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Research Article

RELATION OF SERUM ZINC AND COPPER LEVEL WITH MAJOR RISK FACTOR, THROMBOLYSIS AND ECHOCARDIOGRAPHY INISCHEMIA

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ABSTRACT

Background And Aims: Symptoms of acute coronary syndrome(ACS)may be used in combination with other important information (biomarkers, risk factors, ECG, and other diagnostic tests) in making triage and some treatment decisions in the out-of-hospital and emergency department settings.Copper(cu) and Zinc(zn) is incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. Adequate zinc helps to keep the artery walls flexible. The purpose of the present study is to clarify the association between Zinc & Copper levels with echocardiographic and risk factor in ACS. **Patients and Methods:** Blood samples were collected from patients with ACS (n = 62), and from healthy controls (n =20). Serum Levels of Zinc and copper were determined using atomic absorption spectrophotometry. SerumLevels of glucose and lipid profil were obtained by coloremetry. **Results:** Pooled analysis indicated that subjects with ACShad higher serum Cu levels and lower Zn than healthy controls.ANOVA test revealed there was non significant difference in serum Cu and Zn (mean±SD) levels among five types of acute coronary syndrome patients.The t- test revealed there was no significant difference in serum cu &zn (mean ±SD) levels in the presence(Yes) or absence(NO) of diabetes mellitus, Dyslipidemia, Hypertension, Smoking, Obesity and fibrinolysis treatment(Actilyse).The present study results showed that there was no association between serum Cu&zn levels with Mitral regurgitationseverity, the mean±SD value of serum Zn levels of patients in stage 1 was significantly higher than that in stage 2 and 3 of diastolic dysfunction. There was non significant correlation between the serum Cu concentrations and age,Left Ventricular Ejection Fraction,Isovolumic relaxation time, E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio and Decceleration time.There was significant negative correlation between serum Zn concentrations andLeft atrium diameter, but non significant correlation between serum Zn concentrations and age,left ventricular ejection fraction, Isovolumic relaxation time, E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio and decceleration time. **Conclusion:** Lower serum Zn and heigher Cu concentration were found in patients with ACS but these levels are nonsignificantly different between acute myocardial infarction and unstable angina patients. Also, it seems that risk factor dose not considerably affect serum levels of Zn or Cu in ACS patients.Study revealed a significant correlation between serum zinc concentration and diastolic dysfunction in patients with ACS.Serum zinc&copper werenonsignificantly correlated with parameters of the left ventricular systolic function.

Keywords: ACS , copper, zinc, Echocardiographic.

INTRODUCTION

Symptoms of ACS may be used in combination with other important informations (biomarkers, risk factors, ECG, and other diagnostic tests) in making triage and some treatment decisions in the out-of-hospital and ED settings. The symptoms of myocardial infarction (AMI) may be more intense than angina and most often persist for longer periods of time. The classical symptom associated with ACS is chest discomfort, but symptoms may also include discomfort in other areas of the upper body, shortness of breath, sweating, nausea, vomiting, and dizziness. [1-3]Public education campaigns increase patient awareness and knowledge of the symptoms of ACS, yet have only transient effects on time to presentation.[4,5]For patients at risk for ACS (and for their families), primary care physicians and other healthcare providers should consider discussing the appropriate use of aspirin and activation of EMS system.[6]

Prompt diagnosis and treatment offers the greatest potential benefit for myocardial salvage in the first hours of ST segment elevationmyocardial infarction (STEMI), and early, focused management of unstable angina and non STsegment elevationmyocardial infarction(NSTEMI) reduces adverse events and improves outcome.[7]

Copper is an essential trace element (i.e., micronutrient) that is required for human health.[8] Copper is incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. These enzymes in turn produce cellular energy and regulate nerve transmission, blood clotting, and oxygen transport. the micronutrients is necessary for the proper growth, development, and maintenance of bone, connective tissue, brain, heart, and many other body organs. Copper stimulates the immune system to fight infections, to repair injured tissues, and to promote healing. Copper also helps to neutralize "free-radicals", which can cause severe damage to cells.[9]

