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Estimation of Troponin with Ischemic Disease

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Abstract: 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 .the results observed increasing in Troponin values with Ischemic disease patient.

Introduction

The ischemic heart disease (IHD) is the most common of the cardiovascular disease (CVD) [1], in which there is a blood supply reduction to the myocardium, mostly due to atherosclerosis in the coronary arteries [2]. IHD can be classified into chronic stable angina (SA) and acute coronary syndrome (ACS) that include unstable angina (UA) and myocardial infarction (MI) [3]. The primary prevention of IHD is dependent upon the ability to identify high risk individuals and by studying the cardiac markers long before the development of overt major adverse cardiac events (MACEs) [4].

Troponin

Cardiac troponins are regulatory proteins that regulate the calcium-mediated interaction of actin and myosin, that leads to striated muscle contraction and relaxation. Troponin complex is made up of three subunits: troponin C, which binds calcium, troponin I, which inhibits actin-myosin connections, and troponin T, which connects to tropomyosin and enables contraction. Troponin C is produced by cells in both cardiac and skeletal muscle, whereas troponins I and T have amino acid sequences that are specific to cardiac muscle. This distinction has promoted the creation of quick, quantitative tests for detecting increases in cardiac troponins in serum. Due to its better tissue specificity and sensitivity for MI, as well as its utility as a prognostic indicator, troponin is the recommended biomarker to be used in the diagnosis of acute MI and various CVD diseases such as atherosclerosis[5,6].

Material and Method

Determination of Human cardiac troponin I(cTn-I)

I serum troponin activity was tested using a kit assayed according to the manufacturer's instructions (HCUSABIO Technology Laboratory, Cat. No. CSB-E05139h. USA).

PRINCIPLE OF THE ASSAY:

The quantitative sandwich enzyme immunoassay method is used in this assay. A microplate has been pre-coated with an antibody specific for cTn-I. Pipette standards and samples into the wells, and any cTn-I present is bound by the immobilized antibody. After eliminating any unattached compounds, the wells are incubated with a biotin-conjugated antibody specific for cTn-I. After wash, the wells are treated with avidin conjugated Horseradish Peroxidase (HRP). After removing all unbound avidin-enzyme reagent, a substrate solution is added to the wells, and color develops in proportion to

the quantity of cTn-I bound in the first step. The color development is halted, and the color intensity is measured.

DETECTION RANGE :
47 pg/ml-3000 pg/ml.

SENSITIVITY:
Human cTn-I has a minimum detectable dosage of less than 11.75 pg/ml. The assay's sensitivity, or Lower Limit of Detection (LLD), was determined as the lowest protein concentration which can be distinguished from zero. The mean O.D value of 20 replicates of the zero-standard multiplied by their three standard deviations was calculated.

REAGENT PREPARATION

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.
- It is advised that distilled water be used in the preparation of reagents. Contaminated water or reagent preparation containers will have an effect on the detection result.

1- Biotin-antibody (1x)- Before opening, centrifuge the vial.

Biotin antibody needs a 100-fold dilution. 10 l of Biotin-antibody+ 990 l of Biotin-antibody Diluent is a proposed 100-fold dilution.

2- HRP-avidin (1x)- Before opening, centrifuge the vial.

A 100-fold dilution of HRP-avidin is required. 10 l of HRP-avidin + 990 l of HRP-avidin Diluent is a proposed 100-fold dilution.

3- Wash Buffer (1x)- If crystals develop in the concentrate, bring to room temperature and stir gently until the crystals dissolve entirely. To make 500 ml of Wash Buffer, dilute 20 ml of Wash Buffer Concentrate (25 x) with deionized or distilled water (1 x).

4- Standard

Centrifuge the standard vial for 30 seconds at 6000-10000rpm.

With 1.0 ml of Sample Diluent, reconstitute the Standard. Other diluents should not be substituted. This reconstitution yields a 3000 pg/ml stock solution. Let the standard to rest for at least 15 minutes with moderate agitation after mixing to ensure complete reconstitution before producing dilutions. 250 l of Sample Diluent should be pipetted through every tube (S0-S6). Make a 2-fold dilution series with the stock solution (below). Before the next transfer, properly mix each tube. The high standard (3000 pg/ml) is the undiluted Standard. As the zero standard (0 pg/ml), Sample Diluent is used.

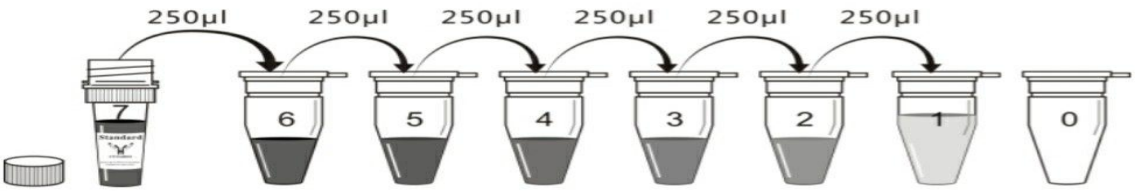

Figure (2-3) preparation of standard solution for troponin

Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	3000	1500	750	375	187.5	94	47	0

