IMPORTANCE OF GLYCOGEN PHOSPHORYLASE ISOENZYME BB, TOTAL SIALIC ACID, OXIDANT AND ANTIOXIDANT PARAMETERS IN THE NONINVASIVE ASSESSMENT OF THROMBOLYTIC THERAPY

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ABSTRACT

Objective: *The aim of this study was to investigate the effect of thrombolytic therapy on oxidant and antioxidant parameters and glycogen phosphorylase isoenzyme BB (GPBB) concentrations in patients with acute myocardial infarction (AMI).*

Material and Method: *This was a prospective study at an urban academic hospital. We enrolled non-traumatized patients arriving at the emergency department within 3 hours after the onset of chest pain suggestive of AMI. The diagnosis of AMI was established according to clinical features, electrocardiographic findings and increased biochemistry cardiac marker levels. The study was carried out on a total of 75 patients with AMI (50 patients received thrombolytic therapy and 25 patients did not receive thrombolytic therapy), and 45 controls. Blood samples were collected before, and, 2 and 24 hours after tissue-type plasminogen activator therapy. Serum malondialdehyde (MDA) and vitamin E were analyzed by high-performance liquid chromatography. Serum total sialic acid and other oxidant and antioxidant parameters were studied spectrophotometrically. Determination of GPBB concentration is based on immunoenzymometric assay.*

Results: *Successful reperfusion was followed by increased serum MDA, protein carbonyls, total sialic acid and GPBB concentrations and by decreased antioxidant vitamins and enzymes.*

Conclusion: *These findings indicate the significance of free radical generation processes in reperfusion injury in AMI patients, and suggest the potential involvement of antioxidants in the management of AMI treated by thrombolysis. Also, these parameters especially GPBB can be used for the noninvasive assessment of the effectiveness of thrombolytic therapy.*

Key Words: *Thrombolytic therapy, glycogen phosphorylase isoenzyme BB, sialic acid, paraoxonase, malondialdehyde.*
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TROMBOLİTİK TEDAVİNİN NONİNVAZİV DEĞERLENDİRMESİNDE GLİKOJEN FOSFORİLAZ İZOENZİM BB, TOTAL SİALİK ASİT, OKSİDAN VE ANTİOKSİDAN PARAMETRELERİN ÖNEMİ

ÖZET

Amaç: Bu çalışmanın amacı, akut miyokard infarktüslü (AMI) hastalarda trombolitik tedavinin oksidan ve antioksidan parametreler ve glikojen fosforilaz izoenzim BB (GPBB) konsantrasyonları üzerine etkisini araştırmaktır.

Materyal ve Metod: Bu bir prospektif çalışmadır. Çalışmaya, AMI'yı düşündüren bir göğüs ağrısının başlamasından sonra 3 saat içinde acil servise başvuran ve travmaya uğramamış hastalar alındı. AMI tanısı klinik özellikler, elektrokardiyografik bulgular ve artmış biyokimyasal kardiyak marker düzeylerine göre konuldu. Çalışmaya AMI'lı total 75 hasta (50 hasta trombolitik tedavi aldı, 25 hasta trombolitik tedavi almadı) ve 45 kontrol alındı. Kan örnekleri doku tipi plazminojen aktivatör tedavisinden önce ve tedaviden 2 ile 24 saat sonra alındı. Serum malondialdehid (MDA) ve E vitamini düzeyleri yüksek performanslı sıvı kromatografisi (HPLC) ile analiz edildi. Serum total sialik asit ve diğer oksidan ve antioksidan parametrelerin düzeyleri spektrofotometrik olarak ölçüldü. GPBB konsantrasyonu immünoenzimometrik ölçüm ile belirlendi.

Bulgular: Başarılı reperfüzyon sonrası serum MDA, protein karbonilleri, total sialik asit ve GPBB konsantrasyonları arttı, antioksidan vitamin ve enzim düzeyleri ise azaldı.

Sonuç: Bu bulgular AMI'lı hastalarda reperfüzyon hasarında serbest radikal oluşumunun önemini göstermekte ve trombolitik tedavi uygulanan AMI'lı hastaların tedavisinde antioksidanların da yer almasının gerekliliğini belirtmektedir. Aynı zamanda bu parametreler, özellikle GPBB, trombolitik tedavinin etkinliğinin noninvaziv olarak değerlendirilmesi için de kullanılabilir.

