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## **Evaluation of the effect of using different doses of levothyroxine on some biochemical parameters in hypothyroid patients**

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**Abstract**--The current study included the collection of ninety serum samples to evaluate the effect of using different doses of levothyroxine on some biochemical parameters in patients with thyroid disorders. Samples were collected for the period between 15/9/21 to 10/1/2022. Sixty samples were taken from patients with hypothyroidism(G1) who subdivided into two subgroups; 26 of them took the levothyroxine dose of 200 µg/day (G2), and 34 of them took the levothyroxine dose of 100 µg/day (G3), and 30 samples were taken as a control group, their ages ranged between (46-60) years .The level of hepcidin hormone and the activity of the alkaline phosphatase-ALP, Alanine amino transferase-ALT, and Aspartate amino transferase-AST enzymes were evaluated in the serum of hypothyroid patients and healthy subjects as a control group. The results showed that the level of hepcidin hormone significantly ( $P\leq 0.05$ ) elevated in serum of patients in group (G3) than the control group and the rest of the other groups. The results showed a significant increase( $P\leq 0.05$ ) in the activity of aspartate aminotransferase enzyme, alanine transferase enzyme and alkaline phosphatase enzyme activities in the serum of patients with hypothyroidism compared to the control group.

**Keywords**--hepcidin hormone, hypothyroidism, levothyroxine, ALT, ALP, AST.

## Introduction

Hypothyroidism, or underactive thyroid, is characterized by impaired perception and metabolism. It is a pathological condition that results from a deficiency in the activity of the thyroid gland and results in decrease in the concentration of thyroid hormones T3 and T4 with an increase in the level of TSH in the blood (1,2). Thyroid hormones increase oxygen consumption by directly affecting the mitochondria in many tissues, especially the liver. These hormones affect the oxidative phosphorylation process (3). Among the most important symptoms caused by a lack of thyroid hormones are reduced metabolic processes in the body, deepening of the voice, weight gain, water retention, delaying growth and mental development in children (4). As well as a slower heart rate, decreased appetite, skin dryness, poor lung efficiency, and difficulty in pregnancy (5) in addition to the role of hypothyroidism in increasing hyperlipidemia, and contributing to cardiac and metabolic diseases (6).

Thyroid disorders may lead to disruption of liver function, and there is a relationship between thyroid disease and liver disease, as patients with liver disease suffer from hypothyroidism, thyroiditis, or hyperthyroidism, as well as patients with thyroiditis or hyperthyroidism may suffer from disturbances in the functions of the liver (7). When the liver malfunctions, it releases enzymes into the blood, which include Alanin amino transaminase - (ALT), Aspartate amino transferase (AST) and alkaline phosphatase (ALP). It is related to liver disease and an imbalance occurs in the levels of the effectiveness of liver enzymes in the serum, which are usually used to conduct liver function tests, and a high level of these enzymes is an indication of liver damage (8).

Hepcidin is a peptide hormone that is produced by hepatocytes (9). It consists of 25 amino acids and is the main hormone that participates in controlling iron balance when iron is more than the normal limit (10). The gene expression of hepcidin hormone is regulated by a group of signals based on the body's need for iron, meaning that the main regulator of hepcidin level is iron and inflammation, as iron excess stimulates gene expression of hepcidin hormone, while iron deficiency prevents this gene expression as if it works by the feedback mechanism for maintaining normal iron levels in the body (11,12). The hepcidin hormone regulates the iron balance in the body's systems by controlling its release from the intestinal cells in the duodenum responsible for absorbing food, phagocytes and hepatocytes that store it (13) it has been assumed that the hormone disrupts the movement of iron from phagocytic cells and promotes its conversion to foamy forms and thus the occurrence of atherosclerosis, so research suggests that iron and hepcidin hormone play a role in the occurrence of atherosclerosis and cardiovascular diseases (14). Levothyroxine is a treatment primarily used to treat hypothyroidism. Oral administration of levothyroxine has been approved by the US Food and Drug Administration for the treatment of primary and secondary hypothyroidism (16,15). Primary hypothyroidism is caused by a disorder in the thyroid gland, and the cause the most common is an autoimmune condition (Hashimoto thyroiditis) and iatrogenic thyroiditis (after thyroidectomy). Secondary hypothyroidism occurs when the disorder occur is in the pituitary gland (from adenomas to surgery), and there is usually a decrease in TSH production. Hypothyroidism is rare, and the problem lies in the hypothalamus with a decrease

in the secretion of thyroid hormone (TSH) (1). Levothyroxine is a synthetic version of the body's natural thyroxine (T4), the hormone thyrotropin-releasing hormone (TRH) stimulates the anterior pituitary gland to release TSH, which subsequently stimulates the thyroid gland to secrete 80% of T4 and 20% of T3. From T3, triiodothyronine, then 50% of thyroxine T4 is converted to its active T3 receptor, then thyroid hormones act by binding to thyroid receptor proteins located in the cell nucleus (17).

