Some Biochemical Risk Factors for Myocardial Infarction in Babylon City.

Maha F. Al-Smaisim Clinical Biochemistry Dept., College of Medicine, Babylon University Hilla P.O.Box. 473, IRAQ.

الخلا صه

تضمنت الدراسة الحالية قياس بعض المتغيرات الكيميائية الحياتية وتحديد عوامل الخطورة في مصل المرضى المصابين باحتشاء العضلة القلبية لا ديهم تاريخ بمرض داء السكري النوع الأول وارتفاع ضغط الدم وبمرض داء السكري+ارتفاع ضغط الدم+التدخين). أربع وستون مريض من المصابين باحتشاء العضلة القلبية تم إدخالهم في مستشفى مرجان التعليمي في مدينة الحلة و32 من الأشخاص الأصحاء ذكور وإناث تم استخدامهم كمجموعة ضابطة. بالقياسات الكيميائية تم إجراء في كل من: الكوليسترول ذات الكثافة العالية والدهون الثلاثية، السيريلوبلازمين، البروتين الكلي، الالبومين، الكلوبيولين، الكوليسترول، الكلي البيليروبين، الترايكليسيرايد وكذلك نسبة الالبومين/الكلوبيولين و نسبة الترايكليسيرايد/الالبومين والفعالية والفعالية النوعية لانزيم السيريلوبلازمين و نسبة تركيز النحاس/فعالية إنزيم السيريلوبلازمين في مصول الأصحاء ومرضى المصابين باحتشاء العضلة القلبية. أظهرت النتائج زيادة عالية المعنوية مقارنة مع مجموعة السيطرة وعند المستوى (p<0.001) في كل من السيريلوبلازمين, البروتين الكلي, الكلوبيولين, الترايكليسيرايد, البيليروبين الكلي نسبة الالبومين/الكلوبيولين و نسبة الترايكليسيرايد/الالبومين و الفعالية والفعالية النوعية لانزيم السيريلوبلازمين و نسبة تركيز النحاس/فعالية إنزيم السيريلوبلازمين في مصول المرضى المصابين باحتشاء العضلة القلبية(بمرض داء السكري+ ارتفاع ضغط الدم+التدخين) زيادة معنوية في مصول المرضى المصابين باحتشاء العضلة القلبية (بمرض داء السكري او ارتفاع ضغط الدم). وتركيز الألبومين اظهر انخفاض معنوي في مصول المرضى المصابين باحتشاء العضلة القلبية بمرض داء السكري+ ارتفاع ضغط الدم+التدخين)(p<0.001)), (بمرض داء السكري)(p<0.01) أو ارتفاع ضغط الدم].
(p<0. 01) الإيجاد المهم في هذه الدراسةِ إن عوامل الخطر الكيميائية الحياتية تَظْهرُ للحَدَث في المرضى المصابين بمرض داء السكري+ ارتفاع ضغط الدم+التدخين أكثر من المرضى الذين لديهم تاريخ بمرض داء السكري, (النوع الأول) أو ارتفاع ضغط. قَدْ يَكُون عاملَ خطر أكثر بروزاً لاحتشاء العضلة القلبية. ارتفاع الترايكليسيرايد يرتبط مع حدوث احتشاء العضلة القلبية وليس ارتفاع الكولسترول. نسبة الترايكليسيرايد/الالبومين كدليل أفضل لخطر أمراض القلب الوعائية من الكولسترول.
الفعالية والفعالية النوعية لانزيم السيريلوبلازمين و نسبة تركيز النحاس/فعالية لإنزيم السيريلوبلازمين كمقياس كيميائية حياتية جديدة وعوامل خطورة لذلك هذه النتائج تعتبر كمؤشر أساسي في احتشاء العضلة القلبية .

