INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

# THE TIME COURSE EFFECT ON THE PLASMA AND NEUTROPHIL`S LEVELS OF THE ENZYMATIC OXIDANTS AND ANTIOXIDANTS IN UNSTABLE ANGINA AND MYOCARDIAL INFARCTION

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**ABSTRACT**

**Objectives:** To determine whether oxidative stress occurs in unstable angina and to examine the state of oxidative stress during reperfusion state following myocardial infarction and bouts of recurrent ischemia in unstable angina. **Methods:** Blood samples were taken from 16 with acute myocardial infarction (AMI), 11 with unstable angina (UA) and 34 healthy volunteers. The concentration of plasma and neutrophil SOD, catalase, MDA, ceruloplasmin, $Cu^{+2}$, $Zn^{+2}$ and thiols were determined at first and seventh day of onset of chest pain following complete clinical response. **Results:** The means of serum total creatine phosphokinase enzyme activity and the neutrophil count were significantly increased ($P<0.001$) with increase in both plasma $Cu^{+2}$ and ceruloplasmin concentration ($P<0.01$) and significant decrease in the mean neutrophil SOD activity in AMI group within the first day of onset of chest pain in comparison to the control mean values ( $P<0.05$). The means of plasma $Cu^{+2}$, and neutrophil $Cu^{+2}$ were increased by 26.8, and 13.7%, respectively after seven days of onset of chest pain ($P < 0.01$) in UA group. The activity of neutrophils catalase revealed 14% decrease in UA at day seven as compared to those of the first day of chest pain. In AMI group, the catalase activities showed a near significant reduction (18.4, $P < 0.07$), on the seventh day, while the mean of SOD activities was significantly increased to 8.4% over the first day reported mean enzyme activity ($P < 0.01$). Significant decrease in thiols and MDA (10.3% , 22.6%.$P<0.01$) in UA and MDA values was increased in AMI to 25.7% ($P<0.01$) as compared to the day one mean concentration. **Conclusion:** There was more alleviation in the oxidative stress effect (MDA and thiols, SOD, catalase, ceruloplasmin) in UA as compared to AMI at reperfusion state.

**Keywords:** Reperfusion, superoxide dismutase, catalase, ceruloplasmin, Zinc.

## INTRODUCTION

Ischemic heart disease is the major cause of morbidity and mortality in the Western world. Ischemia-reperfusion injury may induce cardiomyocyte cell death by necrosis or apoptosis. The heart can be adapted to tolerate an ischemic event by preceding brief episodes of ischemia and reperfusion, called preconditioning. Preconditioning protects the heart when it is directed towards the heart itself either immediately before or several days before an induced ischemic event. Adaptation

by preconditioning can even be achieved in other organs, and preconditioning one organ can protect another organ. Evidence suggests that preconditioning may be a naturally occurring adaptive process in vivo, and in humans unstable angina before acute myocardial infarction may represent the phenomenon[1].The injury induced by reperfusion of the ischemic myocardium could result, in part, from the cytotoxic effects of oxygen free radicals .Various trace elements are involved in several of the reactions leading to free radical production[2]**.** The associations between serum ceruloplasmin (Cp) level and the subsequent incidence of myocardial infarction and stroke were studied in a nested case-control study for the period 1968–1972 in Finland[3].

Ischemia causes alterations in the defense mechanisms against oxygen free radicals, mainly a reduction in the activity of mitochondrial superoxide dismutase and a depression of tissue content of reduced glutathione. At the same time, production of oxygen free radicals increases in the mitochondria and leukocytes and toxic oxygen metabolite production is exacerbated by re-admission of oxygen during reperfusion. Oxidative stress, in turn, causes oxidation of thiol groups and lipid peroxidation leading first to reversible damage, and eventually to necrosis[4].

Epidemiological data that suggests that serum Cp may be an important risk factor predicting myocardial infarction and cardiovascular disease. Biochemical studies have shown that Cp is a potent catalyst of LDL oxidation in vitro. The pro-oxidant activity of Cp requires an intact structure, and a single copper atom at the surface of the protein, near $His^{426}$, is required for LDL oxidation[5].

Since various trace elements are involved in several of the reactions leading to free radical production, we have measured plasma levels of copper, zinc, and to examine the alterations in the defense mechanisms against oxygen free radicals (SOD, catalase, and ceruloplasmin) during reperfusion periods after (seven days) following myocardial ischemia and infarction.

