INTERNATIONAL JOURNAL OF RESEARCH IN MEDICAL SCIENCES & TECHNOLOGY

e-ISSN:2455-5134; p-ISSN: 2455-9059

Creatine Kinase-MB and Beta-Hydroxybutyrate Dehydrogenase with Antioxidant Agent in Heart Disease Patients

***Eman AW Al-Hyali, **Mahmoud AM Fakhri**

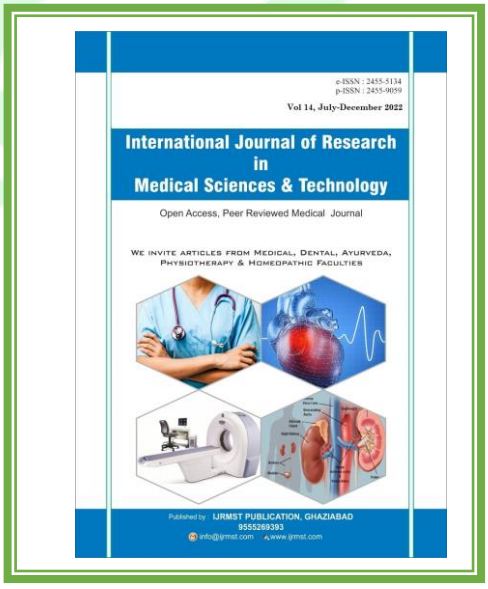
*Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq
**Department of Medical Physics, College of Science, University of Mosul, Mosul, Iraq

Paper Received: 16 August 2022; **Paper Accepted:** 04 November 2022;
Paper Published: 20 December 2022

DOI: http://doi.org/10.37648/ijrmst.v14i01.017

How to cite the article:

Eman AW Al Hyali, Mahmoud AM Fakhri, Creatine Kinase-MB and Beta-Hydroxybutyrate Dehydrogenase with Antioxidant Agent in Heart Disease Patients, IJRMST, July-December 2022, Vol 14, 140-153, DOI: http://doi.org/10.37648/ijrmst.v14i01.017



ABSTRACT

The research Measures the activity of CK-MB & BHBDH enzymes with some biochemical parameters and TAO for the heart Patients group (147), also to the control group (94). The results showed an increase in activity for both enzyme CK-MB (33.29±4.96 U/L) and BHBDH (224.12± 0.82 U/L) in serum for the heart Patients group compared to the control (8.67±0.41 U/L, 136.73±0.95 U/L) respectively. The results also showed a significant difference in the activity of CK-MB and BHBDH in serum for the angina pectoris group with the myocardial infarction group. The results indicated that there was a significant increase activity of peroxidase in serum for the heart Patients group (92.33 ± 4.68 U/L) compared to the control group (47.66± 0.60 U/L), while there was a significant decrease in the activity of Catalase and TAO concentration in serum for heart patients group (25.53 ±0.47 U/L, 0.89 ±0.01 µmol/ml) compared to the activity of Catalase and TAO concentration in serum for the control group (53.11±0.50 U/L, 1.261 ± 0.02 µmol/ml) respectively. Potassium, iron, and copper concentrations in the serum of the heart patients group were significantly higher than those in the serum of the control group (4.07 0.06 mmol/l, 122.45 0.95 g/dl, and 103.95 1.46 g/dl, respectively). Zinc concentration was also significantly lower in the serum of the heart patients group (73.48 2.08 g/dl) than in the control group. Although there was no discernible difference in the serum calcium concentration between the cardiac patient's group (8.53 0.08 mg/dl) and the control group (8.545 0.06 m/dl). Finally, the findings in the group of individuals with heart disease demonstrated a substantial negative correlation between the activity of serum CK-MB and serum calcium levels. It was also found that there was a substantial negative correlation between the serum CK-MB activity and the serum peroxidase and TAO concentrations, and a significant positive correlation between the serum catalase and iron concentrations.

Keywords: *Myocardial Infarction, Angina Pectoris, Creatine kinase–MB, BHBDH, TAO, Minerals*

INTRODUCTION

Heart disease(HD) is a comprehensive term that describes a wide range of disorders that affect the heart's composition and operation, including blood vessels and various organs that supply the heart with blood and oxygen and is considered the main cause of death (1).