Abstract

The present comprehensive study was designed to investigate the changes in some biochemical parameters and determine the risk factors in sera samples of patient with myocardial infarction (MI) [history of diabetes mellitus (DM) type 1, hypertension (blood pressure BP), and DM+ BP+smoking].Sixty four patients with MI attending to Merjan Teaching Hospital in Hilla City, were included in the present study. Also thirty two healthy individuals of matched age and gender were utilized as control.The main measurements included the following biochemical measurements:-Ceruloplasmin (CP), copper(Cu),total protein(TP) ,albumin(Alb.),globulin(Glo),total cholesterol(TC), triglyceride(TG), and total bilirubin(TB) concentrations, also Alb./Glo. ratio, TC/Alb ratio, CP oxidase activity, CP oxidase specific activity and Cu conc. / CP oxidase activity ratio in sera of healthy (control) and patients with MI (history of DM , BP and DM+BP+smoking).The results revealed a highly significant increase($p<0.001$) in the CP, TP, Glo, TG, TB concentrations, Alb./Glo. ratio, CP oxidase activity, specific activity and Cu conc./CP oxidase activity ratio in sera of patients with MI (DM+ BP+smoking),while the same parameter referred significant increase($p<0.01$) in patients with MI(DM or BP) in comparisom with control group. But Alb. concentration show significant decrease in the all groups ($p<0.001$), ($p<0.01$) respectively.

An important finding in this study is that biochemical risk factors appeared to occur in patients with DM+BP+smoking more the patients with DM or BP. This observation suggests that DM+BP+smoking may be more prominent risk factor for MI. Hypertriglyceridemia, but not hypercholesterolemia, is associated with MI.

TC/Alb ratio is considered a better index of cardiovascular risk than serum cholesterol. CP conc., CP activity and specific activity and Cu Conc. / CP oxidase activity anew biochemical parameters and risk factors, therefore this results it is regarded us a primary indicator to MI.

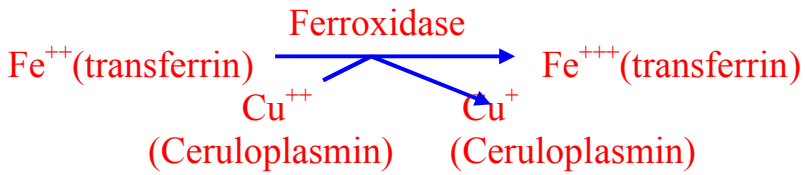
Introduction

Myocardial infarction (MI),also known as coronary thrombosis, is one of the commonest causes of mortality and morbidity in adults[1,2]. MI is usually diagnosed by a history of crushing chest pain, characteristic ECG changes and cardiac muscle enzyme release. MI occurs when the supply of blood to the coronary muscle is reduced below a critical value, usually as result of atheromatous plaque rupture and overlying thrombosis.

This may be presaged by less catastrophic episodes of chest pain (angina pectoris) due to reduction of coronary perfusion caused by the narrowing of the arteries by atheromatous plaque .

Biochemical tests are used as a complementary diagnostic procedures to ECG findings. Three enzymes are commonly used in the diagnosis and follow-up of the MI. These are: creatine kinase(CK), aspartate aminotransferase(AST) and lactate dehydrogenase(LDH).Those enzymes lost their utility due to lack of specificity and limited sensitivities[3].Among the currently available markers cardiac troponins are the most widely used due to their improved sensitivity, specificity, efficiency and low turn around time[3,4].The combination of myoglobin with cardiac troponins I and T has further improved the accuracy in the diagnosis of acute coronary syndromes [3]. Among the other new markers of early detection of myocardial damage, heart fatty acid binding protein,ischemic modified-albumine, lipoprotein(a), remnant lipoprotein-cholesterol, glycogen phosphorylase BB and myoglobin /carbonic anhydrase III ratio seem to be the most promising[3,5,6]. Many risk factors, such as diabetes mellitus(DM) ,hypertension(HP), and hyperlipidemia contribute to the developing of coronary artery disease (CAD) and MI [7].Lipid profile help to determine risk of(CDA) [8].