The majority of blood copper (or serum copper) is bound to ceruloplasmin. The proportion of ceruloplasmin-bound copper can range from 70-95% and differs between individuals, depending, for example, on hormonal cycle, season, and copper status.[10][11][12]High intakes of zinc can significantly decrease copper absorption. the association between blood copper and heart attack risk is that inflammation increases copper in the blood.[13,14] Blood copper level correlates strongly with the marker of inflammation C-reactive protein (CRP) in humans, yet substantially increasing copper intake doesn't increase CRP.[15,16] This suggests that elevated blood copper is likely a symptom of inflammation, rather than its cause, and presents an explanation for the association between blood copper level and heart attack risk.

Zinc (Zn) is one of the important essential trace metals that are required for many cell events. Zn is not only an important nutrient, cofactor of numerous enzymes and transcription factors, but also acts as intracellular signaling mediator.[17] One of the mechanisms by which Zn supplementation protects vascular system from oxidative damage may includethe induction of metallothionein (MT) expression.[13,14] MTs are cysteine-rich metal-binding proteins with several biological roles including antioxidant property. It had been indicated the significant protection of MT against diabetes and diabetes-induced cardiovascular damage.[14-18] MT is ubiquitously expressed in mammalian tissues and also highly inducible by a variety of reagents such as Zn; therefore, the protective effect of Zn supplementation on diabetic heart and kidney was noticed before.[13]Zinc is required for the synthesis of protein structures.Adequate zinc helps to keep the artery walls flexible. Zinc deficiency is associated with increased brittleness and hardening of the arteries. Hardening increases blood pressure, and increases the chances for strokes and aneurysms.Low zinc allows the tissue sodium level to rise, which can contribute to high blood pressure and fluid retention.[19]

SUBJECTS AND METHODS

Protocols

The study conformed to the guiding principles of theethics committee of Baghdad University. All patientsgave their written informed consent to participate.The study was a historical cohort study. We included 62patients with ACS from January 2013 to December 2015 in Baghdad Teaching Hospital, ghazi AlharereHospital and Baghdad University. Exclusion criteria were nohistory of renal disease or receiving anycu and zn supplementation and history of familial auto immune disease because of its probable confounding effect. All patientscame from the same geographical area and had a similarsocioeconomic and ethnic background.

Patient Data

At admission, 2 independent observers collected data onmedical history, physical examination, and results of laboratoryexamination. Clinical history taking andphysical examination paid special attention to cardiovascular riskfactors: age, sex, smoking, hypertension, diabetes mellitus, dyslipidemia,renal failure, heart failure, history of using diuretic drugs,previous acute myocardial infarction and previous

coronary arterybypass grafting. In case of discrepancies, bothinvestigators reevaluated the data to achieve consensus.
Body mass index (BMI) wascalculated as weight (kg) divided by height (m) squared. Prevalentdiabetes was defined as a fasting serum glucose .126 mg/dl orcurrent use of any diabetes medication. Prevalent hypertensionwas defined as seated diastolic blood pressure .90 mmHg, systolicblood pressure .140 mmHg or use of anti-hypertensive drugs, dyslipidemia was defined as atherogenic index > 3.2. Current smokers were defined as selfreportedregular smoking or drinking. We obtained venous blood samples for determining serum cu, zn, glucose and lipid profil level within 24h from admission for patients with unstable angina orafter the onset of myocardial infarction asdescribed [15,16]. Serum cu and zn concentration were determinedby atomic absorption spectrophotometer (Schmiadzu AA5646) .

Echocardiographic parameters were measured in all patientsby consultant cardiologists at echocardiographic unit/ Baghdad Teaching Hospital, they involve: Diameter of left aterium, Diastolic dysfunction stage, Mitral regurgitation,E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio, Isovolumic relaxation timeDecceleration time and Left ventricular Ejection Fraction.