One of the diseases of the heart is myocardial infarction, which is the occurrence of necrosis or death in part of the heart muscle cells as a result of ischemia deficiency due to the presence of a blood clot that blocks the artery feeding the heart muscle cells, where the blockage is complete or partial(2). Angina is the narrowing of the heart's blood vessels and the decrease in the amount of oxygen that travels through the arteries to nourish the tissue, so the ability of the heart muscle to contract and relax is reduced, which causes chest pain, as it occurs in the case of physical activity or psychological agitation(3).

CK-MB It serves as a potential adjuvant test in clinical and forensic medicine and is a biomarker of heart damage (4), biomarkers of the myocardium, such as creatine kinase MB (CK-MB) in the early identification of myocardial infarction, Within 4 to 8 hours of the commencement of chest discomfort, CK-MB levels can be found in the blood; they increase when the heart muscle cells are injured, and they return to normal after (48) hours. (5).

Enzyme Beta-hydroxybutyrate Dehydrogenase (BHBDH) (EC 1.1.1.30) is one of the redox enzymes that belong to the dehydrogenase class(6). It is the first enzyme to degrade ketone bodies, and the last enzyme to form ketone bodies. Also, the reaction of BHBDH is not essential for the formation and decomposition of ketone bodies from a physiological point of view, because the liver can produce acetoacetate into the blood circulation in the period of acidity of the blood, and the enzyme BHBDH is more abundant than acetoacetate in the circulation, and this confirms that The enzyme is important for the natural flow of ketone bodies to produce energy(7).

CAT enzyme It is an enzyme oxidoreductase (EC 1.11.1.6) that plays an important role in quenching reactive oxygen species (ROS) as hydrogen peroxide, which often forms by-products of aerobic respiration(8). Catalase (a quaternary protein consisting of a 60 kDa monomer) is a metal enzyme that catalyzes the degradation of H_2O_2 (a harmful oxidizing agent). Catalase (CAT) is expressed in all major organs (especially in the liver and kidneys) and in red blood cells to protect cells from oxidative damage (9).

The oxidoreductase enzyme (EC 1.11.1.7), also known as peroxidase, is a heme protein or iron porphyrin. It is a member of a large family of enzymes that are involved in a wide range of crucial processes, including oxidation and

reduction reactions by the mechanism of free radicals that convert many compounds into oxidized products. The name is derived from the components of peroxide that are oxidized throughout the procedure. (10)

Total1 antioxidant capacity (TAO) One of the most common strategies for assessing the balance of free radicals in chemical and biological systems is TAO determination (11). Antioxidants can prevent types of free radicals from oxidizing lipids, proteins, and DNA(12). Free radicals are substances that contain one or more unpaired electrons. Unpaired or free electrons are responsible for the interactions of free radicals with various biomolecules (13).

Minerals are the chemical element that is formed in the soil and cannot be produced by living things, but they are needed by living things as a vital nutrient to perform essential activities for life. The soil provides minerals to plants. The majority of the minerals in the human diet originate from drinking water or eating plants and animals. (14).

MATERIALS AND METHODS:

Study subjects: The study was conducted at Al Salam Hospital and Mosul Cardiac Center The study was conducted on Al-Salam Hospital and Heart Center in Mosul, 147 blood samples from people with heart conditions were taken, including (68) myocardial infarctions (40 men and 28 women) and (79) angina pectoris (46 males and 33 females). Al-Salam and the Mosul Center for Cardiac Medicine and Surgery, whose ages ranged from 80 to 30, and (94) blood samples from individuals who appeared to be in good health, including (34) men and (60) women whose ages ranged from 67 to 30. After coagulation, samples were centrifuged and kept for later examination.

MEASUREMENT OF PARAMETERS:

Creatine kinase–MB activity was estimated using a Cobas device from Roche, Germany, using the ultraviolet immunofluorescence method, which tracks the course of the reaction at 340nm wavelength(15). Beta-hydroxybutyrate dehydrogenase activity in serum was estimated by method (16), nicotinamide adenine dinucleotide solution (30 mM) and sodium salt of -hydroxybutyrate (160 mM) (the substrate) was utilized. The reaction mixture was kept at pH 7.8, a buffer of 100 mM Tris-HCl, and a temperature of 37 °C.

