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The relationship of iron-regulated hepcidin hormones in patients with atherosclerosis

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ABSTRACT

The purpose of this study is to evaluate the levels of the hepcidin hormone in patients with atherosclerosis. The study started from December to June 2022. 120 samples were obtained for research purposes. They were divided into 5 groups, each group had 30 samples, the first 4 groups included patients (anemic group, second group with atherosclerosis due to smokers, second group with diabetes mellitus). 3rd group of hypertension, 4th group of hypertension). The fifth and final group was for normal (normal) healthy individuals) and hormone tests were performed on them after the samples were collected for the purpose of comparison with the rest of the study, showed a significant increase in the level of probability of hepcidin hormone concentration in the serum of atherosclerotic patients in the anemia group (A), the smoker group (B), and the diabetes group (C) ($P \leq 0.05$). were compared with the healthy group (E). Also, concentrations of the hormones adrenaline and cortisol in atherosclerotic patients in the smoking group (B) and blood pressure (D) compared to other disease groups and healthy groups were at probable levels ($P \leq 0.05$). (E).

Key word: Atherosclerosis, Hepcidin hormone

Introduction

The hepcidin hormone was discovered in 2000 (Casu et al., 2016). They were first detected in human urine and blood (Kemna et al., 2008) and divided into two groups as antibacterial agents. Krause and his group were able to isolate this peptide from plasma because it is the product. It has hepatic antibacterial properties and was originally called a peptide (LEAP-1), an antibacterial peptide that expresses the hepatic antibacterial protein liver-1 (Krause et al., 2000). In 2001 Park and his group isolated this peptide from human urine associated with inflammation and named it hepcidin, which was observed to be produced in the liver. However, small amounts are collected in other tissues such as adipocytes (Park et al., 2001).

The hepcidin hormone consists primarily of a tightly folded peptide chain composed of 32% of the β -sheet, with a pin structure anchored by four disulfur bridges. supply of iron to the blood circulation through its influence on the absorption process by enterocytes (Ganz, 2013). cells in which it is stored (Krause et al., 2000). It is also a cysteine-rich liver bactericidal protein (Rauf, 2020). Hepcidin levels in the heart are 30-fold lower than in the liver, but appear to play an important role in heart function (Lakhal-Littleton et al., 2016). It should be borne in mind that in addition to the liver, the heart is the organ with the highest hepcidin expression. This relatively high expression of hepcidin in the heart raises questions about the role

of cardiac hepcidin in cardiac function (Krause et al., 2000).

The amount of hepcidin produced by the liver is affected by serum iron levels. This is because low iron levels increase hepcidin production and high iron levels decrease hepcidin production (Schmidt et al., 2008). Atherosclerotic sites Arteries are blood vessels with muscular walls that carry blood from the heart to the rest of the body. It is flexible and absorbs sudden changes in blood pressure, allowing blood to pass freely. This is normal. In the case of atherosclerosis, it is mainly due to increased amounts of fat, especially cholesterol and triglycerides, deposited in the lining of arteries (Gaziano et al., 2011; Miao et al., 2020). This makes the arteries less elastic, causing arteriosclerosis and fat deposits that form ulcers in the arteries, making it easier for blood clots to form in the arteries, which gradually form. and cause partial or complete blockage of arteries. A serious complication of this situation is that clots that have formed detach and pass with the blood to reach small-diameter arteries, blocking their passage and causing clots (Boon et al. 2006).

Materials and working methods

Patients Group

After collecting blood samples, (120) samples from patients with atherosclerosis, their ages ranged from 38 to 72 years.

control group

Blood samples were taken from healthy, disease-free individuals, and this group was considered a control group, during which they were compared with a group of patients.

Determination of hepcidin hormone

The concentration of hepcidin was determined using an enzyme-linked immunosorbent immunoassay (ELISA) by using a special hepcidin kit prepared by Shanghai Biotechnology, China, with number YHB1535Hu.

Working Principle

This assay is based on an enzyme-linked antibody to hepcidin assay by ELISA. This method is based on the principle that a solid phase represented by a monoclonal antibody present in a microplate reacts with a standard solution and a sample representing hepcidin protein to bind to the antibody, and then the binding enzyme, which is an antibody, is added. It is based on. Hepcidin hormone marked with biotin. Unwanted enzymes are removed after an incubation and washing period to bind horseradish peroxidase HRP forming an immune complex, substrate is added and the solution turns blue to green under the influence of acid before being measured.