Statistical Analysis
Analysis of normality of continuous variables involved theKolmogorov-Smirnov test. Data for normally distributed continuousvariables are expressed as mean±SD. Differences wereanalyzed by ANOVA, Kruskal-Wallis test, or chi-square test asappropriate. A two-sided p,0.05 was considered statistically significant. All analyses involved use of SPSS 17.0 for Windows (SPSS, Inc.,Chicago, IL, USA).

RESULTS

The (62) patients with ACS divided to two groups according to gender 44 males & 18 females. The mean±SD value of patients age with ACS was significantly higher when compared with the mean±SD value of healthy control age ($p \leq 0.05$), as shown in (table 1).

Table 1: Distribution of the studied sample according to the age

Age(year)	Number of subjects (n)	Minimum	Maximum	Mean±Std. Deviation
patients	62	25.00	76.00	57.90±12.72
control	20	20.00	58.00	34.10±14.84
P- value				0.023 *

Means compared using Student's unpaired 't' test. * (P≤0.05) Significant.

Table 2 show the mean ±SD level of serum Cu (µg/dL) in patients with acute coronary syndrome(ACS) was significantly higher when compared with the mean value of healthy control ($p \leq 0.001$) ,the mean ±SD level of serum Zn (µg/dL) in patients with acute coronary syndrome(ACS) was significantly lower when compared with the mean value of healthy control ($p \leq 0.01$).

Table 2: The mean±SD level of serum CU and Zn (µg/dL) in patients with acute coronary syndrome(ACS) and Control

Group	Mean ±SD of serumCu(µg/dL)	Mean ±SD of serumZn(µg/dL)
Control	123.5±10.26	97.7±12.4
patients	156.0±6.97	81.1±7.0
P-Value	0.0001***	0.01**

Results expressed as Mean (± SD). ** (P≤0.01),*** (P≤0.001)

ANOVA test revealed there was nosignificant difference ($p > 0.05$) in the value of serum Cu,Zn mean±SD levels among five types of acute coronary syndrome,Table 3.

Table 3: Distribution of the mean±SD level of serum Cu,Zn (µg/dL) according to type of acute coronary syndrome(ACS)

Type of acute coronary syndrome	Number	mean±SD level of serum Cu (µg/dL)	mean±SD level of serum Zn (µg/dL)
Extensive MI	14	152.85±5.17	77.714±11.75
Anteroseptal MI	6	166.33±4.72	75.66±2.08
Anterior MI	8	158.50±3.41	74.0±6.97
Inferior MI	6	158.33±15.53	75.33±1.15
Unstable angina	28	154.50±8.10	79.21±8.24
P-value		0.14**NS**	0.59 **NS**

Results expressed as Mean (+ SD). NS: Non-significant (p >0.05).
(ANOVA) Mean were significantly different at p ≤ 0.05 at 95% confidence limit. NS: Non-significant (p >0.05)

The t- test revealed there was non significant difference (p > 0.05) in the serum cu &zn mean±SD levels in the presence(Yes) or absente(NO) of diabetes millitus,Dyslipidemia, Hypertension,Smoking, Obesityandfibrinolysis treatment(Actilyse) as shown in table 4.

Table 4: Efect of risk factor and fibrinolysis treatment(Actilyse) on serum cu and zn level

Risk factor	NO.	Mean±SD of serum Cu(µg/dL)	P-Value	Mean±SD of serum Zn(µg/dL)	P-Value
Diabetes millitus					
NO	30	155.93±7.85	0.614	77.26±6.61	0.70
YES	32	156.37±8.84	NS	77.68±9.56	NS
Dyslipidemia					
NO	56	155.89±8.33	0.95	77.17±8.13	0.67
YES	6	158.66±8.38	NS	80.33±9.29	NS
Hypertension					
NO	20	155.20±8.1	0.76	81.10±9.04	0.36
YES	42	156.61±8.3	NS	75.76±7.26	NS
Obesity					
NO	38	155.78±9.44	0.083	77.63±9.22	0.54
YES	24	156.75±6.21	NS	77.25±6.41	NS
Smoking					
NO	46	157.39±7.84	0.5	75.95±6.44	0.02
YES	16	152.62±8.84	NS	81.8750±11.11	NS
Actilys					
NO	52	155.66±8.66	0.69	77.66±8.71	0.60
YES	10	158.60±8.26	NS	80.00±6.40	S

Results expressed as Mean (± SD). NS: Non-significant(p **>** 0.05).