The catalase enzyme's activity was calculated using the conventional approach because it relies on the peroxidase enzyme's enzymatic oxidation of hydrogen peroxide to produce a coloured material whose absorption intensity can be measured at a wavelength of 410 nm (17). The activity of the peroxidase enzyme was estimated using the method of Nelson & Kulkarni, 1990. This method relies on the enzymatic oxidation of hydrogen peroxide by the peroxidase enzyme to produce a coloured substance whose absorption

intensity can be measured at a wavelength of 470 nm(18).

TAO in serum was estimated using a ready-made analysis kit from the Chinese company Solarbio, where antioxidants and antioxidant enzymes are detected in the samples, and the principle of action is the blue-coloured reduction of the ferric ion Fe+3 to the ferrous ion Fe+2 (19). By using the flame atomic absorption method, which is a sensitive method for the determination of more than 60 elements, iron, potassium, zinc, and copper were assessed in blood serum. (20), and are frequently employed in worldwide laboratories to calculate traces of components in various models. This method is precise in determining trace element concentrations. Where great accuracy, high sensitivity, high sensitivity, and selectivity have their limits (21).

Statistical Analysis

Quantitative data were analyzed in SPSS version 22.0. All data were presented as means ± standard error values. Correlation between disease status and measured dependent variables was evaluated by determining the correlation coefficient (r).

RESULTS AND DISCUSSION:

Clinical parameters were examined in the cardiac patient's group and compared to the healthy group, as shown in Table 1. When compared to the control group, the heart patient group showed a large increase in CK-MB enzyme activity, which is explained by the death of cardiac cells and myocardial necrosis, which causes the enzyme to be released into the blood. According to the researchers (22).

The results in Table 1 indicated that there was a significant increase in serum activity of BHBDH at P≤ 0.001in heart patients compared to group control, As in heart patients, BHB Substance increases, and thus the effectiveness of the BHBDH will increase, as indicated by the researchers(23) As well as damage to some heart cells, which leads to increase in serum activity of BHBDH (24)

The outcomes in table 1 also demonstrated a notable rise in serum activity of peroxidase at P≤ 0.001 in heart patients compared to the control group, As indicated by the researchers (25). This is due to the increase in serum activity of peroxidase due to the increase of patients' oxidative stress and the increase in the active oxygen species.

While the results presented in Table 1 serum activity indicated a substantial decline. Both catalase and TAO at P≤ 0.001 compared to the group control, and this is congruent with what was discovered in the group cardiac patients. (26) Where the reason for the decrease in serum activity of catalase &TAO concentration is attributed to the increase in oxidative stress in heart patients.

Also, the results in table 1 indicate that there is no significant difference in calcium concentration in group heart patients compared to group control at (P > 0.05).

According to Table 1's findings, the potassium content in the group of heart patients increased significantly at P 0.001 when compared to the group control, which is consistent with the researcher's findings. (27). The reason for the increase in potassium in heart patients is due to physiological disorders that lead to its liberation from the liver cells into the plasma, causing weakness in the heart muscle.

As indicated by the results in table 1, the concentration of iron was significantly higher in the group of heart patients compared to the group controls, with a P value of 0.001(28). This is because iron stimulates the oxidation of LDL and its capture by phagocytic cells to form foamy cells, which worsens heart disease.

According to table 1's findings, there was a significant rise in copper content in the group of heart patients compared to the group controls at (P0.001); Fenton's theory for this is that copper has an oxidative effect. reactions that result in cell death and serve as a catalyst for the enzymes he mentioned that catalyze the oxidation of LDL-C molecules (29The reason is due to the rise in oxidative stress, as zinc is considered a catalyst for many enzymes involved in metabolic processes to reduce oxidative stress, as he indicated, even though observed results showed that there was a significant decrease in zinc concentration at (P 0.001) in group heart patients compared to group control. (30).

Table1. Clinical parameters of heart patients group compared to control group.