### **Materials and Methods**

**Study samples:** The study was conducted on 90 serum samples, 60 samples from people with hypothyroidism with 30 serum samples from healthy people as a control group, their ages ranged between (40-60) years for patients and healthy people, samples were collected from external laboratories in the city of Baghdad, for the period between 9/15/2021 to 01/10/2022.

#### **Determination hepcidin hormone concentration in the blood serum**

Hepcidin concentration was determined by enzyme-linked immunosorbent assay (ELISA). This method depends on the principle of the solid phase represented by the monoclonal antibody present in the microplate and the standard solutions and samples that represent the hepcidin protein to react and bind with those antibodies, and by adding the conjugated enzyme which is the antibody to the hepcidin hormone labeled with the substance Biotin combines with Horseradish peroxidase HRP (18).

#### **Determination the activity of serum AST enzyme**

##### **The Principle**

The enzyme activity of AST in the serum was determined by colorimetric method to measure the enzyme activity by measuring the concentration of hydrazone formed by the reaction of oxalosuccinate with 2,4-Dinitrophenyl-hydrazine by the action of the enzyme (19).

#### **Determination the activity of serum ALT enzyme**

##### **The principle**

The activity of the ALT enzyme in the serum was determined using the colorimetric method by measuring the concentration of hydrazone formed by the reaction of Pyruvate with 2,4-Dinitrophenyl hydrazine (19).

#### **Determination the activity of serum ALP enzyme**

##### **Principle**

The activity of alkaline phosphatase enzyme in the blood serum was estimated by using the colorimetric method (20) and as in the following equation:

**Disodium Phenyl Phosphate**  $\frac{\text{Alkaline Phosphatase}}{\text{PH 10}}$  **Phenol + phosphate**

Statistical analysis:

The statistical program (SPSS) was used, using the Duncan test to compare between a group of patients suffering from hypothyroidism and a group of healthy people as a control group. Significance was calculated when performing the statistical analysis of all data at the probability level ( $p \leq 0.05$ ).

### Results and Discussion

The results show that the mean  $\pm$  standard deviation of the level of hepcidin in the serum of patients with hypothyroidism was  $(17.996 \pm 3.556)$  ng/ml compared to  $(20,451 \pm 5.994)$  ng/ml in healthy controls as shown in Table (1).

Table (1) The mean  $\pm$  standard deviation of hepcidin concentration in the patients and healthy groups

Parameter	Mean $\pm$ SD
	Hepcidin(ng/ml)
C	20.451 $\pm$ 5.994 b
G1	17.996 $\pm$ 3.556 bc
G2	18.047 $\pm$ 4.121 bc
G3	28.669 $\pm$ 5.088 a

Different letters mean that there are significant differences .Similar letters mean there are no significant differences. Group of patients with hypothyroidism, which their total number was 60 samples (G1), were subdivided into two subgroups ;26 of them took the levothyroxine dose of 200  $\mu$ g/day (G2), and 34 of them took the levothyroxine dose of 100  $\mu$ g/day (G3), It is clear from the above results that the hepcidin hormone was significantly higher ( $P \leq 0.05$ ) in (G3) than the control group and (G2) groups as in Figure (1).

