

Comparative Study of Serum lactic Acid, Lactate Dehydrogenase and Lipid Profile in Ischemic Heart Disease Patients and Healthy Control

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Abstract

The term ischemic heart disease (IHD) defines a disease spectrum of diverse etiology, with the common factor being on imbalance between myocardial oxygen supply and demand . Fifty patients (30 male and 20 female) attending Ibn- Al- betar cardiac center, the mean age of male was 65 years and 58 years for female were included in the present study , Thirty healthy subjects (15 male and 15 female) of matched age were used as control groups.

Some biochemical parameters including lipid and lipoprotein, total cholesterol (TC) , triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL), in addition to lactic acid and lactate dehydrogenase (LDH) activities , were evaluated in the sera of IHD patient groups and control group.

The results indicate a significant increase in all parameters except HDL which showed a significant decrease in the sera of male and female patient groups compared with matched sex and age control group.

The results indicate the importance of using the above parameters as risk factors in addition to new biomarkers for differential dignosis and evaluation of the severity of IHD.

Introduction

Ischemic heart disease involves a progression of pathologic conditions that include erosion and rupture of coronary artery plaques, activation of platelets and thrombi. This progression is termed acute coronary syndrome and ranges from unstable angina to extensive tissue necrosis in acute myocardial infarction[1]. Coronary heart disease is caused by a lack of nutrients and oxygen reaching the heart muscle and resulting in myocardial ischemia. Ischemia is a reduced blood supply to one area of the heart and is often a result of atherosclerosis, thrombosis, spasms, or embolisms but may also be a result of anemia, carboxyhemoglobinmia, or hypotension, which

causes reduced blood flow to the heart. Most frequently, ischemia is the result of abnormal coronary arteries, usually caused by an obstruction in one or more of these arteries. Atherosclerosis is a thickening and hardening of the artery walls caused by deposits of cholesterol-lipid-calcium plaque in the lining of the arteries [2].

Lactic acid is a by-product of an emergency mechanism that produces a small amount of ATP when oxygen delivery is severely limited. Pyruvate is the normal end product of glucose metabolism (glycolysis). The conversion of pyruvate to lactate is activated when a deficiency of oxygen leads to an accumulation of excess NADH. As a result, only 2 ATP are produced for each mole of glucose metabolized to lactate, with the excess lactate released into the blood. This case has a clinical importance because the accumulation of excess lactate in blood is an early, sensitive, and quantitative indicator of the severity of oxygen deprivation.

As oxygen delivery decreases below a critical level, blood lactate concentration rises rapidly and indicates tissue hypoxia earlier than PH. Measurements of blood lactate are useful for metabolic monitoring in critically patients, for indicating the severity of the illness, and for objectively determining patient prognosis. There are two types of lactic acidosis. Type A is associated with hypoxic condition, such as shock, myocardial infarction, severe congestive heart failure, pulmonary edema, or severe blood loss. Type B is of metabolic origin, such as with diabetes mellitus, severe infection, leukemia, liver or renal disease, and toxins (ethanol, methanol, or salicylate poisoning)[3]. Lactate dehydrogenase (LDH) is an enzyme that catalyzes the interconversion of lactic and pyruvic acids. It is a hydrogen-transfer enzyme that uses the coenzyme NAD^+ .

Lactate dehydrogenase is widely distributed in the body. High activities are found in the heart, liver, skeletal muscle, kidney, and erythrocytes; lesser amounts are found in the lung, smooth muscle, and brain. Because of its widespread activity in numerous body tissues, LDH is elevated in a variety of disorders. Increased levels are found in cardiac, hepatic, skeletal muscle, and renal diseases, as well in several hematologic and neoplastic disorders[4].

An increased serum cholesterol concentration has been shown to have a strong association with atherosclerosis. Lowering the serum cholesterol, especially the low-density lipoprotein (LDL) cholesterol fraction, has been shown to decrease the incidence of coronary artery disease and slow the progression of coronary atherosclerosis[5].