Clinical parameters	Control group Mean ± S.E	heart Patients Group Mean ± S.E	p-value
CK-MB (U/L)	8.67 ± 0.41	33.29 ± 4.96	≤ 0.001 p
BHBDH (U/L)	136.73±0.95	224.11 ± 0.82	≤ 0.001 p
Peroxidase (U/L)	47.66 ± 0.60	92.33 ± 4.68	≤ 0.001 p
Catalase (U/L)	53.11 ±0.50	25.53 ± 0.47	≤ 0.001 p
TAO (µmol/ml)	1.261 ± 0.02	0.89 ± 0.01	≤ 0.001 p
Ca^{+2} (mg/dl)	8.54 ± 0.06	8.53 ± 0.08	> 0.05 p
K^{+1}(mmol/l)	4.07 ± 0.06	6.70 ± 0.23	≤ 0.001 p
Fe^{+2} (µg/dl)	122.45 ± 0.95	185.40 ± 0.98	≤ 0.001 p
Cu^{+2} (µg/dl)	103.95 ± 1.461	217.73 ± 5.90	≤ 0.001 p
Zn^{+2} (µg/dl)	92.48 ± 1.326	73.48 ± 2.08	≤ 0.001 p
Note: A very significant difference P≤ 0.001, no significant difference p> 0.05			

CK-MB enzyme activity was measured in the serum of patients with myocardial infarction and angina pectoris and compared with the control group, as shown in Figure 1. It was observed that there was a significant increase in the activity of CK-MB at P≤0.001. in both patients (myocardial infarction & angina pectoris) within group control, and a significant increase in serum activity of CK-MB at P≤0.01 in patients with angina pectoris compared to patients with myocardial infarction, and the reason is that enzyme is one of the important diagnostic indicators, as it acts as an indicator of myocardial infarction, and the reason for the high activity of CK-MB is necrosis Cardiac muscle and heart cell death, so the enzyme is released into the blood (24). The activity of CK-MB in angina pectoris is less than that of myocardial infarction, The reason is that angina pectoris occurs without major changes in heart enzymes, as mentioned by the researcher (27).

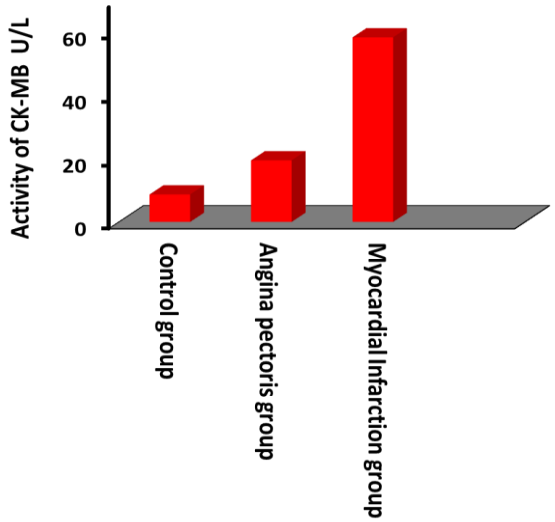


Figure 1. The activity of serum CK-MB in the control group, angina pectoris group and myocardial infarction group.

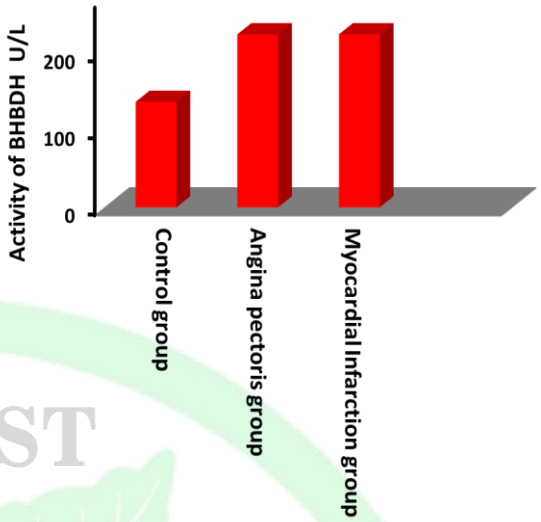


Figure 2. The activity of serum BHBDH in the control group, angina pectoris group and myocardial infarction group

It is evident from Figure 2 that there is a significant increase in the BHBDH enzyme activity in patients with angina pectoris and heart attack compared to the control group, as well as a significant increase in the enzyme at (P0.001) in angina pectoris compared to myocardial infarction, and this is consistent with previous research. BHBDH enzyme activity was measured in the serum of patients with myocardial infarction & angina pectoris and compared with the control group (23), wherein damage to some heart cells and an increase in BHB in patients with heart disease both increase the serum activity of BHBDH, as mentioned by (31).