Anahtar Kelimeler: Trombolitik tedavi, glikojen fosforilaz BB, sialik asit, paraoksonaz, malondialdehid.
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INTRODUCTION

Acute revascularization is now a standard practice for patients with acute myocardial infarction (AMI). The objective for thrombolytic therapy is to recanalize occluded arteries and to diminish the infarct size and major cardiac complications. Thrombolytic therapy has been effective in the early hours following an acute myocardial infarction to reestablish blood flow and reperfuse ischemic myocardial tissue.[1] However, reperfusion with enhanced oxygen delivery to the ischemic myocardium may actually contribute to further tissue and cell damage inducing a process termed reperfusion injury. Therefore, successful reperfusion may result in transient or permanent myocardial injury (reperfusion injury), assumed to be free oxygen radical-mediated.[2]

Free radicals can be produced during ischemia and reperfusion by several different biochemical pathways. When produced, free radicals can potentially damage myocytes or endothelial cells through peroxidation of membrane lipids or damage to proteins or nucleic acids.[3] Whether oxidation of membran lipids play an important role in postischemic injury is still uncertain. Several studies have demonstrated increased lipid peroxidation after reperfusion, while some studies have failed to show evidence for accumulation of lipid peroxidation products.[4-7] Malondialdehyde (MDA), a stable metabolite of the free radical-mediated lipid peroxidation cascade, is widely used as marker of oxidative stress.[8] On the other hand, growing evidence has suggested that proteins are the major targets for free radicals in postischemic injury. These studies have shown that ischemia and reperfusion result in carbonyl formation; their level in tissues and plasma is a relatively stable marker of oxidative damage.[9,10] Although reports are available on serum MDA levels of patients to whom thrombolytic therapy has been administered, the relationship between serum protein carbonyl levels and thrombolytic therapy is not well known.[11,12]

Sialic acids are a family of amino sugars that are commonly found as terminal oligosaccharide residues on glycoproteins and glycolipids.[13] Although a close relationship has been described between total sialic acid and ischemic cardiovascular diseases in recent years the role of sialic acid in ischemia and reperfusion damage and in noninvasive assessment of thrombolytic therapy is not clear.[14,15] Therefore, we aimed to look for the changes in serum total sialic acid levels after reperfusion.

The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants. Antioxidant activity is achieved by the antioxidant scavenger system which includes enzymes and antioxidant vitamins. The extent of free oxygen radical-induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense →

Table 1: Clinical baseline characteristics and lipid profiles of the study groups

	AMI (n=75)	Control (n=45)
Age (years)	59±10	56±10
Male/Female	46/29	29/16
BMI (kg/m^2)	27.8±4.3	27.2±4.0
Risk factors*		
Diabetes (%)	11	7
Hypertension (%)	44#	4
Hyperlipidemia (%)	42#	9
Smoking (%)	45#	20
Family history (%)	20	15
Serum lipids, mmol/L		
Total Cholesterol	4.97±0.90	4.81±0.70
HDL- Cholesterol	1.14±0.19#	1.35±0.19
LDL- Cholesterol	3.22±0.44#	2.83±0.49
Triacylglycerol	1.89±0.44#	1.36±0.31
Total C / HDL-C	4.37±0.49#	3.58±0.39
Apolipoprotein AI (g/L)	1.22±0.19‡	1.33±0.16
Apolipoprotein B (g/L)	1.25±0.18#	1.09±0.15
Lipoprotein(a)** (g/L)	0.22±(0.02-1.41)‡	0.11 (0.01-0.58)

AMI: Acute myocardial infarction, BMI: Body mass index, HDL: High density lipoprotein, LDL: Low density lipoprotein, C: Cholesterol,*: chi-square test, **: Median (Range) ‡: p< 0.01, #: p< 0.001

Table 2: Clinical baseline oxidant and antioxidant parameters, total sialic acid and GPBB levels