## MATERIALS AND METHODS

The study was conducted on 27 patients (11 with unstable angina, and 16 with acute myocardial infarction) and 34 apparently healthy controls. The patients were selected from those referred to the Coronary Care Units in Ibn Al-Nafes and Al-Imamain Alkadhemain Teaching Hospitals.The research has been approved by the ethical Committee of Alnahrain College of Medicine . The diagnosis of unstable angina (UA) and acute myocardial infarction (AMI) was established by the senior cardiologist following thorough history, clinical examination, ECG and cardiac enzyme analyses .Only patients who experienced persistent chest pain within the last twenty four hours were selected for this study. No attempt was made to alter the patient's medications. However, among these patients 20 were on calcium channel blockers and seven were taking β- blockers and all were put on glycerine mononitrite. Patients with renal or hepatic insufficiency, alcohol usage, and intake of supplements containing Cu or Zn within 1 week were excluded from the study.

Venous blood samples were aspirated from patients and controls following an overnight fasting and distributed into two test tubes one is EDTA containing tube for blood indices ( total WBC ,total percentage of neutrophils ) and a second tube with sodium heparin for the neutrophil isolation , the plasma was separated by centrifugation at 1000Xg for the determination of plasma cupper ($Cu^{+2}$), ceruloplasmin , malonyldialdehyde (MDA),thiols, and neutrophil intracellular enzymes ( SOD, catalase) and their cofactors ( $Cu^{+2}$ and $Zn^{+2}$).

During hospitalization period, second blood samples were withdrawn from the 27 treated patients at seventh day following the onset of chest pain and when complete clinical response had been reported, as checked by subjective responses (relief of pain, dyspnoea, vomiting, sweating... etc), clinical assessment, together with ECG findings. Blood samples were processed as previously reported to define and correlate the pattern of antioxidant activities and lipid peroxidation products (MDA) with the achieved clinical responses.

$Cu^{+2}$ and $Zn^{+2}$ levels have been assayed with atomic absorption spectrophotometry. The neutrophil SOD, catalase and plasma ceruloplasmin and thiols concentrations were measured as previously published methods[6-9].

Statistical analysis was performed with the SPSS 16 software using independent sample t test for comparison between different studied parameters. A $P< 0.05$ is considered statistically significant.

## RESULTS

Table 1 revealed a highly significant increase in the mean serum total creatine phosphokinase enzyme activity and total WBC count in patients with acute myocardial infarction within the first day following the onset of chest pain($p<0.001$) as compared to

the unstable angina mean values .In AMI , there were 70% increase in the neutrophil count.

Table 2 shows the means (±SEM) of neutrophil catalase, SOD, $Cu^{+2}$, and $Zn^{+2}$ levels together with the mean plasma values of ceruloplasmin, $Cu^{+2}$ in 11 patients with UA at first and seventh day following the onset of chest pain. The means of plasma $Cu^{+2}$ at first day of chest pain were significantly elevated whereas the mean plasma $Zn^{+2}$ decreases in UN group as compared to the respective mean values in the control group. ($P<0.01$,$P<0.05$). Furthermore, only the mean neutrophil SOD activity showed significant reduction as compared to mean values ($p<0.05$). The means of plasma $Cu^{+2}$, and neutrophil $Cu^{+2}$ increased by 26.8, and 13.7%, respectively after seven days of onset of chest pain ($P < 0.01$).The activity of neutrophils catalase revealed 14% decrease in day seven as compared to those of the first day of chest pain. The observed percentage of variations in all of other parameters showed insignificant deviations from the mean first day values which is partly due to the small sample number.

The means (±SEM) of neutrophil catalase, SOD, $Cu^{+2}$, and $Zn^{+2}$ levels together with the mean plasma values of ceruloplasmin, $Cu^{+2}$ in 16 patients with AMI are listed in Table 3. There were statistically significant increase in both plasma $Cu^{+2}$ and ceruloplasmin concentration($P<0.01$) and significant decrease in the means of neutrophil $Zn^{+2}$ and SOD activity in AMI group within the first day of onset of chest pain in comparison to the control mean values ($P<0.01$, and $P<0.05$). Results of catalase activities showed a near significant reduction (18.4, $P < 0.07$), on the seventh day, while the mean of SOD activities was significantly increased to 8.4% over the first day reported mean enzyme activity ($P < 0.01$). The percentage of elevation in means of plasma ceruloplasmin did not differ significantly from the first day respective mean values. Similarly, the percentages of rise in means of plasma and neutrophil $Cu^{+2}$ values (10.4, and 0.7, respectively) were statistically in-significant.