The activity of peroxidase was measured when compared to the control group, in the serum of patients with myocardial infarction and angina pectoris. A significant increase was observed at ($P \leq 0.001$) in serum activity for both groups (myocardial infarction and angina pectoris) compared to group control as shown in figure 3, the reason for the increase in serum activity peroxidase is due to the increase in oxidative stress and the increase in active oxygen species in both patients with group control. This is consistent with (25). While there was no significant difference in serum activity of peroxidase at $p>0.05$ between angina pectoris and myocardial infarction.

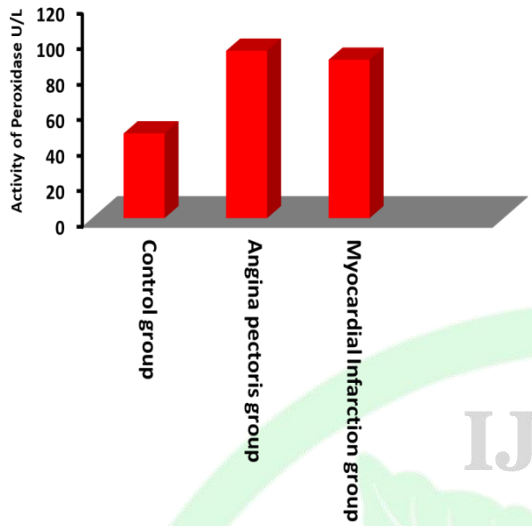


Figure 3. The activity of serum peroxidase in the control group, angina pectoris group and myocardial infarction group.

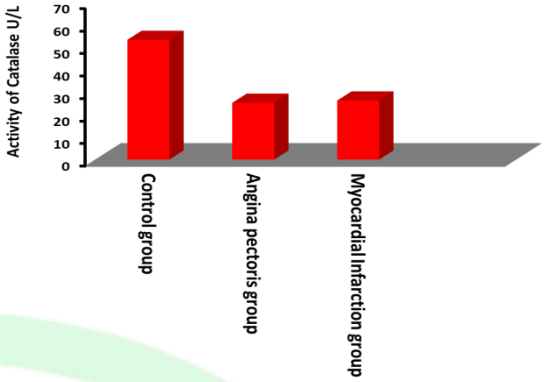


Figure 4. The activity of serum catalase in the control group, angina pectoris group and myocardial infarction group.

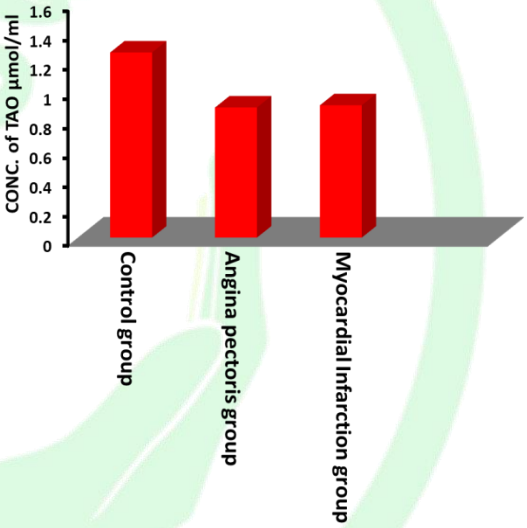


Figure 5. The activity of serum TAO in the control group, angina pectoris group and myocardial infarction group.

The findings in figures 4 and 5 demonstrated a substantial decrease in serum catalase activity and total antioxidant capacity in the blood serum of patients with angina pectoris and myocardial infarction compared to the control group at (P0.001). The explanation is that the antioxidant effects of the enzymes catalase and TAO, which reduce LDL oxidation, limit its deposition on artery walls, prevent lipid peroxidation, and platelet aggregation, and suppress the inflammatory response, play a significant role in protecting against heart disease. (32,33).

The results shown in table 2 show that there is no significant difference in calcium concentration between the groups of people with angina pectoris and those without it at (P>0.05), but that there is a difference between the groups of people with myocardial infarction and those without it at (P0.05). According to studies, calcium deposition

causes calcification of blood arteries, which explains why an elevated calcium concentration is linked to an increased risk of heart disease, particularly myocardial infarction (34).