Experimental part

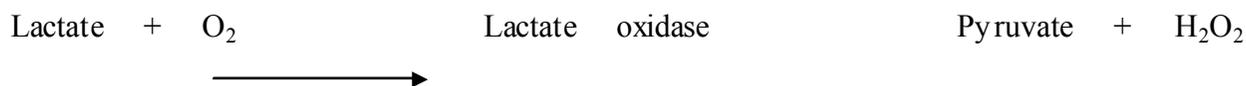
The chosen patients were affected by IHD and were referred to the intensive care unit (ICU) in Ibn- Albetar cardiac center according to their specialist surgeon diagnosis which was confirmed by (ECG) electro cardio graphs, X-ray and echo study, and were subjected to angio-catheterization and found to be angio-positive. This study includes 50 patients 30 male and 20 female. The mean age of male was 65 year and 58 year for female.

Blood samples were collected from 30 healthy subjects to be used as control group (15 male and 15 female), the mean age of the control group was 63 years for male and 55 years for female. Both groups (study and control) have no other medical diseases, which may interfere

with the tests of our study, like viral hepatitis, renal failure, diabetes mellitus, and endocrine disorders. 8ml of blood samples were collected from all subjects by venipuncture, and left for 45 minutes for clotting centrifuged to get the serum, which was refrigerated unless worked immediately.

The method used is modified from that of classical method by David.(1963).

Lactate concentration was determined by using a couple enzymatic reaction, and was monitored colorimetrically .First lactate oxidized to pyruvate and H₂O₂



Secondly peroxide in the presence of p-chlorophenol and

4-aminoantipyrine yield quinoneimine, a red complex absorbing light at 500 nm.



Totale serum LDH activity was measured by utilizing a colorimetric method (Wroblewski and Due,1955), where a readymade kit from Randox laboratories England is used, this method is based on the reduction of pyruvate to lactate in presence on NADH by the action of LDH.



The pyruvate that remains unchanged reacted with 2,4- dinitrophenyl hydrazine to give the corresponding phenylhydrazone, which is determined colorimetrically in alkaline medium by measuring maximum absorbance at 520 nm. Enzyme activity was expressed in U/L.

Determination of total serum cholesterol [12] involves the use of three enzymes , cholesterol esterase , cholesterol oxidase and peroxidase . In the presence of the former mixture (N- ethyl propyl - m - anisidine) and 4 - amino - antipyrine are condensed by hydrogen peroxide to form quinoneimine dye proportional to the concentration of cholesterol , when the absorbance of the samples was measured against the reagent blank within 60 minutes at 500 nm.

The Triglycerides were determined after enzymatic hydrolysis with lipases . The indicator is aquinoneimine formed from hydrogen peroxide , 4-aminophenazone , and 4 – chlorophenol under the catalytic influence of peroxidase .

The absorbance was measured for test and standard against the reagent blank within 6 minutes at 500 nm [13].

In determination of high density lipoprotein – cholesterol HDLc (14), the method uses a selective precipitations of chylomicrones and the apolipoprotein containing lipoprotein VLDLc and LDLc by addition of 4% phosphotungstic acid solution , which contains 10 % magnesium chloride PH 6.2 . Sedimentation of the precipitant is by centrifugation, and subsequent enzymatic analysis of HDLc as residual cholesterol remaining in the clear supernatant, from which the cholesterol can be determined as described above according to [12]. Low density lipoprotein

cholesterol LDLc was determined by using empirical Friedwald formula which was based on the assumption that VLDLc is present in serum at a concentration equals to one fifth of the TG concentration. This formula is as follow [15]: when all concentration are given in milligrams per deciliter:-

$$\text{LDLc(mg / dl)} = \text{Total cholesterol} - (\text{HDLc} + \text{VLDLc})$$

$$\text{VLDLc} = 1/5 \text{ TG}$$

$$\text{LDLc (mmol/l)} = \text{Total cholesterol} - (\text{HDLc} + \text{TG}/2.2)$$

Statistical Analysis of Data

To compare the significance of the differences in the mean values of any two groups student's t-test was applied and P value less than 0.05(p<0.05) was considered statistically significant.

Results and Discussion

Lactic acid concentration and lactate dehydrogenase activity in all groups participated in this study are shown in Table (1).

A significant increase in lactic acid in serum of patient groups (male and female) compared to control groups (male and female) was noticed, while no significant differences between male control group compared to female control group was found. On the other hand, a significant increase in serum lactic acid for female patient group compared to that for male patient group was found (252.7±18.7 vs. 227.3±29.3 mg/dl, <0.05).