The pattern of change in the levels of plasma thiols and LPO product (MDA) in unstable angina and AMI groups are listed in table 4. The means of serum thiols in AMI and unstable angina groups was significantly lower than the control mean values with a significant decrease in only unstable angina at day seven as compared to day one mean values. The MDA values at the first day of chest pain in both AMI and unstable angina patients were significantly elevated above the mean control values ($P<0.01$).Yet, in unstable angina patients the mean MDA values after seven days of follow up showed significant reduction as compared to the first day values (22.6%, $P<0.01$), whereas in patients with AMI the mean MDA values increase to 25.7%($P<0.01$) as compared to the day one mean concentration.

## DISCUSSION

It has been suggested that myocardial production of oxygen free radicals above the neutralizing capacity of the myocytes is an important cause of reperfusion damage. An oxygen free radical-mediated impairment of mechanical function also occurs during reperfusion of the human heart[10].

In the present study, we recorded highly significant decrease in the mean of MDA values ($P < 0.01$), with significant rise in neutrophil SOD ($P < 0.01$ mean values after seven days of ischemia as compared to their respective mean concentration at the first day of onset of chest pain.

The low activities of SOD and catalase reported in these patients (as compared to mean normal activities) compromise the first defense mechanism of cells against superoxide anion radical. This mechanism of SOD inactivation by OH radical explains the relative reduction observed in catalase enzyme activities after seven days of ischemia in patients with UA .Also it might be incriminated for the low intracellular and extracellular thiols. It was reported that the increase in MnSOD mRNA in failing hearts pinpoints that the increased oxidative stress in failing myocardium may lead to increase the transcription of antioxidant enzymes[11].

Tsan et al. (1991) displayed an induction in the activities of catalase, SOD, and GSH-Px in rat lung under high oxygen tension, and they documented the protective role offered by the administration of interleukin-L[12]. Ultra structural studies revealed that hypoxia alter the morphology of mitochondria isolated from type II rats' alveolar living cells. The morphological changes involve elongation of mitochondria, increase in numbers of ribosome, and dilatation of cisternae of endoplasmic reticulum. These structural modifications were consistent with the elevation in the mitochondrial SOD activities during hypoxia. At 85 of O2, SOD induction might be saturated or gradually exhausted and the excess oxygen anions formed would alter H2O2- decomposing enzymes leading to low catalase and GSH-Px activities. The reduced activity of these enzymes raises the H2O2

concentration to a level that would inactivate SOD enzyme as shown below[13].

Experimental studies conducted on canine hearts showed that repeated pretreatment with brief periods of ischemia and intervening reperfusion triggers adaptive changes that protect myocardium from the effect of subsequent prolonged ischemic insult compared with that of controls. Moreover, the size of myocardial infarction fall by three forth[14]. This induced tolerance to ischemia was known as ischemic preconditioning[15]. The exact mechanisms underlying this powerful endogenous form of myocardial protection is not clear, but current researches had stressed the role of inducibility of antioxidant defenses in this preconditioning process[16] . These differences in antioxidant activities and indicators of oxidative damage to lipids (MDA values) and proteins (thiols) reported herein probably reflect the underlying persistence of wall motion dysfunction caused by the generated FRs and oxidants[17] .

Dwivedi (2006) showed that the activities of anti-oxidant enzymes (viz. SOD, catalase and glutathione reductase) are significantly decreased whereas there is significant increase in the levels of malonyldialdehyde (a marker of free radical-mediated damage) in the patients. The findings points out that ischemic myocardial disorder are associated with excessive free radical generation and free radical-mediated damage of lipids[4].

Garelnabi et al. measured platelets aggregation, malonyldialdehyde (MDA), plasma-ionized $Ca^{+2}$, and antioxidant enzymes, i.e., glutathione peroxidase and superoxide dismutase in healthy volunteers and patients with myocardial infarction, unstable and stable angina and concluded that the increased oxidative stress in these patients was accompanied by platelet activation and impaired antioxidant enzymes activity[5].

Subset analysis conducted by Chandra et al. (1994) revealed that in unstable angina and acute myocardial infarction, superoxide anion, malonyldialdehyde and glutathione reductase were elevated while superoxide dismutase and catalase levels were reduced. However, this alteration was less marked than in unstable angina and acute myocardial infarction. In the post myocardial infarction group there was no alteration in any of these parameters[18,19].

The mean $Cu^{+2}$ level of the ischemic cardiomyopathy group (1.54 +/- 0.52 mg/L) was found to be significantly more than the $Cu^{+2}$ levels of the healthy volunteers (1.31 +/- 0.24 mg/L; p = 0.048). Whereas the $Zn^{+2}$ level of ischemic cardiomyopathy patients was not significantly different in comparison with the healthy volunteers[20].

