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Study of relationship between Homocystine and Atherosclerosis

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Abstract: In this study the effect of amino acid metabolism via determination of homocystiene blood glucose to determination sugar in blood. The study conducted on too Iraqi patient with chronic atherosclerosis at age rang (29-70years) in Baquba teaching hospital in (center care unit) during the period from 25 September 2020to may 2021. The patients divided in (55 mans and 45 patients) the total patient divided in tow groups according the treatment (30) of total patient under go to treatment for two to three days after atherosclerosis and 70 patient in same day of diagnosis of atherosclerosis. Atherosclerosis effect on HCY value where the results observed decreasing compare with control.

1. Introduction

The German surgeon Felix J. Marchand (1846–1928) established the term "atherosclerosis" from the Greek words "athere" (gruel) and "scleros" (hard) [1]. Atherosclerosis is a chronic illness that affects the blood vessels. It is distinguished by a loss in elasticity as a consequence of the constriction and stiffness of the blood vessel walls produced by the accumulation of lipids, cholesterol, calcium, and other substances (minerals, cellular debris, and so on) in the inner layer of medium and large-sized arteries. Arteriosclerosis raises blood pressure, reduces blood flow to various bodily organs, and causes severe tissue damage. Furthermore, the plaque developed in the blood artery walls may rupture, resulting in the production of blood clots (thrombus), that could cause catastrophic obstructions in situ or elsewhere. The most significant health implications are ischemic heart disease or coronary heart disease (CHD) (inadequate supply of oxygen-rich blood to the heart muscle) and stroke (decrease in blood flow to brain tissue due to blood vessel obstruction or intracranial hemorrhage)[2].

Cardiovascular disease (CVD) is one of the most important causes of mortality worldwide [3]. It is expected that CVD will remain as the most important cause of death (36%) until 2020. It accounts for 17 million deaths per year, and one-fourth of the 40 million annual deaths in developing countries. CVD is also the most common cause of death in Iran [4]. Many risk factors have been proposed to be associated with CVD. The main risk factors include family history, smoking, hypertension, history of diabetes, hyperlipidemia and obesity. However, new factors have been recently proposed and taken into consideration [3, 5]. These include blood homocysteine, fibrinogen, plasma factor VII activity, lipoprotein (a) and several other factors [3]. Although many studies are available on the impact of hyperhomocysteinemia on CVD, the adverse effects of this condition on development and exacerbation of diseases should not be ignored. This review has aimed to report the effects of homocysteine on the cardiovascular system, and the role of this amino acid in other

diseases. vitamin B12 can increase blood homocysteine level [6]. Defective homocysteine metabolism increases its plasma level. The defect could be genetic similar to cystathionine-β-synthase (CBS) deficiency and 5, 10- methylenetetrahydrofolate reductase (MTHFR) or acquired similar to inadequate uptake of folate and vitamin B6/B12 that are cofactors for enzymes necessary for the homocysteine metabolism [7]. In early reports in 1964, Mudd et al. showed that the accumulation of homocysteine in blood and urine is due to CBS deficiency [5]. Normal homocysteine level ranges between 5 to 15 µmol/L, and hyperhomocysteinemia is classified as mild (15-30 µmol/L), moderate (30-100 µmol/L) and severe (more than 100 µmol/L) [8]. According to some reports, 5- 10% of individuals in a population have mild to moderate hyperhomocysteinemia (15-40 µM plasma homocysteine) [9]. It is estimated that two thirds of hyperhomocysteinemia cases are due to vitamin B12, vitamin B6 and folate deficiency, while folate appears to be more important in this regard [10]. Homozygous CBS subjects or individuals with hereditary defect of cobalamin metabolism have very high levels of homocysteine, and are often subjected to severe and premature atherosclerosis [11].

2. Material and method

Table (1) Instruments with their company and the origin

Equipment	Company	Origin
Centerifuge	Heraeus-Christ Gmbh	Germany
Eppendorf tube	AFICO-DISP	Jorden
Micropipette	Gilson	France
Multi-channel micropipette	Gilson	France
Deep freeze	Gorenje	Serbia
ELISA Human reader shaker and washer	Teco diagnostics	USA
Gel Tube	Xinle	China
Tips	AFICO-DISP	Jorden
Vacuum EDTA Tubes	AFICO-DISP	Jorden

Determination of Human HCY (Homocysteine):

Principle of the Assay:

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with HCY. During the reaction, HCY in the sample or standard competes with a fixed amount of HCY on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to HCY. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of HCY in the samples is then determined by comparing the O D of the samples to the standard curve.

Reagent Preparation and Storage:

1. Wash Buffer: Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.
2. Standard:

A. 500pmol/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 minutes and mix them thoroughly.

B. 250pmol/ml→7.8pmol/mlof standard solutions: Label 6 Eppendorf tubes with 250pmol/ml, 125pmol/ml, 62.5pmol/ml, 31.25pmol/ml, 15.625pmol/ml, 7.8pmol/ml , respectively.

Instruction manual: Aliquot 0.3 ml of the Sample/Standard dilution buffer into each tube. Add 0.3 ml of the above 500pmol/ml standard solution into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.

