibrahim, 2022). Ferritin is a protein that is central to many vital body organs, processes, functions, and diseases. It is implicated in coronary artery disease and malignancy and has been directly involved in iron-deficiency anemia, neurodegenerative disorders, inflammation, phagocytic syndrome, hyperlipidemia, diabetes mellitus and blood pressure. (Khan, et al 2016). Also, ferritin levels in the blood are independently correlated with LDL and VLDL levels and age. (Nasif, et al 2018). So as to thyroid hormones are primarily involved in energy balance, lipid and glucose metabolism, and high blood pressure as people with low normal thyroid function are at increased risk for cardiovascular disease. (Özer, et al 2015)

Materials and methods

Measurement of the concentration of Thyroid Hormones in the blood

Principle

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation

50 μL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

1st incubation: 15 μL of sample, a biotinylated monoclonal T3, T4-specific antibody and a monoclonal T3, T4-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2nd 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 substances are then removed with Pro Cell /Pro Cell M. 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 or e-barcode.

Reagents working solutions

The reagent rackpack is labeled as TSH, T4, T3.
M: Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL, preservative.
R1: Anti-TSH-Ab~biotin (gray cap), 1 bottle, 14 mL:

Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L;
Phosphate buffer 100 mmol/L, pH 7.2; preservative.
R2: Anti-TSH-Ab~Ru(bpy) (black cap), 1 bottle, 12 mL:
Monoclonal anti-TSH antibody (mouse/human) labeled with
Ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.2;
Preservative.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel. Li-, Na-, NH -heparin, K3-EDTA, sodium citrate and sodium fluoride/potassium oxalate plasma. Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95. Stable for 7 days at 2-8 °C, 1 month at -20 °C.7 Freeze only once. The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube

Manufacturer.
Centrifuge samples containing precipitates before performing the assay.
Do not use heat-inactivated samples.
Do not use samples and controls stabilized with azide.
Ensure the samples, calibrators and controls are at 20-25 °C prior to

Measurement

- After 7 days when using the same reagent kit on the analyzer
- As required: e.g. quality control findings outside the defined limits.

Calculation

The analyzer automatically calculates the analyte concentration of each
Sample either in pmol/L, ng/dL or ng/L.
Conversion factors: pmol/L x 0.077688 = ng/dL
ng/dL x 12.872 = pmol/L
Pmol/L x 0.77688 = ng/L. (Roch, 2018)

Measurement of the concentration of Ferritin level in Blood

Test principle

The method for measurement of Ferritin on the cobas® e601 is a sandwich principle with a total duration time of 18 minutes. The 1st incubation uses 10 µL of sample, a ferritin-specific antibody and a labeled ferritin-specific antibody to form a sandwich complex. The 2nd incubation occurs after the addition of microparticles that cause the complex to bind to the solid phase. 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.

Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials: Equipment and Instrumentation

Reagent Preparation

All reagents are supplied by Roche Diagnostics in a ready-for-use unit that cannot be separated. Store the reagent kit upright in order to ensure complete availability of the microparticles. Bring the cooled reagents to approximately 20°C (45 minutes at room temp) and open the lids slightly before placing on the reagent disk of the analyzer. The reagent kit is stable until the expiration date or up to 12 weeks at 2-8°C after opening, whichever comes first. The Ferritin reagent pack can only be stored on-board the e601 for a maximum of 6 weeks so the reagent pack is generally removed from the instrument and stored at 2-8°C when all samples are completed.

Standards Preparation

Elecsys Ferritin CalSet is supplied by Roche Diagnostics in liquid form. Store the standards at 2-8°C until the expiration date of the kit.

Preparation of Quality Control Materials
Roche controls

Elecsys Preci Control Varia can be used for quality control of the Elecsys Ferritin immunoassay on the e601 analyzer. This is a lyophilized control serum based on human serum matrix in three concentration ranges. The lot specific values need to be entered into the cobas® 6000 before analysis. Use Class A volumetric glassware if volumetric glassware is specified in the package insert.

To reconstitute the PC Varia, carefully dissolve the contents of one bottle of each level by adding exactly 3.0 mL of distilled water and allow to stand closed for 30 minutes. Mix carefully, avoiding the formation of foam. Transfer aliquots of the reconstituted controls into appropriately labeled empty snap-cap vials avoiding cross contamination. Aliquots intended for storage at <-20°C should be frozen immediately and are stable for 1 month (freeze only once). Controls stored at 2-8°C are stable for 3 days. Ensure the controls are at ambient temperature before use.

CDC QC pools

Quality control materials for this assay are prepared in-house from blood products acquired from blood banks or from other volunteer blood donors. After screening the pools for Ferritin, the serum is pooled to obtain the desired QC levels. All pools are filtered through gauze to remove debris before being dispensed. Serum (usually 750 µL) is aliquoted into labeled 2.0-mL Nalge cryovials, capped, and is typically stored at <-70°C. The QC pools are stable for at least 3 years.