Kazi et al.(2008) observed a decrease in the concentrations of $Zn^{+2}$ in whole blood and scalp hair samples in AMI patients as compared to normal subjects. They concluded that deficiency of zinc and high concentration of $Cu^{+2}$and iron may play a role in the development of heart disease[21]. The increase in serum levels of $Cu^{+2}$ and Fe and the decrease in serum levels of $Zn^{+2}$ and Se in patients with higher levels of troponins and CK-MB imply that trace element levels are related to the degree of myocardial damage and thus may play a role in the pathogenesis of ischemic heart disease[22].

Altekin and co researchers measured the plasma levels of $Cu^{+2}$, $Zn^{+2}$ , selenium, and iron in 18 patients (mean age 60 years) subjected to thrombolytic therapy within 6 h after the onset of a myocardial infarction and recorded an increase in plasma $Cu^{+2}$ levels from day 4 to day 10 post infarction whereas a decrease in plasma zinc levels was observed and was maximum 12 h after the onset of the thrombolytic treatment[23].

The changes in serum $Zn^{+2}$ concentration and distribution during the 15 days following acute myocardial infarction were studied in 21 patients. The method is based on ultrafiltration and electrothermal atomic absorption spectrometry. Serum $Zn^{+2}$ ($P < 0.00001$) and exchangeable $Zn^{+2}$ (mainly albumin-bound $Zn^{+2}$, $P = 0.01$) declined within the first 3 days and then returned gradually to reference ranges. This result could be related to an increase of $Zn^{+2}$ uptake by tissues[24] .

Fluorescence microscopic examination of the in intact cardiomyocytes suggests that $Zn^{+2}$ influx is through sarcolemmal $Ca^{+2}$ channels and that significant stores of intracellular $Zn^{+2}$ may be released quickly (<1s) into the cytosol. The $Zn^{+2}$ acts as a modifier of $Ca^{+2}$ regulation or as a possible signaling messenger for gene expression[25].

Ceruloplasmin levels were measured in stored serum samples from 104 myocardial infarction or stroke cases occurring during a median follow-up of about 11 years and from 104 individually matched controls. High serum ceruloplasmin levels were significantly associated with higher future odds of myocardial infarction but not of stroke[3] .

In 11 patients with UA, we recorded non-significant elevations in both neutrophil SOD, plasma ceruloplasmin activities. Yet, in 16 patients with AMI there were no change in ceruloplasmin mean values as compared to first day mean values. Ziakas and coworkers investigated the time course and prognostic

value of ceruloplasmin (CP) in addition to fibrinogen (Fib), C-reactive protein (CRP), and interleukin-6 (IL-6) in patients with severe unstable angina. They reported that the mean CP levels were significantly higher in patients with complications during follow-up ($P < .05$) from 6 hours until 6 months they concluded that only CP levels were related to 12-month follow-up prognosis[26].

## CONCLUSION

Oxidative stress can be evidenced in patients with unstable angina or acute myocardial infarction. Yet, less marked changes were seen in patients with unstable angina. Prolonged ischemia reduces the naturally occurring defense mechanisms of the heart against oxygen free radicals, particularly catalase, SOD and ceruloplasmin, and the intracellular pool of $Cu^{+2}$ and $Zn^{+2}$ .Consequently, reperfusion results in severe oxidative damage, as evidenced by accumulation and release of MDA. After seven days of the preconditioning, the decrease in the FR effect may spare cardiac myocytes function as evidenced by the decrease in the thiols and MDA values.

## ACKNOWLEDGMENTS

I would express my sincere appreciation to all medical staff at Ibn Al-Nafes and Al-Imamain Alkadhemain Teaching Hospitals for their support and help in sample collection and support during the laboratory analysis.

**Table 1: Some clinical and biochemical data of the studied groups**

| Variables | Unstable angina | Acute Myocardial infarction |
|---|---|---|
| Number | 11 | 16 |
| Age(year) | 54.6±8.14 | 55.4±10.04 |
| Time of onset of chest pain (hours) | 18.0±6.02 | 17.8±6.20 |
| Pulse rate/ minutes | 78.9±16.09 | 82.8±20.07 |
| Diastolic blood pressure (mmHg/Hr) | 82.8±12.58 | 83.6±15.04 |
| Fasting serum Glucose (mM/L) | 5.873±2.798 | 6.984±3.716** |
| Serum total creatine phosphor kinase (IU/L) | 9.4±6.449 | 37.9±24.162*** |
| Total WBC ($x10^9$ /L) | 7.944±1.991 | 12.370±4.534*** |
| Total blood Neutrophils ($x10^9$ /L) | 5.7±1.788 | 9.0±3.362** |
| % of neutrophilia | 16% | 70.5%*** |