Ceruloplasmin is a copper-containing, which has enzymatic activities It is also called Ferroxidase 90% or more of total serum copper is associated with ceruloplasmin[9]and the remaining 5-10% of copper is believed to be fairly loosely attached to albumin and histidine and only a trace of copper is present as free Cu^{++}[10, 11].CP, synthesized in the liver is multifunctional protein it can help transferrin to bind iron[12,13].



The antioxidant protection of ceruloplasmin is derived mainly from its ability to oxidize highly toxic ferrous iron to the relatively non toxic ferric form[14], and that helps in preventing oxidative damage of proteins, lipids, and DNA[15].

The aim of our study is to evaluate the risk factors to the new parameters such as ceruloplasmin oxidase activity, ceruloplasmin (CP) concentration , copper(Cu) concentration , mean value of Cu concentration / CP oxidase activity ,total protein ,albumin, globulin concentrations, albumin/ globulin ratio in sera of healthy (control) and patients with MI (history of (DM) , (BP) and DM+ BP +smoking).

Patients and Methods

Chemicals: All laboratory chemical and reagent in this work were of analar grade and imported from BDH Co. and SIGMA Co

Samples:(64) patients with MI were included in this study, (divided three groups; **1**-History of DM, type 1 ,no=21),**2**-History of BP,systolic blood pressure(BP) more than 160 mm Hg and diastolic less than 70 mm Hg, no=19. **3**-DM + BP + smoking, no=24). Admitted to Merjan Teaching Hospital in Hilla City, over period of about eight months from March 2007 to September 2007. The diagnosis of MI was established an clinical ground. All the subjects were examined clinically and informations pertaining to age, habits and health status were recorded in special case proforma. Other group included (no=32) apparently healthy (control) with no history of MI and other disease. Venous blood samples were collected from both controls and patients(in the first day, after MI) for the estimation of serum parameters.

Total Protein Estimation: The protein content of all samples was determined by Lowery method [16] using bovine serum albumin as standard protein for all samples.

Albumin Concentration Estimation : Serum albumin was determined by dye-bindin method[17] using kit manufactured by bioMerieux.

Globulin Concentration Estimation: The concentration of globulin in serum samples of control and patients groups was calculated, using the following equation:

$C_{glob.} = C_{Tp} - C_{alb}$, $C_{glob.}$ = concentration of globulin , C_{Tp} = concentration of total protein and C_{alb} = concentration of albumin

Cerulopalsmie Oxidase Activity Assay: The activity of ceruloplasmin oxidase was determined in serum using, the modified Rice method[18] where as ceruloplasmin catalyzed the oxidation of p-phenylenediamine (substrate) to give blue-violet color that measured at ג = 525 nm

Copper Concentration Estimation: The copper concentration determined in serum by the atomic absorption spectrophotometery[19].

Total cholesterol, triglyceride and total bilirubin were determined by using commercially available kits (Biomergreb kit, iMorocco kit)**.**

Statistical Analysis: The data of the study was subjected to statistical analysis is expressed as mean ± SD [20].Statistical analyses were done on a computer by using the Student't' test.

Results

Table 1 shows a significant increase in serum concentration of total protein (TP), and globulin (Glo.)Concentration between healthy and MI (DM, $P<0.01$), MI (BP, ($P<0.01$) and MI (DM+ BP + smoking, $P<0.001$) patients. In contrast, the results revealed a highly significant decease ($P<0.001$) and ($P<0.01$) of albumin(Alb.) concentration in the case of MI (history of DM+ BP +smoking) and MI(history of DM+ BP) respectively in comparison to that of the control group. Also Alb./ Glo. ratio show a highly significant increase ($P<0.001$)in all patient groups.

Table 1: Total Protein (TP), Albumin (Alb.), Globulin (Glo.)Concentrations and Alb. / Glo. ratio in Sera of Control and MI Patients (Mean Value ± SD).