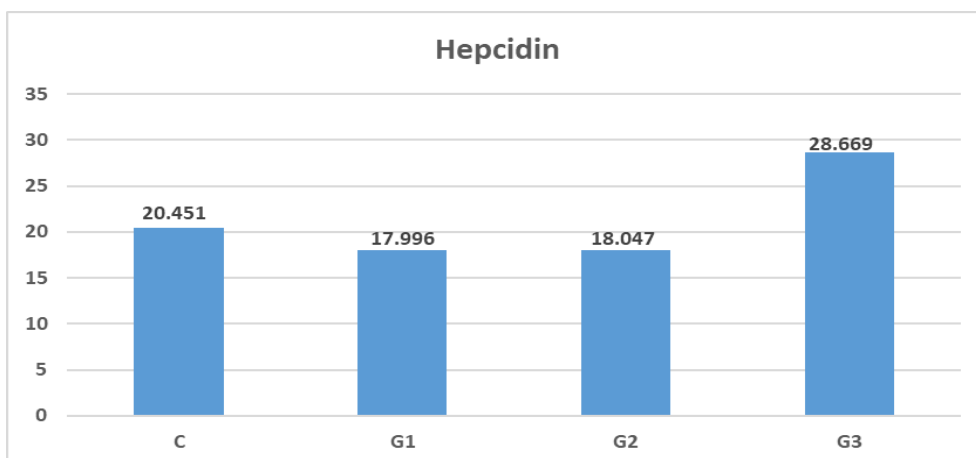


Figure (1) The level of hepcidin hormone concentration in serum of the study groups

The results of the current study showed an increase in the level of hepcidin hormone in group(G3) compared with the rest of the groups, and thus the results of the current study agree with the results of previous study of Filipowicz et al (21) who showed a decrease in the level of hepcidin hormone concentration after treatment in patients with thyroiditis. Thyroiditis is characterized by increased levels of hepcidin and decreased after treatment. The results of Liu et al. (22) and Bruinstroop et al. (23) demonstrated a beneficial effect of LT4 substitution therapy on non-alcoholic fatty liver disease-NAFLD in patients with subclinical hypothyroidism-SCH, and those with hyperlipidemia, with a decrease in the prevalence of NAFLD and liver enzymes in the blood. The level of hepcidin is regulated by iron ions in the blood serum and various stimulating and inhibitory factors. In most cases, low iron concentration (eg. hemolysis or hemorrhage) leads to a decrease in hepcidin production. Conversely, acute IL-6-mediated inflammation, cancers, chronic diseases, and autoimmune diseases lead to increased levels of hepcidin (34).

Previous experiments confirmed the increase of hepcidin by inflammatory and infectious stimuli via the interleukin-6 (IL-6) pathway (25). The study of Bartalena et al., (26) showed an increase in IL-6 in patients with thyroiditis. Thus, the level of hepcidin hormone rises in hypothyroidism before treatment through inflammatory and infectious stimuli through the interleukin-6 (IL-6) pathway, and this is consistent with the results of the current study. The use of levothyroxine treatment led to a decrease in the level of hepcidin to levels comparable to the healthy group, which indicates the benefit of this drug in modifying hormone levels in studygroups.

#### **Liver function test of the groups under study**

Liver function was evaluated by measuring the activity of the liver enzymes Alkaline phosphatase-ALP, Alanine amino transferase-ALT, and Aspartate amino transferase-AST, as in Table (2) which shows the mean  $\pm$  standard deviation of the activity of these enzymes.

Table (2) mean  $\pm$  standard deviation of Liver function test in the study.groups

Parameter	Mean $\pm$ SD		
	ALP (U/L )	ALT(U/L )	AST(U/L )
C	62.564 $\pm$ 15.656 b	35.802 $\pm$ 8.726 b	25.410 $\pm$ 6.395 c
G1	106.222 $\pm$ 33.67 a	72.746 $\pm$ 17.926 a	45.781 $\pm$ 13.327 ab
G2	98.481 $\pm$ 30.981 a	71.585 $\pm$ 19.113 a	40.721 $\pm$ 11.863 b
G3	97.484 $\pm$ 27.578 a	71.437 $\pm$ 17.482 a	47.444 $\pm$ 12.131 ab

### ALP enzyme activity

Table (2) shows that the mean  $\pm$  standard deviation of ALP activity was  $(106.222 \pm 33.67)$  IU/L in patients with hypothyroidism, while it was  $(62.564 \pm 15,566)$  IU/L in control group. It is clear from the results that the activity of alkaline phosphatase was significantly ( $P \leq 0.05$ ) increased in the group of patients compared to the control group as in Figure (2).