Table 2. Mineral assessment in a group of myocardial infarction and angina pectoris and compared to the control group.

minerals	Controlagroup Mean ± S.E	Myocardial infarction group Mean ± S.E	Angina Pectoris group Mean ± S.E
Ca^{+2}	8.545±0.060 A	8.171±0.138 B*	8.730±0.092 A
K^{+}	4.079±0.057 A	7.201±0.593 B***	6.424±0.125 B***
Fe^{2}	122.45±0.952 A	185.91±1.459 B***	185.11±1.295B***
Cu^{+2}	103.95±1.461 A	224.564±12.059 B***	213.930±6.299B***
Zn^{+2}	92.489±1.326 A	73.436±4.351 B***	73.503±2.175B***
*** A very significant difference P≤ 0.001 , *aa significant difference p≤ 0.05			

According to the results presented in Table 2, both groups of patients with angina and myocardial infarction had significantly higher potassium concentrations than the control group ($P \leq 0.001$). In particular, as we indicated earlier, the heart to physiological disorders that lead to its release from the liver cells into the blood plasma causing weakness of the heart muscle. (27). The results also indicated that there was no difference in potassium levels between the group with angina pectoris and those with myocardial infarction ($P > 0.05$).

According to the findings in Table 2, both patient groups with angina pectoris and myocardial infarction had significantly higher iron concentrations ($P \leq 0.001$) than the group serving as the control. The cause is thought to be iron, which increases LDL oxidation and macrophage capture of LDL to form foam cells. This makes heart disease more prevalent, and this is consistent with (28). The results also showed that there was no significant iron concentration ($P>0.05$) in the group of angina pectoris compared to myocardial infarction.

The findings in Table 2 revealed a significant increase at ($P\leq0.001$) in the concentration of copper for both groups (myocardial infarction patients and angina pectoris) compared to group control, and the reason is due to the oxidative effect of copper by Fenton reactions that

destroy cells and act as a catalyst for enzymes that catalyze oxidation LDL-C particles and this is consistent with (29). The results also showed that there was no significant copper concentration (P>0.05) in a group of angina pectoris compared to myocardial infarction.

The results showed that there was a significant decrease in zinc concentration at (P≤0.001) in both groups (myocardial infarction patients and angina pectoris) due to increased oxidative stress, as zinc, is a catalyst for many enzymes involved in metabolic processes to reduce oxidative stress as well (30). The results also showed that there was no significant zinc concentration (P>0.05) in a group of angina patients compared to myocardial infarction.

Table 3. The relationship Between clinical parameters with CK-MB and BHBDH enzymes in heart Patients group.

Clinical parameters	CK-MB		BHBDH	
	r- value	p- level	r- value	p- level
Peroxidase	----------	---------	-0.245*	≤ 0.05
Catalase	----------	----------	0.822**	≤ 0.01
Fe	----------	----------	0.803**	≤ 0.01
TAO	----------	----------	-0.406**	≤ 0.01
Ca	-0.276	≤ 0.01	--------	--------

The results in table 3 showed a linear correlation between the activity of CK-MB and BHBDH in serum with clinical parameters in the heart patients group, there was a very strong inverse link observed between the activity of BHBDH and activity of peroxidase in serum at P≤0.05, while there was a significant positive relationship between the activity of BHBDH with the activity of catalase in serum at P≤0.01. The reason for that BHBDH reduces oxidative stress, prevents lipid peroxidation and protein oxidation, increases antioxidant protein levels, and improves mitochondrial respiration and ATP production(35).

The results showed in table 3, there was a positive significant relationship at P≤0.01 between the activity of BHBDH and iron concentration in the serum of heart patients group, and the reason is that BHBDH increases in heart patients (23), in addition,

iron concentration increases in heart patients, and this is consistent with (28).

Table 3 observed a significant negative correlation between the activity of BHBDH and TAO concentration in serum of the heart patients group at P≤ 0.01, so that TAC protects against oxidative stress which agrees with(33), While an increase in BHB in the heart patients leads to an increase in activity of BHBDH enzyme and thus an increase in oxidative stress, as indicated with(23).

A significant negative correlation was observed in table 3 between the activity of CK-MB with calcium concentration in the serum of the heart patients group at P≤ 0.01. This agrees with (36), and the reason is that calcium has an important role in the electrical activity of the heart and its function in pumping blood, as the pumping of blood to the heart muscle decreases, and therefore due to a lack of calcium concentration.

CONCLUSION

We conclude from the research that there is an increase in the activity of CK-MB, and BHBDH in serum for the heart Patients group (angina pectoris, myocardial infarction groups) compared to the control group.