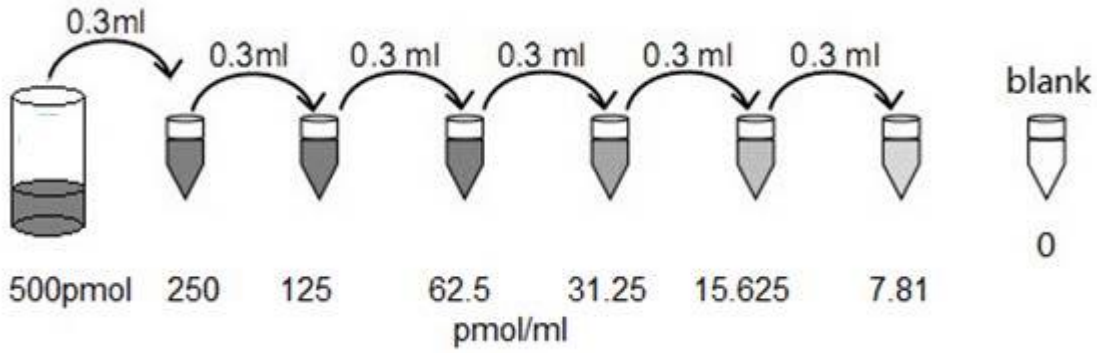

Figure (1) preparation of standard solution OF HCY (Homocysteine)

3. Preparation of Biotin- labeled Antibody Working Solution: Prepare it within1 hour before experiment.

A. Calculate required total volume of the working solution: 0.05 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

B. Dilute the Biotin- labeled Antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl of Biotin- labeled Antibody into 99μl of Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

A. Calculate required total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

B. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC dilution buffer.)

Assay Procedure:

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (zero) wells!

2. Add Sample and Biotin- labeled Antibody: Add 50μL of Standard, Blank, or Sample per well. The blank well is added with Sample/Standard dilution buffer. Immediately add 50μL Biotin-labeled Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C. (Solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can.)

3. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.

4. HRP-Streptavidin Conjugate (SABC): Add 100μL SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.

5. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time.

6. TMB Substrate: Add 90μl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 15-20 minutes.(The reaction time can be shortened or extended according to the

actual color change, but not more than 30minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)

7. Stop: Add 50µL Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. OD Measurement: Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution.

3. Results

The HCY showed decrease significant in patients (10.33 ± 1.46), when compared with control (62.45 ± 4.11) p-value <0.05, while several studies observed that the CVD an in depended of HCY as Paul Ganguly. *et al.*(2015)[12] were observed Hyper homocysteinemia may lead to an enhancement of the adverse effects of risk factors like hypertension, smoking, lipid and lipoprotein metabolism, as well as promotion of the development of inflammation. The prevalence of hype rhomocysteinemia may vary significantly between populations, and most likely depend on age, diet, and genetic background as well. Increasing age, male sex, smoking, coffee consumption, high blood pressure, unfavourable lipid profile, high creatinine and faulty diet are some of the factors associated with increased homocysteine levels figure (2) table (2).

Table (2) serum HCY in study groups

Parameters	Group	Mean ± Std.	P-value
HCY	Control	62.45 ± 4.11	0.000
	Patients	10.33 ± 1.46	

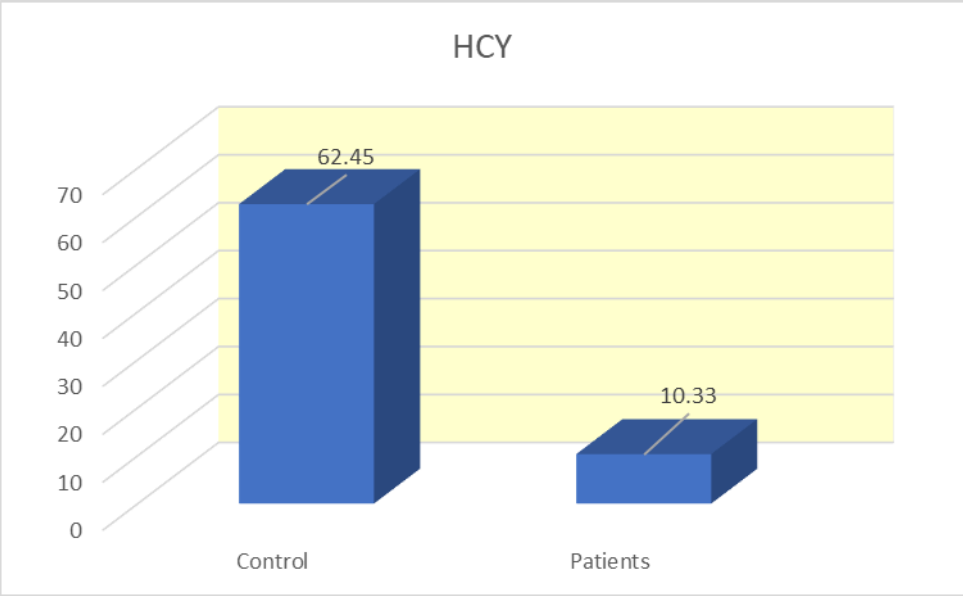

Figure (2) serum HCY level in study groups

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