Groups	Sex	No.	Age(Y)	Variations			
				TP(g/dl)	Alb.(g/dl)	Glo.(g/dl)	Alb./ Glo.
Control	M	18	38.6±19.2	7.74±1.24	4.43±0.61	3.21±0.61	1.12±0.31
	F	14	37.4±18.2	7.56±0.94	4.33±0.53	3.33±0.53	1.10±0.51
MI(DM)	M	11	41.3±15.2	8.99±1.2*	3.53±0.4*	5.36±0.4**	0.64±0.4**
	F	10	40.5±13.2	8.94±1.4*	3.53±0.3*	5.44±0.3**	0.65±0.4**
MI (BP)	M	10	37.3±12.2	8.67±0.9*	3.63±0.5*	5.14±0.5**	0.72±0.3**
	F	9	38.2±91.2	8.77±1.2*	3.53±0.2*	5.14±0.2**	0.67±0.2**
MI(DM+ BP) +smoking)	M	16	39.9±12.2	10.31±1.2**	2.23±0.4**	8.18±0.4**	0.27±0.4**
	F	8	41.3±10.2	10.22±1.3**	2.31±0.3**	7.81±0.3**	0.29±0.3**

*(P<0.01), ** (P<0.001)

Table 2 shows that the serum triglyceride concentration and total bilirubin significantly increased in patients with Ml [DM,BP($p<0.01$) and DM+ BP +smoking($p<0.001$)] in comparison with healthy .On the other hand there was no significant difference in serum total cholesterol(TC) concentration between patients with Ml and controls . The ratio (TC/Alb) found that patients with Ml (DM and DM+ BP +smoking) should a highly significantly increase ($p<0.01$, $p<0.001$ respectively) in comparison with control, while a highly significant decrease ($p<0.001$) in patients with MI (BP).

Table(2):Total cholesterol(TC), Triglyceride(TG) ,Total bilirubin (TB)and Cholesterol to albumin ratio(TC/Alb) in Sera of Control and MI Patients(Mean Value ± SD).

Groups	*Sex*	*No.*	*Age(Y)*	*Variations*			
				TC mmol/L	*TG mmol/L*	*TB µmol/L*	*TC/Alb.*
Control	M	18	38.6± 19.2	5. 42±1.41	1.58±0.81	15.61±3.1	1.22±0.5
	F	14	37.4± 18.2	5. 32±1.21	1.56±0.77	15.56±2.7	1.22±0.4
MI(DM)	M	11	41.3± 15.2	5. 33±1.21	2.87±0.57 *	28.3±2.5*	1.50±0.4 *
	F	10	40.5± 13.2	5. 40±1.31	2.98±0.46 *	29.1±1.2*	1.52±0.5 *
MI(BP)	M	10	37.3± 12.2	5. 41±1.41	2.82±0.45 *	26.1±1.4*	0.67±0.4 **
	F	9	38.2± 91.2	5. 33±1.11	2.13±0.22 *	26.3±1.2*	0.66±0.5 **
MI(DM+ BP +smoking)	M	16	39.9± 12.2	5. 41±1.21	3.97±0.2**	32.9±2.2**	2.42±0.7 **
	F	8	41.3± 10.2	5. 39±1.22	3.88±0.4**	32.8±2.4**	2.33±0.6 **

***(P<0.01), ** (P<0.001)**

Table 3 shows a highly significant increase (P<0.001) in ceruloplasmin(CP) concentration and serum copper for MI (history of DM, BP and DM+ BP +smoking) patients in comparison to that of control.

Table (3): Ceruloplasmin (CP) Concentration and Copper (Cu) Concentration in Sera of Control and MI Patients (Mean Value ± SD).

