



Estimation of the level of homocysteine and vitamin B₁₂ in serum of patients with hypothyroidism

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ABSTRACT

In this study, homocysteine and vitamin B₁₂ levels were measured in patients with hypothyroidism in Salahdin Governorate. The study was conducted at the Tigris Educational Hospital in Tikrit. Blood samples were taken from patients with hypothyroidism (40 cases) with thyroid deficiency and 30 healthy (non-infected) subjects. Their ages ranged between 25-35 years. The study found that there was a significant increase $p < 0.05$ in the homocysteine level compared to the healthy Individuals as control group. The study also showed a significant decrease in the level of vitamin B₁₂ for patients $p < 0.05$ compared to healthy individuals .

Introduction

The thyroid gland is one of the largest endocrine glands in the human body. It is the only organ which is capable of synthesizing iodine in large quantities to use in hormone synthesis. Its location is appeared in the bow tie in front of the neck [1,2] and under Adam's apple, it consists of two lobes that bind together strongly to the trachea by means of an isthmus [3].

That hypothyroidism disease is a decrease in the function of the thyroid gland, therefore, there is a lack of thyroid hormones in the blood, and deficiency either to be in the thyroxine tetra-iodine T₄ or triode iodine T₃ or in both. There should be a compensate for any deficiency in the formation of hormones increases the secretion of thyroid hormone, which in turn swells the gland and enlarged were the disease is called a (Goiter) [4]. There is a condition called (Endemic Goiter) which is found in areas that have lack iodine and may cause other elements such as selenium. There are also areas within the region where the gland disease is spread but it is not because of iodine deficiency, but because of lack of other elements such as selenium [5]

Homocysteine (Hcy) is naturally occurring amino acid containing sulfur, α(2-amino-4-mercaptobutanic acid) that is free or associated (but not in proteins; Not the essential amino acid constituents of proteins) and plays important roles in the metabolic processes of mammals [6]. The normal metabolism of homocysteine requires the supply of vitamins (Folic acid B₉), (copalminevitamin B₁₂), (pyridoxine vitamin B₆) and (Riboflavin B₂), as these levels are conversely

proportional to Hcy distribution levels [7] . Elevated levels of homocysteine are independent risk factor for myocardial infarction, type 2 diabetes, high blood pressure, cholesterol elevation and increasing body mass index [8] .

Vitamin B₁₂ (Copalamin) is a water soluble vitamin as part of the B-complex group of B vitamins obtained from animal sources such as liver, meat, eggs, milk and its derivatives [9] . This is called an external factor. As this vitamin is absorbed into the body, the cyanide group is transformed into two effective enzymatic accompaniments, which are converted into (coenzymes), namely Methylcobalamin (B₁₂), Deoxy adenosyl cobalamin (DAB₁₂). These coenzymes act as temporary carriers of the alkyl group. [10. 11] Vitamin B₁₂ is absorbed in the last part of the small intestine in the ileum, which requires absorption of a protein produced in the lining of the stomach called Intrinsic factor. [12.13] Vitamin deficiency is due to the lack of internal treatment due to damage to the cells of the stomach due to ulcers or surgeries that occur in the stomach or may be due to infection with intestinal worms, which lead to non-absorption and put it with feces and symptoms of vitamin deficiency and neurological disorders and gastrointestinal disorders [14] B₁₂ is acting by reducing the level of homocysteine in the human body, which is a risk factor for heart disease and arteries [15] by adding methyl to homocysteine remethylation of homocysteine to convert to methionine. [16].

Materials and methods

The study is conducted at Degla Teaching Hospital in Tikrit city on patients with hypothyroidism. The samples were divided into 40 infected male samples and 30 samples of healthy people aged between 25 and 35 years. The sample where collected by withdrawing the blood from the vein from the front attachment package with a 5 ml plastic syringe , with the volume of the blood drawn (3-4) ml . The serum was obtained from the drawn blood by leaving the blood in a plastic tube free of anticoagulant and at a temperature (25 °c) until it is clotted and then placed in the centrifuge at a speed of 242 RPM and for 15 min. The serum was then withdrawn by a micropipette and placed in clean, sterile tubes and kept in a freezing state at -20 °c Until the use and conduct tests .

Measuring serum homocysteine level

The level of homocysteine in serum is estimated using the(Kit) provided by CRYSTAL CHEM, INC. USA in an enzymatic process. The principle of the test is based on analysis of the auxiliary substrate and its transformation into a product. Co-Substrate is a molecule that is not the base material Which is converted by the enzyme, and does not contain homocysteine in this analysis, and the normal ratio is (7-12 $\mu\text{mol/L}$). [17,18]

Measuring the concentration of vitamin B₁₂

The level of vitamin B₁₂ is assessed in patients and healthy blood using the kit processed by Sigma-Aldrich, USA. The principle of working to add a specific concentration of dissolved vitamin B₁₂ to the model and to measure the difference in concentration from reading the optical density of a device Spectrophotometer at a wavelength of 546 nm and a normal ratio of (300-600 p mole / L). [19]

Results and discussion

Result of this study showed that there was an increase in the concentrations of homocysteine in patients with hypothyroidism while there was a significant decrease of vitamin B₁₂ compared with control .