Table (5) shows the mean(±SD) value of serum Cu,Zn levels according to diastolic dysfunction stages, 52 patients had diastolic dysfunction, the mean(±SD) values of serum Cu levels of patients in stage 2 was non significantly higher than that in stage 1 and 3 (p-value = 0.578), the mean(±SD) values of serum Zn levels of patients in stage 1 was significantly higher than that in stage 2 and 3 (p-value = 0.02).

Table 5: The mean±SD of serum Cuand Zn levels (µg/dL) according to diastolic dysfunction Stages

Diastolic dysfunction Stages(DDS)	Number of patients=62	Mean ± SD of serum Cu(µg/dL)	Mean ± SD of serum Zn(µg/dL)
NO D.D.	10	150.33±7.76	**69.20±9.62**
1	28	156.50 ±10.27	**81.28±7.76**
2	18	158.22 ± 6.41	**77.00±5.61**
3	6	154.33 ± 5.50	**75.00±2.62**
P-value	----	0.578 NS	0.02*

Results expressed as Mean (± SD).
(ANOVA) Mean were significantly different at p ≤ 0.05 at 95% confidence limit. NS: Non-significant(p **>**0.05),
* significant(p ≤ 0.05)

The present study result showed, there was no association between serum Cu&zn level with MR severity as shown in table (6).

Table 6: The mean±SD of serum Cuand Zn levels (µg/dL) according tomitral regurgitataion(MR) severity

Mitral regurgitataion (MR)	Number=62	Mean ± SD of serum Cu(µg/dL)	Mean ± SD of serum Zn(µg/dL)
NO	36	155.11±9.19	78.55 9.00±
Mild	18	155.66±7.71	±74.667.33
Moderate	6	163.004.35±	76.66± 2.30
sever	2	159.00±0.0	86.00 ±2.30 .
P-value	.	0.4	0.5

(ANOVA) Mean were significantly different at p ≤ 0.05 at 95% confidence limit.
NS: Non-significant(p >0.05).

Table 7: The Mean (± SD) of echocardiographic parameters

Echocardiographic parameters	Mean±	Std. Deviation
LAD	3.71	8.52
E/e	9.33	0.72
IVRT	96.96	3.47
DT	198.41	48.12
EF	50.06	65.71

Table (6) shows that there was non significant positive correlation between the serum Cu concentrations and the value of Age (r=0 .04,P=0 .83) , Diameter of left atrium(r=0 .09,P= 0.63). However, there was non significant negative correlationbetween serum Cu concentrations and Left Ventricular Ejection Fraction(r =-0.15) (P = 0.39), Isovolumic relaxation time (r = - 0.03) (P= 0.52),E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio(r =- 0.03) (P = 0.86) and Decceleration time (r =- 0.12) (P = 0.52).

There was significant negative correlation between serum Zn concentrations andLeft atrium diameter, but non significant negative correlation between serum Zn concentrations andthe value of Age (r=0 .14, P=0 .43) and Left Ventricular Ejection Fraction(LVEF) (r =-0.16) (P = 0.38). There was non significant positive correlation between serum Zn concentrations and the value of Isovolumic relaxation time (r = 0.18) (P= 0.32), E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio (r = 0.07) (P = 0.71) and Decceleration time (r = 0.15) (P = 0.42).