Groups	Sex	No.	Age(Y)	Variations	
				CP(mg/dl)	Cu (µmol/l)
Control	M	18	38.6±19.2	48.31±2.34	5.73±0.81
	F	14	37.4±18.2	48.34±3,42	5.68±0.81
MI(DM)	M	11	41.3±15.2	72.64±4,42**	32.85±0.92**
	F	10	40.5±13.2	72.45±3,42**	31.84±0.22**
MI(BP)	M	10	37.3±12.2	65.44±3,32**	25.86±0.34**
	F	9	38.2±91.2	65.32±3,23**	26.75±0.41**
MI (DM+ BP +smoking)	M	16	39.9±12.2	76.64±3,13**	35.99±0.64**
	F	8	41.3±10.2	76.54±3,13**	37.97±0.46**

** (P<0.001)

The results in fig.(1) presented the mean value of ceruloplasmin oxidase activity and the specific activity fig.(2) in sera samples. The results reflect the presence of a significant increase (P<0.001) in CP oxidase activity of MI(history of DM+ BP +smoking), and significant increase (P<0.01) in MI (history of DM and BP), in comparison to that of the control group(fig.1).

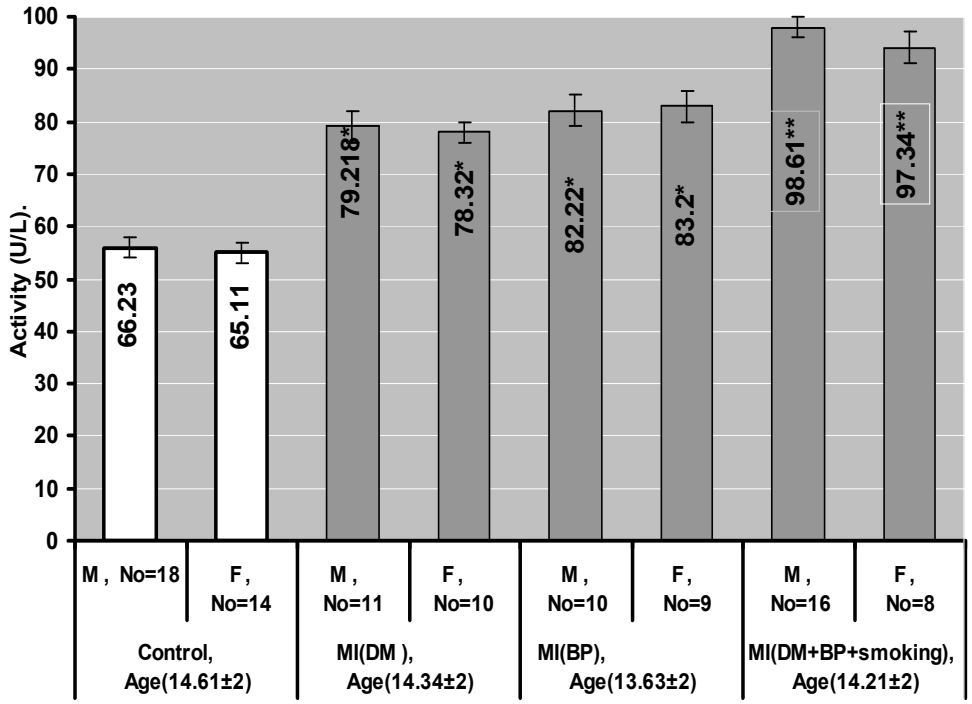


Fig. (1)*: Ceruloplasmin Oxidase Activity(U/L) in Sera of Control and MI Patients (Mean Value ± SD,*(P<0.01), (P<0.001).***

Regarding the specific activity, significant increase (P<0.01) was found in both MI(history of BP and DM+ BP +smoking) in comparison to that of the control (fig.2)