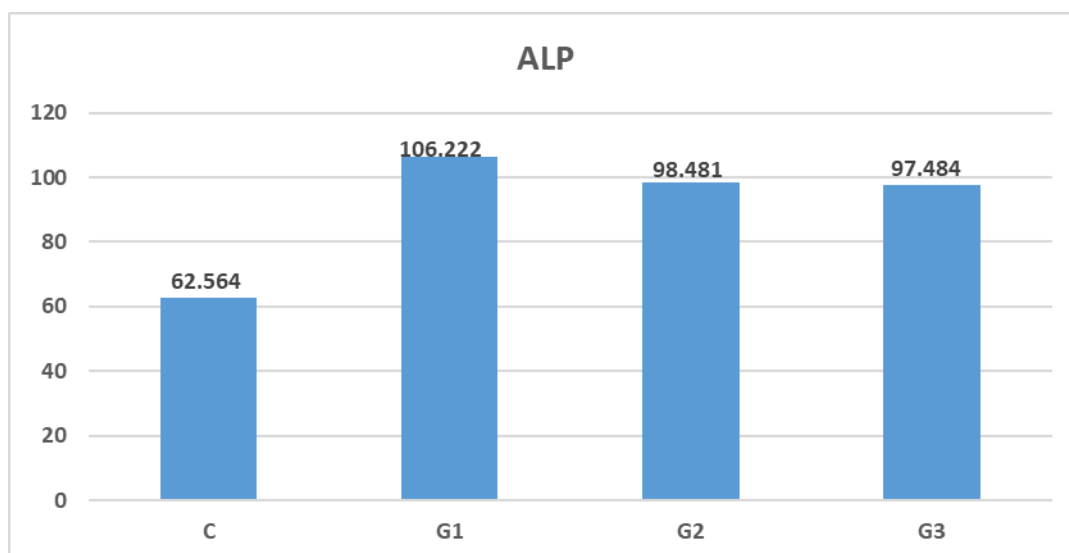


Figure (2) The activity of serum ALP enzyme in the study groups.

### ALT enzyme activity

Table (2) shows that the mean  $\pm$  standard deviation of alanine aminotransferase enzyme activity was  $(72.746 \pm 17.926)$  IU/L in patients with hypothyroidism, while it was  $(35.802 \pm 8.726)$  IU/L in control group. The results indicated that the activity of alanine aminotransferase was significantly ( $P \leq 0.05$ ) increased in patients with hypothyroidism compared to the control group, as shown in Figure (4).

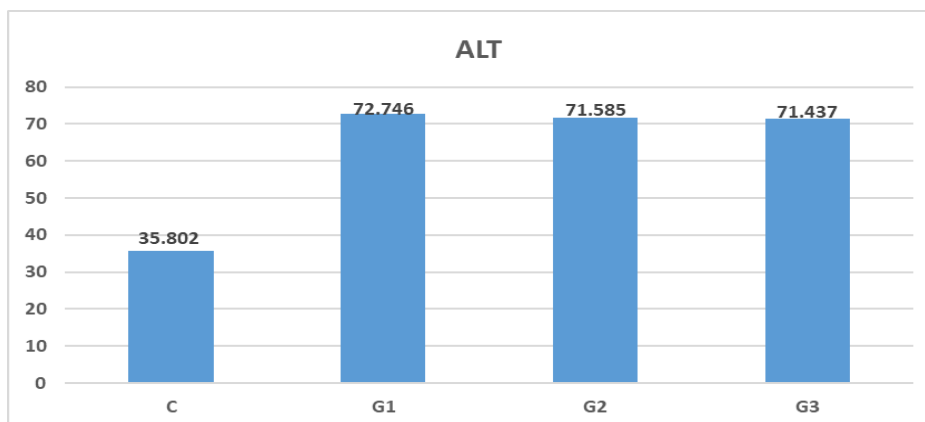


Figure (3) The activity of serum ALT enzyme in the study groups.

### AST enzyme activity

Table (2) shows that the mean  $\pm$  standard deviation of the activity of aspartate aminotransferase enzyme was  $(45.781 \pm 13.327)$  IU/L in patients with hypothyroidism, while it was  $(25.410 \pm 6.395)$  IU/L in healthy subjects as a control group. It is clear from the above results that the level of aspartate aminotransferase significantly ( $P \leq 0.05$ ) increased in the group of patients compared to the control group, as shown in Figure (4).