Table 8: correlation (r) between serum Cu,Zn (µg/dL) with Age, left atrium diameter, E/e, Isovolumic relaxation time, and LVEF% for patients groups

parameters	Age (year)	Left atrium diameter (cm)	E/e	Isovolumic relaxation time(ms)	Decceleration time(ms)	LVEF%
Cu(µg/dL)	r=0.04 P=.83 NS	r=0.09 P=0.63 NS	r=0.03 P=0.86 NS	r=-0.03 P=0.52 NS	r=-0.12 p= 0.52 NS	r=-0.15 P=o.39 NS
Zn(µg/dL)	r=-0.14 p=0.43 NS	r=-0.43 p=0.019* S	r=0.07 p=0.71 NS	r=0.18 p=0.32 NS	r=0.15 p=0.42 NS	r=-0.16 p=0.38 NS

NS: Non-significant(p > 0.05).
E/e = (left ventricular inflow velocity) / (tissue doppler velocity) ratio

DISCUSSION The pathogenesis of some heart diseases has been associated with changes in the balance of certain trace elements.The results of current study showed thatthe mean ±SD level of serum Zn(µg/dL) in patients with acute coronary syndrome(ACS) wassignificantly lower when compared with the mean value of healthy control (p ≤ 0.01).Zinc is involved in many enzymatic reactions, cellular signaling mechanisms, and other essential functions in the cell.[6] And although it is not an antioxidant itself, zinc can exhibit antioxidant effects indirectly through the activation of other

molecules.[2,6] Low zinc levels are associated with less comprised antioxidant defenses that normally protect the heart, leading to a susceptibility to oxidative stress.[3,6]Zinc deficiency is also associated with cell death (apoptosis) in heart tissues, as zinc normally suppressesapoptosis.[2,3]Zinc supplementation is, therefore, an effective way to protect the heart from injury.[6]Liu B et al.[20] concluded in thier meta-analysis that there is a significant association between Zn deficiency and MI.Kazi TG et al.[21] found the concentrations of Zn in whole blood samples were lower in MI patients as compared to normal subjects, theyconcluded that Deficiency of zinc and high concentration of copper and iron may play a role in the development of heart disease.

The mean ±SD level of serum Cu (µg/dL) in patients with acute coronary syndrome(ACS) was significantly higher when compared with the mean value of healthy control, it is important to note that serum copper largely reflects serum ceruloplasmin and is not a sensitive indicator of copper nutritional status.[22] Serum ceruloplasmin levels are known to increase by 50% or more under certain conditions of physical stress, such as trauma, inflammation, or disease. Because over 90% of serum copper is carried in ceruloplasmin, which is increased in many inflammatory conditions, elevated serum copper may simply be a marker of inflammation that accompanies atherosclerosis. In contrast to the epidemiological findings linking increased serum copper levels to heart disease.[23] Additionally, the copper content of white blood cells has been positively correlated with the degree of patency of coronary arteries in CHD patients.[24,25] Further, patients with a history of myocardial infarction (MI) had lower concentrations of extracellular superoxide dismutase (SOD) than those without a history of MI.[26]ANOVA test revealed there was non significant difference ($p > 0.05$) in the value of serum Zn&Cu mean±SD levels among five types of acute coronary syndrome,table 3. Giannoglou GDet al.[27] reported: In vitro studies attribute antiatherogenic properties to zinc (Zn). However, only a few conflicting clinical data exist concerning the relationship between Zn and coronary artery disease (CAD). They studied 72 patients without prior history of myocardial infarction or revascularization procedures, who underwent coronary angiography for evaluation of chest pain. Coronary artery disease severity was estimated using 3 angiographic scores. Zn in serum and 24-hour urine, as well as serum Zn/24-hour urine Zn ratio were determined. Serum Zn was not associated with CAD prevalence severity.Zinc opposes the absorption and redox activity of copper via induction of the antioxidant protein metallothionein, which binds copper tightly.

Risk factors

Diabetic mellitus

The present study result showed the mean(± SD) value of serum zn concentrations was non significantly higher in non diabetic group than diabetic group,the cause may be due to all diabeticpatients were treated with insulin-zinc mixture. Giannoglou GD et al. (27) reported that Serum Zn/24-hour urine Zn ratio was inversely associated with CAD, as well as with diabetes mellitus prevalence, fasting glucose, and glycated hemoglobin levels.[28]

The mean(± SD) value of serum Cu concentrations was non significantly higher in diabetic group than non diabetic group,the cause may be due to that elevated blood copper is likely a symptom of inflammation, rather than its cause.