The method of work:

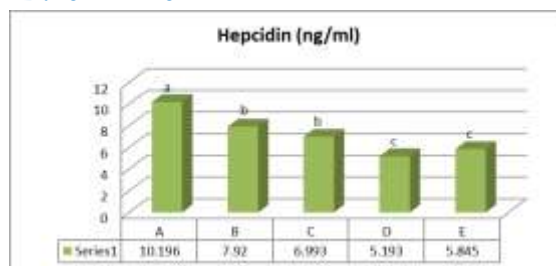
1. Dilute the standard with the diluent to 5 dilutions and take the diluent standard as the concentration number (0).
2. Add 50 microliters of the prepared standard to the corresponding wells.
3. Add 40 μ l of sample to the corresponding pits, then add 10 μ l of Antibody, add 50 μ l of HRP to each, mix well and cover with special glue.
4. Infuse for 1 hour at 37°C
5. The plate should be washed and diluted with the washing solution prepared in the test kit (washing solution is diluted 1:30 with distilled water).
6. Dry the remaining wash solution from the plate with filter paper.
7. Add 50 μ l of Chromogenic Reagent from Substance A to all pits, then add 50 μ l of Chromogenic Reagent from Substance B back to all pits.
8. When the etching solution turns blue, incubate at 37 °C for 10 minutes.
9. Add 50 microliters of stop solution and mix well (turns yellow).
10. Read the plate using ELISA technology at a wavelength of 450 nanometers and get the results.

statistical analysis

Analysis of variance (ANOVA) was used to statistically analyze the results using the statistical program (Mini tab). Comparisons between group arithmetic means were made using Dunkin's test for probability levels ($p \leq 0.05$) and ($p \leq 0.01$) (Al-Rawi, 1989).

Results and discussion

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The Figure shows the mean±standard deviation of the hepcidin hormone concentration in the Anemia Group (A) Smokers group (B), Diabetic Group (C), and Blood Pressure Group (D) for patients with atherosclerosis compared to the Control group (E).

And as shown in the figure, the results show a significant difference in the levels of hepcidin in atherosclerotic patients in the anemic group (A), the smoker group (B), and the diabetic group (C) ($P \leq 0.01$). showed a significant increase in concentration. compared to the healthy group (E). Comparing the disease groups, we found that the anemia group (A) recorded a significant increase at a significant level compared to the other disease groups ($P \leq 0.01$). This is due to the fact that the amount of hepcidin produced by the liver is affected by serum iron levels, with low iron levels increasing hepcidin production and high iron levels decreasing hepcidin production. increase. (Schmidt et al., 2008).

Also note from the figure that the hepcidin concentration is significantly increased in the smoker group (B) compared to the healthy group (E). It leads to stimulation of the degradative processes of cells and the development of inflammation. Therefore, the main regulators of hepcidin hormone levels are iron and inflammation (Cornelissen et al., 2019). The figure also showed that the hepcidin concentration was significantly elevated in the diabetic patient group (C) compared to the healthy subjects. Diabetes mellitus shown in many studies (Jiang et al., 2011).

(Aignr et al., 2013) evaluated the effects of glucose on blood iron and hepcidin. A control group (healthy) receiving only water without glucose. Elevated serum hepcidin is independently associated with intimal thickening of the common carotid artery, a marker of atherosclerosis. (Li et al., 2015) as found (Biljana Ilkovska et al., 2014) people with high blood sugar levels have elevated levels of hepcidin compared to healthy people, an explanation for this is In patients, the proportion of iron stores is increased and, since the function of hepcidin is to regulate the proportion of iron in the body, the secretion of hepcidin hormone is increased to balance iron in the body (AlexAregbesola et al. 2015).

These results are consistent with investigators' findings (Yayar et al., 2018) showing that hepcidin is effective in cardiovascular mortality and the pathophysiology of atherosclerosis in dialysis patients. These results were also consistent with the researchers' findings (Wang et al., 2018). They found an association between baseline hepcidin after

follow-up and atherosclerosis and other vascular risk factors.

The results differed from the findings of (Oguz et al., 2006). The results do not support the hypothesis that hepcidin levels as an indicator of chronic

inflammation may be elevated in atherosclerotic cardiovascular disease.

As shown in the figure, the results also showed that there was no significant difference in the level ($P \leq 0.01$) in the blood pressure group (D) compared to the healthy group (E).

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