The level of homocysteine in the serum of patients with hypothyroidism, was (19.19 $\pm$ 5.51 mol/L) compared with the healthy control (8.42 $\pm$ 1.34 mol / L) (p=0.0003) Figure (1) and Table (1). The main reason for the high level of homocysteine is the lack of the enzyme supplements (vitamins) [20] due to slow the metabolic processes. There is no equivalency between anabolism and catabolism ,it leads to the accumulation of homocysteine in the blood, due to a decrease in the level of thyroxin , which has a negatively affects on the absorption of vitamin B₁₂ by the bowel and thus affects the process of re-methylation the other reason is because of a genetic mutation in the level of thyroxin, which leads to rise homocysteine level[21]. High homocysteine level also attributed to the lace of reactive enzyme pathogens by homocysteine reintroduction to methionine, or a deficiency of the sulfonic transmission pathway .This deficiency is due to the

thyroid hormone, which leads to vitamin B₁₂ deficiency and thus weak the enzymes of these two pathways [22,23] hypothyroidism reduces the levels of liver enzymes involved in the re-methylation of homocysteine pathway and thus lead to an increase in the level of homocysteine [24], And that the relationship between homocysteine and thyroid hormones is the result of either low levels of vitamins (folic acid, B₁₂, B₆) from dietary sources, or a direct effect of hypothyroidism on homocysteine metabolism and synthesis [25] and the first cause can be explained by malnutrition, which is associated with the thyroid gland and partly due to hypothyroidism and loss of appetite as well as associated with weakness, fatigue and weight gain which is due to low metabolic rate [26,27,28].

Table 1 showed the concentration of homocysteine and vitamin B₁₂ in patients with hypothyroidism.

Parameter	Control n=30 Mean $\pm$ SD	Patiant's n=40 Mean $\pm$ SD	t.Test	p.value
Homocysteine $\mu\text{mol/L}$)	8.44 $\pm$ 1.34	19.66 $\pm$ 5.51	7 ^{**} .45	0.0003
B ₁₂ pmol/l	582 $\pm$ 102	341 $\pm$ 166	5 ^{**} .81-	0.0002

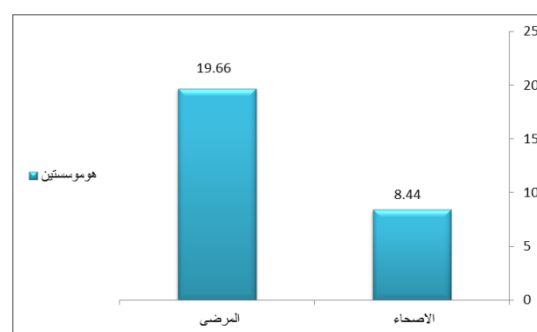


Figure 1 The concentration of homocysteine in patients with hypothyroidism compared with control .

The study showed a significant decrease in vitamin B₁₂ in patients with hypothyroidism (341 $\pm$ 166 p mol / l) comparing with healthy control (582 $\pm$ 102 pmol / l) (P=0.0002) as shown in table (1) and figure, it was found that the main cause in decreasing vitamin B₁₂ is due to complications in Absorption for that vitamin [29], it was found that the level of homocysteine is effecting by the nutrient materials , containing vitamin B₁₂ and folate because they regulate the metabolism of homocysteine and help in reducing its level [30,31].

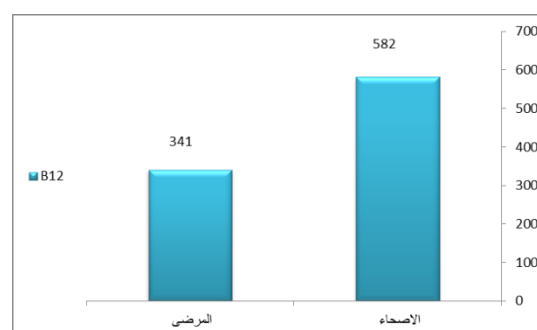


Figure (2) shows the concentration of B₁₂ in patients with hypothyroidism compared with control

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تقدير مستوى الهوموسستين وفيتامين B₁₂ في مصل دم المرضى المصابين بقصور الغدة الدرقية

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الملخص

تم في هذه الدراسة قياس تركيز الهوموسستين وقياس فيتامين B₁₂ لدى المرضى المصابين بقصور الغدة الدرقية في محافظة صلاح الدين. حيث اجريت الدراسة في مستشفى دجلة التعليمي في تكريت, وقد قسمت عينات الدراسة الى 40 حالة مرضية مصابين بقصور في الغدة الدرقية و 30 عينة من الاصحاء (الاشخاص الغير مصابين) وتركزت اعمارهم بين (25-35) سنة. وقد توصلت الدراسة الى ان هناك ارتفاع معنوي عند مستوى احتمالية $p < 0.05$ في مستوى الهوموسستين مقارنة بالاصحاء كما اظهرت الدراسة ان هناك انخفاض معنوي في مستوى B₁₂ للمرضى عند مستوى احتمالية $p < 0.05$ مقارنة بالاصحاء.