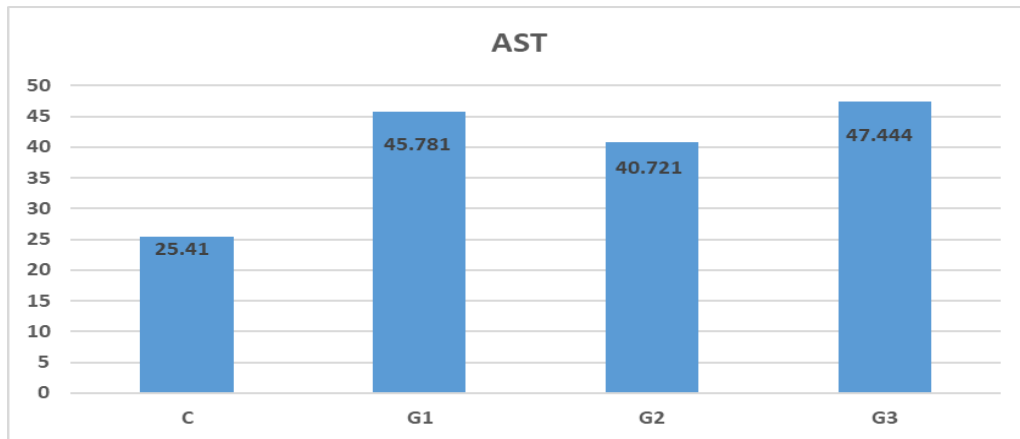


Figure (4) The activity of serum AST enzyme in the study groups.

The liver is an essential organ of the metabolism of thyroid hormones, and hepatocytes are often affected by hypothyroidism. Thyroid disorders are often accompanied by abnormal levels of liver enzymes and disturbances in liver function. Neglecting these findings may lead to an over or underdiagnosis of liver disease. And the thyroid gland and therefore causes errors in patient care, and the results of the current study agree with the results of several studies, as they indicated that people with hypothyroidism have high levels of AST, ALT and ALP compared to the healthy ones (27, 29).

The results of the recent study conducted in the city of Najaf in Iraq showed an increase in the thyroid hormone "TSH" in patients with thyroidectomy. The study also revealed a significant increase in liver enzymes AST, ALT in patients with thyroidectomy, as well as a high blood lipid level, as hypothyroidism is the main consequence of thyroidectomy, and it causes an imbalance in lipid metabolism and liver enzymes, leading to secondary hyperlipidemia and impaired liver function (30).

The increase in the activity of the ALT enzyme is an indication of the leakage of enzymes from the hepatocytes into the bloodstream as a result of hepatotoxicity resulting from the inactivity of the thyroid gland, as the enzyme is an important indicator of liver injuries and a clinical indication of the safety of the liver function. Hepatic enzymes are transferred to plasma as a result of disturbance in liver function (31), and an increase in the activity of ALT is associated with an increase in the activity of both AST and ALP (32). As it is possible that the difference in the concentration of enzymes between patients and healthy people is

due to the excessive breakdown of red blood cells or due to the need to manufacture peptide chains through the activity of these enzymes in the transfer of amino groups to amino acids or both. The activity of the AST enzyme increases in the case of liver diseases that lead to hepatitis, acute pancreatitis, or hypothyroidism, as it is high in people with hypothyroidism, and therefore the metabolite materials leaks from the liver into the plasma and causes many diseases that may lead to death, and that the liver functions it changes with the high concentration of this enzyme and affects bile duct secretion significantly (33,28).

Also, the increase in the concentration of AST enzyme is one of the problems that cause a rapid loss of liver function and the entry into the blood of intracellular enzymes, and because of the breakdown of liver cells and tissue cells in which they are located, increases the concentration of this enzyme in detecting liver damage caused by hepatitis and cirrhosis of the liver. An increase in the concentration of this enzyme increases of neutrophilic leukocyte count (34). Liver impairment resulting from iron overload or chronic viral infections has become another cause that can lead to death (35). Elevated liver enzyme levels are a sensitive indicator of liver toxicity, so measurement of liver enzyme levels ALT, AST, and ALP is used to assess the functional state of the liver. Therefore, serum ALT, AST levels are often used to assess liver damage (36). Or it may be caused by elevated liver enzymes AST, ALT as a result of iron overload, as the main factor for iron balance in the body is the hepcidin hormone, which in turn leads to impairment in liver function, as iron overload is toxic to body tissues and may cause serious damage such as Liver damage, heart disease, and endocrine glands (37,38). Thus, the high level of hepcidin hormone affects iron levels in the body, and iron overload leads to an increase in the levels of liver enzymes, as in the current study.

The effect of a dose of 200 µg/day of levothyroxine on the AST enzyme was clear, as it was significantly lower than the rest of the groups under study, especially the group (G3) who were taking levothyroxine dose of 100 µg/day, which indicates that levothyroxine improved the performance of the liver in Hypothyroid patients.

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