Ischemia (not enough oxygenated blood getting to a certain area), sever oxygen deprivation of tissues results in a switch from aerobic to anaerobic metabolism, Since lactate is the main product of anaerobic metabolism, it accumulates and leads to lactic acidosis. As most of lactate is metabolized predominantly in the liver (60%) and kidney (30%), so any liver disease and renal diseases will lead to disturbance of production of H⁺ (or of the lactate anion) and impairment of its excretion from the body. Also the fact that only the liver and kidney that have the enzymes that can convert lactate to glucose.Orchard,1990(8) reported that the effects of lactic acidosis on the cardiovascular system are particularly pernicious and can include decreased cardiac output, decreased arterial blood pressure, deceased hepatic and renal blood flow, and centralization of blood volume[6].

Yong et al, 1997 reported that ischemia resulted in a significant elevation in lactate levels in blood[7].

The significant differences found between lactic acid in the sera of patient groups (male and female), could be due to excessive production or reduced utilization associated with stage, duration or the severity of the disease. A significant increase in the activity of LDH in the sera of male patient group compared to male control group, also in female patient group to that in female control group was found. Lactate dehydrogenase is among other diagnostic aid of biomarkers for acute myocardial ischemia [8].

Also Adams and Miracle(1998) reported that measurement of cardiac enzymes do not always provide accurate clinical diagnosis, particularly in patient with other concomitant diseases , and alternative biomarkers of cardiac disease should be used such as , cardiac troponins and myoglobin[9].

It has been reported that LDH activity is inhibited in the postischemic myocardium, which is associated with poor glucose oxidation and impaired myocardial performance [10].

A study conducted on rat reported that a three fold increase in LDH content in rat heart compared with liver, the authors also demonstrated that greater basal content of LDH kinase, which inhibits LDH activity , in rat liver compared with heart[11].

Serum levels of total cholesterol and triglycerides in all studied groups are shown in table (2). A significant increase in male patient group compared to male control, also a significant increase in female patient group compared to female control group was found.

Our results are compatible with many studies anticipated that patients with both hypercholesterolemia and hypertriglyceridemia , show impaired endothelium – dependent vasodilation even before hemodynamically significant arterial stenosis develops, and the association between TG and ischemic heart disease events may be related to the presence of atherogenic triglyceride rich particles in the plasma , also elevated levels of cholesterol found to be related to the evolution of ischemic heart diseases which may lead to cardiac death [12,13].

While elevated plasma cholesterol levels are believed to be the major factor in promoting atherosclerosis , it is now recognized that triglycerides are also an independent risk factor [14].

Serum levels of high density lipoprotein and low density lipoprotein in all of the studied groups are shown in Table (3).

A significant increase in LDL levels and a significant decrease in HDL levels in the serum of both patient groups (male and female) compared to that in control groups was found.

The drastic decrease in serum HDL and elevation of LDL level were common features among patient groups compared to control groups in this study which agree with other studies reported that the excess TC present in the form of LDL particles to that the form of HDL can be used to evaluate susceptibility to the development of heart diseases , where LDL/HDL ratio may give a promising evidence when evaluated carefully with sufficient samples , and could be conclusive in this respect , due to protective effect of HDL , which transport circulating cholesterol to the liver for clearance , exerts anti _ atherogenic effect , and the significant decrease in its value in both patient groups , and the high levels of LDL which is more susceptible to oxidation (ox-LDL) , forming foam cells which become trapped in the walls of blood vessels and contribute to the formation of atherosclerosis plaques that cause arterial narrowing and lead to heart attack[15]

Studies conducted on patients with different lipid profile abnormalities , reported that the higher (ox-LDL) and the lower HDL levels showed the severity of clinical symptoms of

endothelial dysfunction indicating that ox-LDL and HDL play the crucial role even in the early stages of atherosclerosis [13][16].

In conclusion, the abnormal lipid profile detected by elevation in TC, TG and LDL could be among other crucial factors in the cascade leading to ischemic damage in the cardium, and prolonged ischemic caused accumulation of non-esterified fatty acid intra- and extra cellularly which may change the permeability of plasma membrane of heart, which may lead to the leakage of cellular substance and enzymes outside the cells, therefore more work is required, considering careful selection of IHD patients, with strict control of the variables which affect the studied parameters, and the time course of the onset and duration of the acute IHD attack.