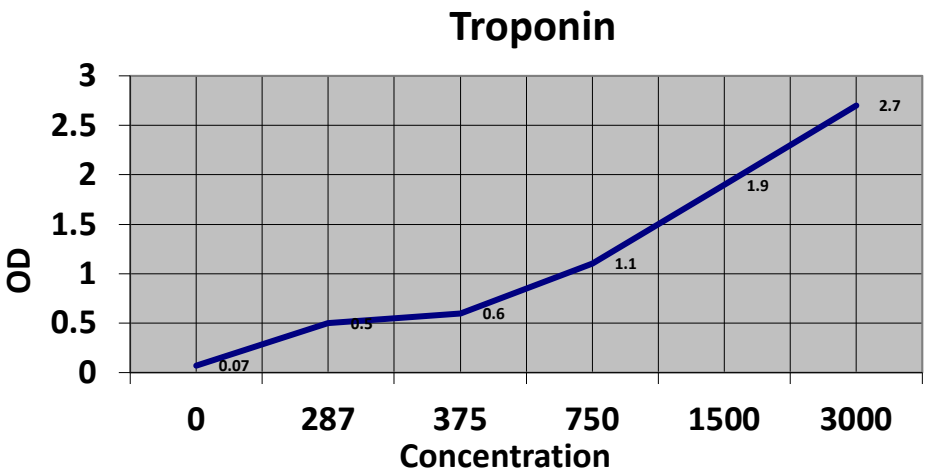

Figure (2-5) standard curve of troponin

ASSAY PROCEDURE

Before using any reagents or samples, bring them to room temperature. Before the test, centrifuge the material again once it has thawed. It is strongly advised that all samples and standards be tested in duplicate.

1. Make preparations for all reagents, working standards, and samples according to the instructions in the preceding sections.
2. Refer to the Assay Layout Sheet to calculate the amount of wells to be utilized, then return any residual wells and desiccant to the pouch and close the ziploc, storing unused wells at 4°C.
3. Pour 100µl of standard and sample into each well. Cover using the included sticky strip. Incubate at 37°C for 2 hours. A plate layout is supplied to record the standards and samples that have been analyzed.
4. Discard the liquid from each well without washing.
5. To every well, add 100µl of Biotin-antibody(1x). Replace the sticky strip. Incubate at 37°C for 1 hour. (Biotin-antibody(1x) could be cloudy. Bring to room temperature and gently stir until the solution seems homogenous.)
6. Aspirate and wash every well, then redo the process two more times for a total of three washes. Load every well with Wash Buffer (200l) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let stand for 2 minutes; full removal of liquid at each stage is critical for excellent performance. Discard any leftover wash Buffer by aspirating or decanting after the last wash. Invert the plate and wipe it cleanly with paper towels.
7. To every well, add 100µl of HRP-avidin (1x). Apply a clean adhesive strip to the microtiter plate. Incubate at 37°C for 1 hour.
8. Repeat the aspiration/wash procedure five times as described in step 6.
9. Fill each well with 90l of TMB Substrate. Incubate at 37°C for 15-30 minutes. Keep out of direct light.
10. Pour 50µl Stop Solution into every well and gently tap the plate to achieve complete mixing.

11. Using a microplate reader set to 450 nm, measure the optical density of every well in 5 minutes. Set the wavelength adjustment to 540 nm or 570 nm if it is provided. Subtract 540 nm or 570 nm values from 450 nm readings. This subtraction will compensate for optical flaws in the plate. Direct readings at 450 nm without adjustment may be higher and less accurate.

Result and discussion

The results of the statistical analysis showed a significant increase in the troponin level in the patients (373.71 ± 24.47) compared to the control (142.18 ± 14.69) group, group p-value < 0.05 , Troponin result was agree with Eduard M. Laufer ,*et.al* .(2010)[7] were observed that a positive correlation between the extent of CAD and the levels of hs-cTnT, result was agree with Ana Rita Castro,et ,al.(2018)[8] . It also increases the uptake of LDL by macrophages. Thus, in the process of inflammatory response CRP is considered both a biomarker and a risk factor for CVDs. As referred before, it slowly returns to basal levels making it a less accurate biomarker in treatment evolution. Thus, there is still a lack of consensus in the use of hsCRP in the clinical practice. figure (1) table (1).

Table (1) serum Troponin in study groups (patients compared with controls)

Parameters	Group	Mean ± Std	P-value
Troponin	Control	142.18 ± 14.69	<0.000
	Patients	373.71 ± 24.47	

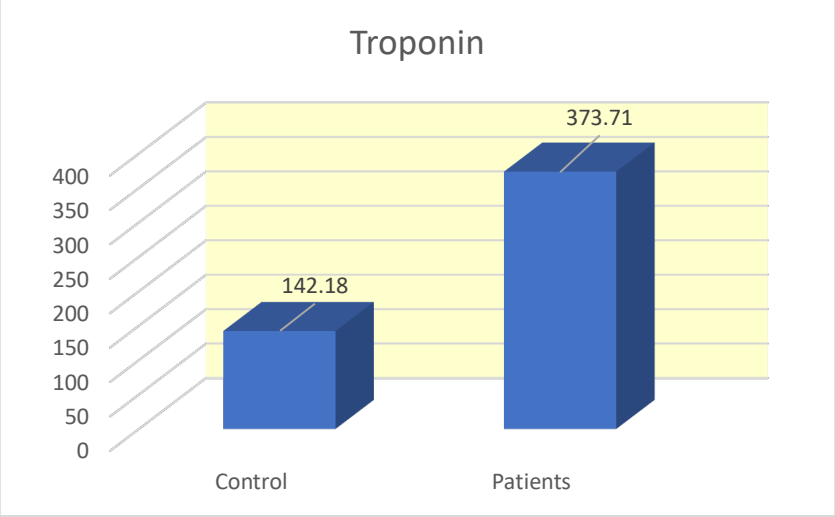

Figure (1)serum Troponin level in study groups

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