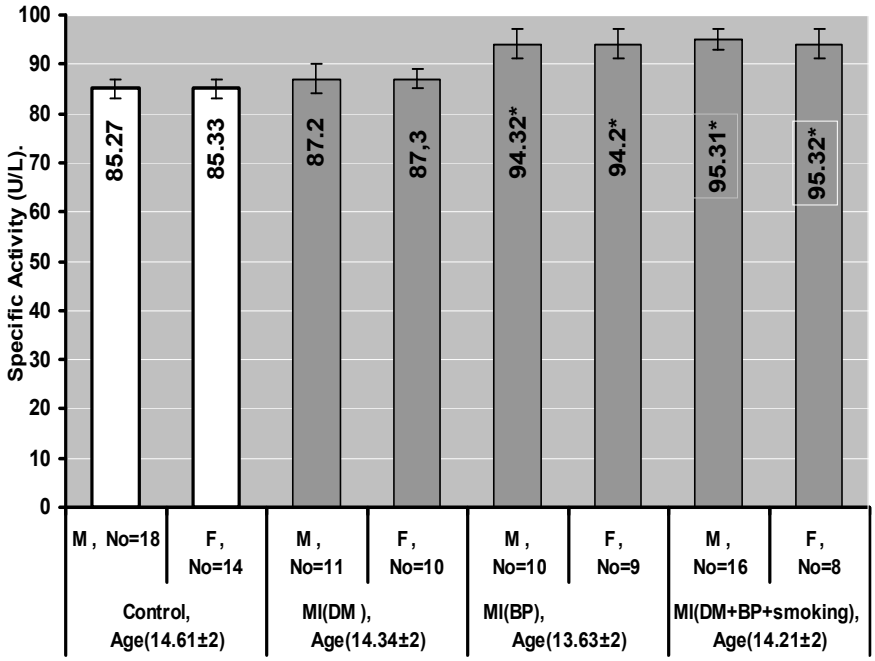


Fig. (2): Specific Activity (U/mg $\times 10^{-3}$) of Ceruloplasmin Oxidase in Sera of Control and MI Patients (Mean Value ± SD, *(P<0.01).

Table 4 shows the mean value of copper concentration / CP oxidase activity in sera samples, and reflect a significant increase (P<0.001), in MI(history of DM + BP + smoking), in comparison to that of the control. Also(P<0.01) in MI(history DM).

Table (4): Copper Concentration / CP Oxidase Activity in Sera of Control and MI Patients (Mean Value ± SD, *(P<0.01), (P<0.001).**

Groups	Sex	No.	Age(Y)	Cu / CP activity
Control	M	18	38.6±19.2	0.236±021
	F	14	37.4±18.2	0.239±022
MI(DM)	M	11	41.3±15.2	0.414±0.13**
	F	10	40.5±13.2	0.405±0.22**
MI(BP)	M	10	37.3±12.2	0.313±0.12*
	F	9	38.2±91.2	0.320±0.21*
MI (DM+ BP +smoking)	M	16	39.9±12.2	0.364±0.13**
	F	8	41.3±10.2	0.402±0.21**

Discussion

The results of this study showed a significant increase in serum total protein , total bilirubin and globulin concentrations between Ml patients and control group. Therefore, it would appear that the increased susceptibility of these biochemical components may be a more prominent for MI patients (DM+BP+smoking) than for patients with MI(DM+BP)risk factors. However, a controlled prospective study is needed to investigate this further.

The reduction in sera albumin concentration(Table 1) observed in the current study may be explained as follows:-The observed reduced albumin level in sera of patient group, may be due to the role of albumin as one of the extracellular antioxidants[21,22] where albumin constitute up to 49% of total plasma antioxidant status[23]. Meanwhile albumin acts as sacrificial antioxidant by inhibiting the generation of free radicals through an immediate attacks of albumin molecule itself, so the radical reaction continue on albumin surface and cause damage to albumin molecule[24,25], such damage is probably biologically insignificant, due to fact that the albumin is present in plasma in high concentration[26].

The other cause of the observed reduced albumin level in sera of patient group, may be due to liver is the site for the synthesis of most proteins including both negative acute phase reactant proteins(APR) (pre albumin and albumin) and positive APR[27]. It is well known that the concentration of positive APR in serum increased in different disease, which mean that liver will be busy with synthesis of this type of APR leaving behind the synthesis of other proteins like albumin [28], also the synthesis of inflammatory cytokines, such as tumor necrosis factor-a (TNF-a) and interlukines and C-reactive protein seems to cause a reduction in albumin concentration[29].