The QC limits for all pools are established by analyzing duplicates of each pool for at least 20 consecutive runs.

Procedure Operating Instructions, Calculations, and Interpretation of Results

Preliminaries

1) Allow Calibrators, QC and patient samples to reach ambient temperature.

2) Ensure that the amount of reagents, diluent, and wash solutions are adequate for the amount of samples to be run. You may place more than one bottle of reagent at a time on the analyzer; however, avoid using more than one lot number of reagent for a single run.
3) Make sure the analyzer and/or tests required are not masked.
4) Check to see if calibration is required for the tests that will be run.
5) If running the same tests on all samples, go to the “Start” global button and set the “default profile”.
6) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.
7) Perform the required maintenance on the e601 system. (Health, 2016)

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means. Person's Coeff. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability in this study. (., 2018)

Results

Table(1) indicates that there were significant weak positive correlations between T3 hormone [r = 0.35, p = 0], TSH hormone [r = 0.37, p = 0.01] with ferritin, while the results in the same table indicate that there were no correlations between ferritin levels with T4 hormone [-0.07].

Table 1 ---: Correlation coefficient between Ferritin and Thyroid functions

Thyroid functions	Correlation coefficient-r with Ferritin	P-value
T3	0.35	NS
T4	-0.07	NS
TSH	0.37	NS
NS: Non-Significant.		

In table [2] there was no significant relation between T3 in control [1.36 ±0.13, p-value = 0.959] and T3 in Overweight [1.32 ±0.06, P-value = 0.959], T3 in Obesity [1.35 ±0.06, P-value = 0.959]. While there was a strong significant relation between T4 in control [113.00 ±6.25, P-value = 0.0417] and T4 in Overweight [103.03 ±4.21, P-value = 0.0417], Obesity [90.00 ±6.73, P-value = 0.0417].

Table 2: *Comparison between difference groups in Hormones level*

Group	Mean ± SE	
	T3 ( )	**T4 ( )**
Control	1.36 ±0.13	113.00 ±6.25 a
Over Weight	1.32 ±0.06	103.03 ±4.21 b
Obesity	1.35 ±0.06	90.00 ±6.73 ab
LSD value	0.283 NS	19.09 *
P-value	0.959	0.0417

Means having with the different letters in same column differed significantly. * (P≤0.05).

So as to in table [3] there was a strong significant relation between ferritin in control [86.67 ±15.53, P-value = 0.0161] and ferritin in Overweight [52.72 ±5.61, P-value = 0.0161], Obesity [41.62 ±8.66, P-value = 0.0161]

Table 3: *Comparison between difference groups in Ferritin*

Group	Mean ± SE Ferritin ( )
Control	86.67 ±15.53 a
Over Weight	52.72 ±5.61 b
Obesity	41.62 ±8.66 b
LSD value	28.82 *
P-value	0.0161

Means having with the different letters in same column differed significantly. * (P≤0.05).

Discussion

As in table [1] our study showed significant weak positive correlations between ferritin to T3 (r=0.35; p=0.00) and between ferritin to TSH (r=0.37; p=0.00). However, there was no correlation between ferritin and T4 (r = - 0.07; p=0.00). This was similar with previous study which showed that there was weak positive significant correlation between serum ferritin and T3, while there was no significant between ferritin and TSH, T4. (Özer, et al 2015) Studies by Dahiya and Araque showed similar results in which there was no significant correlation between ferritin and TSH, (Dahiya K, 2016), (Maldonado-Araque C, 2018) but they could reveal significant correlation between ferritin and FT4 levels in the diabetic population. This might be due to a higher inflammatory state in obesity with diabetes than in obesity without diabetes.

In table No. [2] The mean T3 level in our study (1.36 ±0.13 pmol/mL) and decreased T4 level in obesity (90.00 ±6.73) compare to control (113.00 ±6.25) It may be a subsequent excess in weight gain in patients who become hypothyroid and are replaced with L-T4. (Peter Laurberg1, 2012)

Table 3 Our study showed that about a half subjects [n=10; (20%)] the control, had ferritin levels within the range of reference values, [86.67 ±15.53] while the remaining [n=8; (16%)] the

overweight, had ferritin levels below the reference value, [52.72 ±5.61] and the remaining [n=32; (64%)] the obesity, had ferritin levels below the reference value, [41.62 ±8.66] In the other hand, study from Lecube showed that ferritin levels in obese patients with metabolic syndrome were higher than obese patients without metabolic syndrome. The different finding between study by Lecube and our study was due to the difference in study population, in which the previous study was involving obese patients with metabolic syndrome and diabetes mellitus, while our study did not observe the glucose levels in each subject (Lecube A, 2008).

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