Hypertension

The mean(±SD) values of serum Cu levels in hypertensive patients was non significantly higher than that in non hypertensive patients and vice versa for Zn. A possible explanation for the observed changes in tissue Cu and Zn metabolism is that the stress of the hypertensive condition resulted in an increase in glucocorticoid synthesis and release Glucocorticoids can stimulate synthesis of the Cu containing protein ceruloplasmin in the liver. Once ceruloplasmin is synthesized, it is transported into the blood. Glucocorticoids increase the synthesis of the liver Zn-binding protein metallothionein. An increase in the amount of this protein in liver could result in increased liver Zn concentrations and decreased plasma Zn concentrations.[29]

Obesity

The present study result showed the mean(± SD) value of serum zn concentrations was non significantly higher in non obesegroup than obesegroup. Kennedy and Failla[30] reported that genetically obese (ob/ob) miceabsorbed more and retained more Zn than did lean controls. The Zn content was inversely related to the body mass index.[31]

Dyslipidemia

The results of the present study that the mean(± SD) value of serum Zn concentrations was non significantly lower whileserum cu concentrations was non significantly higher in dyslipidemic groupthan non dyslipidemic group, are dysagreeable with suggestions that zinc can act as an endogenous protective factor against atherosclerosis by inhibiting the oxidation of LDL in the presence of transition metals.[32]Cu and CVD may be associated directly, through a direct effect on the vascular endothelium. [33]

Smoking

The mean of serum copper concentration was non significantly lower in non smokers than in smokers, while the mean of serum Zn concentration was non significantly lower in smokers than in nonsmokers. Kocyigit et al.[34]reported that serum copper concentration was significantly lower in tobacco smokers than in nonsmokers. There was non significant difference in plasma zinc concentrations between the two groups.

Effect of fibrinolytic treatment (Actilyse)

The mean of serum copper & zinc concentration was non significantly heigher in patients with fibrinolytic treatment(Actilyse) than in patients without fibrinolytic treatment. There were no previous studies in this section to be relied on for comparison.

Echocardiography

The present study result showed, there was no association between serum Cu&zn level with MR severity. A significant negative correlation between serum Zn concentrations with Left atrium diameter and Left Ventricular Ejection Fraction(LVEF). There was non significant positive correlation between the serum Zn concentrations and the value of Isovolumic relaxation time, E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio and Decceleration time. The study showed that there was non significant positive correlation between the serum Cu concentrations and the value of Diameter of left atrium. However, there was non significant negative correlation between serum Cu concentrations and Left Ventricular Ejection Fraction(LVEF), Isovolumic relaxation time , E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio and Decceleration time.

The mean(±SD) values of serum Cu levels of patients in stage 2 of diastolic dysfunction was non significantly higher than that in stage 1 and 3, the mean±SD values of serum Zn levels of patients in stage 1 was significantly higher than that in stage 2 and 3 of diastolic dysfunction.There were no previous studies in this section to be relied on for comparison.

Agewas non significant negativly correlate with serum Zn concentrations and non significant positivly correlate with serum Cu concentrations.Zinc levels tend to decline with age, just as the risk for cardiovascular diseases increases.[35]Researchers believe that decreased zinc levels might also increase your risk for cardiovascular disease.[36]

CONCLUSION

Lower Zn and higher Cu serum concentration was found in patients with ACS but these levels are non significantly different between myocardial infarction and unstable angina patients Also, it seems that risk factor dose not considerably affect serum levels of Zn or Cu in patients.Study revealed significant correlation between zinc concentration and diastolic dysfunction in patients withacute coronary syndrome. zinc&copper are non significantly correlat with parameters of the left ventricular systolic function[Diameter of left aterium, Mitral regurgitation, E (left ventricular inflow velocity) / e (tissue doppler velocity) ratio, isovolumic relaxation time Decceleration time and Left ventricular Ejection Fraction].

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