In the current study, globulin concentration showed a significant increase in patient groups and this may attribute to that most of the positive (APR) are globulins (i.e. glycoprotein), so the increased level of globulins is due to the increased synthesis of positive APR in different disease [30].

Table 2 shows that the serum triglyceride concentration in patients with MI(DM,BP and DM+BP+smoking) were significantly higher than that in healthy controls. On the other hand ,there was no significant difference in serum total cholesterol concentration between patients with MI(DM,BP and DM+BP+smoking) and controls, suggesting that hypertriglyceridemia, but not hypercholesterolemia, is associated with MI. For a long time. the association between serum triglyceride and CHD remained unclear. In a large number of both case control and cross-sectional studies, an association between hypertriglyceridemia and myocardial infarction was reported, but only in three of them [31-33] did the dissociation remain significant after controlling for other confounding factors. Recently. Bainton *et al*.[34] reported that serum triglyceride was an independent risk factor for CHD.

The serum total bilirubin in patients with MI(DM,BP and DM+BP+smoking) were significantly higher than that in healthy controls. Since serum total cholesterol concentration to albumin ratio(TC/Alb.) has been reported to be a better index of cardiovascular risk than serum cholesterol[35].

Ceruloplasmin is considered as one of the positive acute-phase reactants (APR). The measured increase in ceruloplasmin concentration in the present study, in sera samples of patient groups . The increased CP concentration in sera samples result from its increased synthesis by liver, as one of the acute phase proteins which their concentration increases upon different diseases including tumors[36].Ceruloplasmin is an enzyme which has a role as an antioxidant [37]. The unbalanced production of reactive oxygen intermediates have been postulated to play a role in the pathogencsis of cancer[36].

In this study, CP oxidase activity and specific activity were increased, The increase of ceruloplasmin activity may be associated with enhanced synthesis of ceruloplasmin in the liver as one of the positive acute phase reactant protein[38] which their levels increased upon malignancies.

It was reported that ceruloplasmin plays an important role in protecting a variety of tissues from free radical injury[39], the antioxidant protection of CP drives mainly from its ability to oxidize polyamines[40], also it can play an important role in preventing the formation of free radicals by controlling the levels of highly toxic iron. Most evidence points to CP ferroxidase activity as an antioxidant activity; conversion of Fe^{+2} to Fe^{+3} may reduce the oxidation by inhibition of the Fenton reaction (which requires reduced metal), by decreasing the amount of the pro-oxidant Fe^{+2}or an Fe^{+2} / Fe^{+3} complex or by causing iron sequestration by apo-transferrin[41,42].

Copper and ceruloplasmin are usually closely correlated with each other, since ceruloplasmin is the major copper- binding protein (over 95% of plasma copper is bound to CP). Copper helps to protect the heart by increasing HDL[43].

The [Cu]/CP activity ratio reflect the concentration of Cu that is either bound to albumin or free copper. This form of Cu is consider to be one of the pro- oxidants in the body, where transition metals such as Fe^{+2} or Cu^{+}, catalyze formation of the hydroxyl radical ($OH^{\cdot}$) from hydrogen peroxide in the nonenzymatic Fenton reaction.