Also, an increase was observed inactivity of peroxidase, the concentration of iron, potassium and copper in serum for the heart Patients group compared to the control group, while a decrease was found in the activity of catalase, concentration of TAO and zinc in serum for heart Patients group compared to control group. Their results showed no significance in the concentration of calcium.

There was also a positive correlation ship between the activity of BHBDH in serum for heart patients with the activity of Catalase and concentration of iron, while a negative correlation ship was found with the activity of peroxidase and concentration of TAO. Finally, There was a negative correlation between the activity of CK-MB in serum for heart patients and calcium.

ACKNOWLEDGEMENT

We would like to thank the administration of Al-Salam Hospital and the Center for Cardiac Diseases in Mosul, as well as the doctors and laboratory staff for their assistance in completing this research, as well as the management of laboratories at the Faculty of Science, Department of Chemistry, and Medical Physics, without whom this research would not have been completed.

Financial support and sponsorship: Nil

Conflict of Interest: None

REFERENCES

1. Mohan, S., Thirumalai, C., & Srivastava, G. (2019). Effective heart disease prediction using hybrid machine learning techniques. IEEE access, 7, 81542-81554
2. Saleh, M., & Ambrose, J. A. (2018). Understanding myocardial infarction. F1000Research, 7
3. Dechend, R., & Predel, H. G. (2022). Exercise in Patients with Chronic Angina Pectoris: Friend or Foe. Cardiology and Cardiovascular Medicine, 6, 364-373.
4. Ay, H., Arsava, E. M., & Sarıbas, O. (2002). Creatine kinase-MB elevation after stroke is not cardiac in origin: comparison with troponin T levels. Stroke, 33(1), 286-289.
5. Carvalho, G., & Rassi, S. (2016). The prognostic value of CK-MB in Acute Myocardial Infarction in developing countries: a descriptive study. Angiology, 4(3).
6. Hoque MM, Shimizu S, Juan EC, Sato Y, Hossain MT, Yamamoto T, Imamura S, Suzuki K, Amano H, Sekiguchi T, Tsunoda M, Takénaka A. Structure of D-3-hydroxybutyrate dehydrogenase prepared in the presence of the substrate D-3-hydroxybutyrate and NAD+. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2009 Apr 1;65(Pt 4):331-5. doi: 10.1107/S1744309109008537. Epub 2009 Mar 26. PMID: 19342772; PMCID: PMC2664752.
7. Otsuka, H., Kimura, T., Ago, Y., Nakama, M., Aoyama, Y., Abdelkreem, E.,&Fukao, T. (2020). Deficiency of 3-hydroxybutyrate dehydrogenase(BHBDH1) in mice causes low ketone body levels and fatty liver during fasting. Journal of inherited metabolic disease, 43(5), 960-968
8. Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol chem, 195(1), 133-140.
9. Rakotoarisoa, M., Angelov, B., Espinoza, S., Khakurel, K., Bizien, T., & Angelova, A. (2019). Cubic liquid crystalline nanostructures involving catalase and curcumin: BioSAXS study and catalase peroxidatic function after cubosomal nanoparticle treatment of differentiated SH-SY5Y cells. Molecules, 24(17), 3058.
10. van Lith, R., & Ameer, G. A. (2016). Antioxidant polymers as biomaterial In Oxidative Stress and Biomaterials (pp. 251-296). Academic Press
11. Fraga, C. G., Oteiza, P. I., & Galleano, M. (2014). In vitro measurements and interpretation of total antioxidant capacity. Biochimica et Biophysica Acta (BBA)-General Subjects, 1840(2), 931-934.
12. Venditti, P., & Di Meo, S. (2020). The role of reactive oxygen species in the life cycle of the mitochondrion. International journal of molecular sciences, 21(6), 2173.
13. Jakubczyk K, Kałduńska J, Dec K, Kawczuga D, Janda K. Antioxidant properties of small-molecule non-enzymatic compounds. Pol Merkur Lekarski. 2020 Apr 22;48(284):128-132. PMID: 32352947.14.
14. Godswill, A. G., Somtochukwu, I. V., Ikechukwu, A. O., & Kate, E. C. (2020). Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. International Journal of Food Sciences, 3(1), 1-32.
15. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med. 2007; 45(9):1240-3. doi: 10.1515/CCLM.2007.254. PMID: 17635066.
16. Tal S, Smirnoff P, Okon Y. Purification and characterization of D (-)-β-hydroxybutyrate dehydrogenase from Azospirillum brasilense Cd. Microbiology. 1990 Apr 1;136(4):645-9. https://doi.org/10.1099/00221287-136-4-645
17. Boriskin, P., Deviatkin, A., Nikitin, A., Pavlova, O., & Toropovskiy, A. (2019, December). Relationship of catalase activity distribution in serum and tissues of small experimental animals. In IOP Conference