Results are expressed as mean ± SEM
ANOVA test: **P<0.01, ***P<0.001

**Table 2: Day One and Seven Changes in the Mean ± SEM Values of Catalase, superoxide dismutase (SOD), Ceruloplasmin, $Zn^{+2}$, $Cu^{+2}$ in Plasma and Neutrophils of Patients with Unstable Angina**

| Concentration of: | Controls (n=34) | Day one (n=11) | Day seven (n=11) | % of difference | P value |
|---|---|---|---|---|---|
| Plasma $Cu^{+2}$(µM/L) | 14.03 ±0.314 | 15.3 [a] ±0.958 | 20.9 ± 1.189 | 26.8% [a] | <0.01 |
| Plasma Ceruloplasmin (mg/L) | 269.6 ±6.085 | 332.6 ±20.9 | 361.4 ±17.7 | 8% [a] | NS |
| Neutrophils $Cu^{+2}$(µM/L) | 60.9 ±2.71 | 56.3 ±6.378 | 64.818 ±8.533 | 13.7% [a] | <0.08 |
| Neutrophils $Zn^{+2}$(µM/L) | 198.3 ±11.097 | 168.2 [b**] ±16.298 | 168.1 ±8.195 | 0% | NS |
| Neutrophils Catalase (units/mg protein) | 57.4 ±2.278 | 55.9 ±0.61 | 48.1 ±0.63 | 14% [b] | NS |
| Neutrophils SOD (units/mg protein) | 0.76 ±0.030 | 0.61 [b*] ±0.047 | 0.63 ±0.05 | 3.8% [a] | NS |

[a] Represent the increase.
[b] Represent the decrease.

**Table 3: Day One and Seven Changes in the Mean ± SEM Values of Catalase, superoxide dismutase (SOD), Ceruloplasmin, $Zn^{+2}$, $Cu^{+2}$ in Plasma and Neutrophils of Patients with Acute Myocardial infarction**

| Concentration of: | Controls (n=34) | Day one (n=16) | Day seven (n=16) | % of difference | P value |
|---|---|---|---|---|---|
| Plasma $Cu^{+2}$(µM/L) | 14.03 ±0.314 | 18.03 [a**] ±1.152 | 20.1 ±1.103 | 10.4% [a] | NS |
| Plasma Ceruloplasmin (mg/L) | 269.6 ±6.085 | 343.0 [a**] ±30.7 | 384.2 ±25.415 | 10.7% [a] | NS |
| Neutrophils $Cu^{+2}$(µM/mg) | 60.9 ±2.71 | 56.7 ±4.006 | 57.1 ±6.249 | 0.7% [b] | NS |
| Neutrophils $Zn^{+2}$(µM/mg) | 198.3 ±11.097 | 146.5 [b**] ±15.345 | 146 ±11.168 | 0.2% [a] | NS |
| Neutrophils Catalase (units/mg protein) | 57.4 ±2.278 | 54.8 ±4.81 | 44.7 ±3.211 | 18.4% [b] | <0.07 |
| Neutrophils SOD (units/mg protein) | 0.76 ±0.030 | 0.68 [b*] ±0.042 | 0.74 ±0.046 | 8.4% [a] | <0.01 |

[a] Represent the increase.
[b] Represent the decrease.

**Table 4: Day One and Seven Changes in the Mean ± SEM Values of Plasma peroxides (MDA) and thiols in Patients with Unstable angina and Acute Myocardial infarction (AMI)**

| Concentration of: | Controls (n= 34) | Unstable angina N=11 | | % Differ. | AMI N=16 | | % of differ. |
|---|---|---|---|---|---|---|---|
| | | Day one | Day seven | | Day one | Day seven | |
| **Plasma Thiols(µM/L)** | 480.3 ± 11.706 | 341.2 [b*] ± 26.866 | 306.2 ± 23.3 | 10.3% [b*] | 311.4 ± 16.303 | 303.5 ± 17.767 | 2.5% [b] |
| **Plasma MDA (nM/ml)** | 3.8 ± 1.57 | 7.8 [a**] ± 1.1 | 6.0 ± 0.40 | 22.6% [b*] | 10.2 ± 1. 62 | 7.6 ± 1.07 | 25.7% [a**] |

[a] Represent the increase.
[b] Represent the decrease.

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