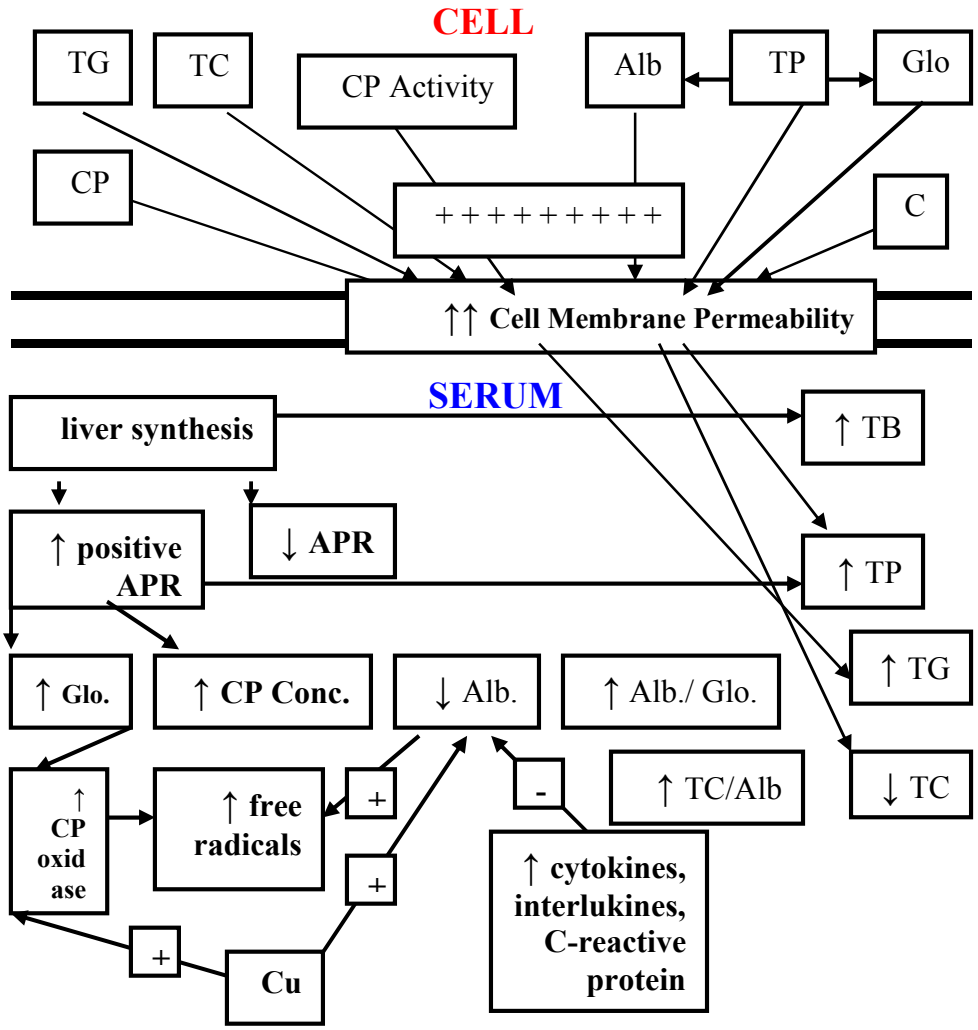
Conclusions

The overall results of the present study that is summarized in the following table:

Parameters	Myocardial Infraction(MI)Patients		
	MI(DM)	MI(BP)	MI(DM+BP+smoking)
Total Protein(TP) Conc.	↑*	↑*	↑**
Albumin(Alb.) Conc.	↓*	↓*	↓**
Globulin (Glo.) Conc.	↑	↑*	↑*
Alb./ Glo. Ratio	↓**	↓**	↓**
Total cholesterol(TC) Conc.	↔	↔	↔
Triglyceride(TG) Conc.	↑	↑*	↑*
Total bilirubin (TB) Conc.	↑	↑*	↑*
Cholesterol / albumin ratio(TC/Alb)	↑	↓*	↑*
Ceruloplasmin(CP) Conc.	↑*	↑*	↑*
Copper (Cu)Conc.	↑*	↑*	↑*
Ceruloplasmin Activity.	↑*	↑*	↑*
Ceruloplasmin Specific Activity	↔	↑*	↑*
Cu Conc. / CP Oxidase Activity	↑*	↑*	↑*

MI(DM): MyocardialInfarction(diabetesmellitus),
MI(BP): MyocardialInfarction(bloodpressure),
MI(DM+BP+smoking): Myocardial Infarction(diabetes mellitus+ blood pressure+ smoking). ↔ no change(as in control**),** ↑ Increase , ↓ Decrease and (*=p<0.05,** = p < 0.001)

The overall conclusions of the present study can be summarized in the following diagram :



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