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Table (1) :Serum Lactic acid and lactate dehydrogenase in all studied groups

Group parameters	Control n=30	Patients n=50	p-value
Lactic acid (mg/dL) Mean ± SD	Male n=15	Male n=30	P < 0.05 (s)
	131.2 ± 19.7	227.3 ± 29.3	P* > 0.05 (NS)
	Female n=15	Female n=20	P < 0.05 (s)
	128.8 ± 27.1	252.7 ± 18.7	P** > 0.05 (s)
Lactate Dehydrogenase (LDH) (U/L) Mean ± SD	Male n=15	Male n=30	P < 0.05 (s)
	182.8 ± 22.3	430.4 ± 30.3	P* < 0.05 (s)
	Female n=15	Female n=20	P < 0.05 (s)
	136.7± 28.6	415.6 ± 34.2	P**< 0.05 (s)

*represent P value between male and female for control group

** represent P value between male and female for patient group

S = significant

NS = non _ significant

N = number of subject

Table (2): Serum triglyceride and total cholesterol in patient and control groups.

parameters \ Group	Sex	Control	patients	P-value
TC (mmole/L) Mean ± SD	Male	3.46 ± 1.21	5.8 ± 1.36	P < 0.05 (s)
	Female	4.2 ± 0.3	5.07 ± 1.26	P < 0.05 (s)
TG (mmole /L) Mean ± SD	Male	1.5 ± 0.47	2.25 ± 0.4	P < 0.05 (s)
	Female	1.4 ± 0.38	2.31 ± 0.48	P < 0.05 (s)

Table (3): Serum lipoprotein levels in patients and control groups.

parameters \ Group	Sex	Control	Patients	P-value
HDL mmole /L Mean ± SD	Male	1.2 ± 0.23	0.98 ± 0.07	P < 0.05 (s)
	Female	1.1 ± 0.28	0.96 ± 0.23	P < 0.05 (s)
LDL mmole /L Mean ± SD	Male	2.3 ± 0.87	3.1 ± 1.001	P < 0.05 (s)
	Female	2.46 ± 0.59	3.73 ± 1.009	P < 0.05 (s)
LDL/HDL Mean ± SD	Male	1.9 ± 0.21	3.16 ± 0.2	P < 0.05 (s)
	Female	2.23 ± 0.17	3.88 ± 0.21	P < 0.05 (s)

دراسة مقارنة لحامض اللبنيك وفعالية أنزيم اللاكتيك دي هايدروجينيز و صورة الدهون في امصال مرضى القلب الزاوية و الأصحاء

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الخلاصة

يطلق مصطلح امراض القلب الزاوية (IHD) على المرض الناشئ من عدم توازن كمية الاوكسجين المطلوبة لعضلة القلب وكمية الاوكسجين المجهزة .

تضمنت الدراسة خمسين مريضاً (30 ذكراً و 20 اناث) يراجعون مستشفى ابن البيطار وكانت معدل اعمار الذكور 65 سنة والاناث 58 سنة وكذلك ثلاثين من الاصحاء (15 ذكراً و 15 اناث) متقاربين في معدلات اعمارهم لمجموعة المرضى مجاميع سيطرة .

قيست بعض الدوال الحيوية وشملت الدهون والبروتينات الدهنية وهي الكولسترول الكلي، والكليسريدات الثلاثية، والبروتينات الدهنية عالية الكثافة، والبروتينات الدهنية واطئة الكثافة فضلا عن حامض اللبنيك وفعالية اللاكتيك دي هايدروجينيز في امصال مجاميع المرضى ومجاميع السيطرة .

دللت النتائج على وجود زيادة معنوية في جميع الدوال الحيوية المدروسة عدا البروتينات الدهنية عالية الكثافة التي ظهر فيها انخفاض معنوي في امصال مجاميع المرضى مقارنة مع مجموعة السيطرة المتماثلة في الجنس والعمر .

اظهرت لنا الدراسة اهمية استخدام الدوال المذكورة اعلاه عامل خطورة مقترناً مع مؤشرات جديدة يمكن استخدامها معيار كيمو حيوي للتشخيص التمييزي وتقييم شدة امراض القلب الزاوية ومتابعة المرضى حيويًا .