Series: Earth and Environmental Science (Vol. 403, No. 1, p. 012113). IOP Publishing.

18. Nelson,J.L. and Kulkarn, A.P.(1990).Partial purification and characterization of peroxidase activity from human placenta.Biochem.J.268:79-747.
19. Pellegrini N, Serafini M, Salvatore S, Del Rio D, Bianchi M, Brighenti F. Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different in vitro assays. Mol Nutr Food Res. 2006 Nov;50(11):1030-8. doi: 10.1002/mnfr.200600067. PMID: 17039458.
20. D'haese, P. C., Lamberts, L. V., Vanheule, A. O., & De Broe, M. E. (1992).Direct determination of zinc in serum by Zeeman atomic absorption spectrometry with a graphite furnace. Clinical chemistry, 38(12), 2439-2443.
21. Sneddon, J. (1997)." Advances in atomic spectroscopy. Elsevier". Minor Higher Education Group Inc., 3:2
22. Aydin S, Ugur K, Aydin S, Sahin İ, Yardim M. Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag. 2019 Jan 17;15:1-10. doi: 10.2147/VHRM.S166157. PMID: 30697054; PMCID: PMC6340361.
23. Chu, Y., Zhang, C., & Xie, M. (2021). Beta-hydroxybutyrate, friend or foe for stressed hearts. Frontiers in aging, 16.
24. Jasim, R. F. (2021). Effect of plasma isolated Orexin-A on the regulation of metabolites in male rats
25. Dubois-Deruy, E., Peugnet, V., Turkieh, A., & Pinet, F. (2020). Oxidative stress in cardiovascular diseases. Antioxidants, 9(9), 864.
26. Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxid Med Cell Longev. 2019 Nov 11;2019:9613090. doi: 10.1155/2019/9613090. PMID: 31827713; PMCID: PMC6885225.
27. Basher ,M.(2008).The Relationship Between The Heart Functions Decline And Some Biochemical Changes Of Vascular System Patients In Karbala.
28. Cornelissen, A., Guo, L., Sakamoto, A., Virmani, R., & Finn, A. V. (2019). New insights into the role of iron in inflammation and atherosclerosis. EBioMedicine, 47, 598-606.
29. Bhattacharya, P. T., Misra, S. R., & Hussain, M. (2016). Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica, 2016.
30. Gać, P., Czerwińska, K., Macek, P., Jaremków, A., Mazur, G., Pawlas, K., & Poręba, R. (2021). The importance of selenium and zinc deficiency in cardiovascular disorders. Environmental Toxicology and Pharmacology, 82, 103553.
31. Kley, R. A., Schmidt-Wilcke, T., & Vorgerd, M. (2018). Differential diagnosis of HyperCKemia. Neurology International Open, 2(01), E72-E83.
32. Di Meo, S., & Venditti, P. (2020). Evolution of the knowledge of free radicals and other oxidants. Oxidative Medicine and Cellular Longevity, 2020.
33. Yaghoubi, N., Youssefi, M., Jabbari Azad, F., Farzad, F., Yavari, Z., & Zahedi Avval, F. (2022). Total antioxidant capacity as a marker of severity of COVID-19 infection: Possible prognostic and therapeutic clinical application. Journal of Medical Virology, 94(4), 1558-1565.
34. Park, B., & Lee, Y. J. (2019). Borderline high serum calcium levels are associated with arterial stiffness and 10-year cardiovascular disease risk determined by Framingham risk score. The Journal of Clinical Hypertension, 21(5), 668-673.
35. Puchalska, P., & Crawford, P. A. (2017). Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. Cell metabolism, 25(2), 262-284.
36. Sutanto, H., & Heijman, J. (2019). The role of calcium in the human heart: with great power comes great responsibility. Front Young Minds, 7(65), 10-3389.