	AMI (n=75)	Control (n=45)
Oxidant parameters		
Malondialdehyde (nmol/ml)	0.75±0.23‡	0.61±0.24
Protein Carbonyls (nmol/mg protein)	0.74±0.16#	0.56±0.21
Antioxidant Vitamins (µmol/L)		
Vitamin E	28.27±5.4#	37.61±5.8
Vitamin C	34.63±8.8#	54.00±9.1
Total Carotenoids	2.36±0.6‡	2.75±0.6
Antioxidant Enzymes		
Paraoxonase (U/L)	201±62‡	244±77
Arylesterase (kU/L)	99±14‡	108±16
Catalase (kU/L)	35.3±12.7‡	42.4±16
Total Sialic Acid (mmol/L)	1.52±0.23‡	1.35±0.19
GPBB* (µg/L)	85.4 (8.7-204)#	11.8 (6.1-53.7)

GPBB: Glycogen Phosphorylase Isoenzyme BB, AMI: Acute Myocardial Infarction, *: Median (Range), ‡: p< 0.01, #: p< 0.001

mechanisms.[16] There is evidence that antioxidants can protect against free radical production which is responsible for reperfusion-induced damage and lipid peroxidation, and may thereby inhibit thrombosis and myocardial damage during AMI.[5,17] Apart from these important antioxidants, paraoxonase appears to have antioxidative properties, as well. Paraoxonase, a high density lipoprotein (HDL)-associated enzyme carried on apolipoprotein AI, is believed to protect lipoproteins against oxidative modification.[18-20] Arylesterase activity born by paraoxonase can be considered as an index of actual protein concentration.[21] Compared with the considerable literature describing the relation between antioxidant vitamins and enzymes and ischemia-reperfusion damage, there is no report about paraoxonase.

The process of myocardial injury and infarction weakens the myocyte membrane wall and permits the intracellular macromolecules, collectively referred to as biochemical cardiac markers or biomarkers, to diffuse into the peripheral circulation.[22] Besides AMI diagnosis and risk assessment, cardiac markers can be used to assess the success or failure of thrombolytic therapy. AMI patients who develop patent coronary circulation will release a bolus amount of enzymes and proteins into the circulation ("washout phenomenon") when compared with AMI patients with permanent occlusions.[23] Glycogen phosphorylase isoenzyme BB (GPBB) is a promising marker for early AMI diagnosis.[24] Up to now, use of various biochemical cardiac markers, including myoglobin, creatine kinase-MB, troponin T, and troponin I, have been extensively studied for noninvasive assessment of reperfusion, but studies about GPBB are not enough.[25,26]

The aim of this study was to investigate the effect of thrombolytic therapy on circulating MDA, protein carbonyls, total sialic acid levels, antioxidant enzymes (paraoxonase, arylesterase, catalase), antioxidant vitamins (vitamin E, vitamin C, carotenoids) and also GPBB concentrations in patients with AMI.

MATERIAL and METHOD

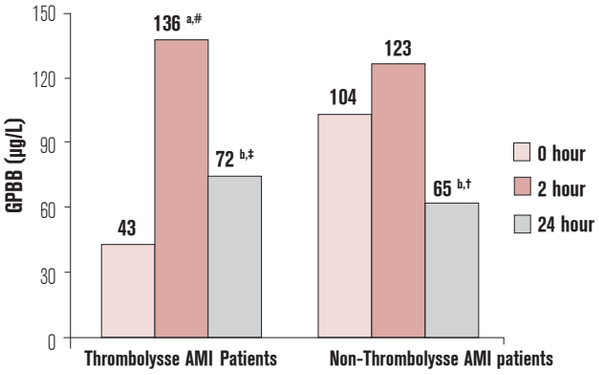
The study was carried out on a total of 75 patients with AMI (50 patients received thrombolytic therapy and 25 patients did not receive thrombolytic therapy due to history of hemorrhagic cerebrovascular event), and 45 controls. The patient population consisted of 46 male and 29 female patients about 59 (mean) years old (range, 35 to 80 years). The control population comprised 29 male and 16 female volunteers, aging 31 to 71 years (mean 56 years). Patients who presented to emergency department within 3 hours of onset of symptoms were followed. Diagnosis was established by clinical and electrocardiographic examination and the measurement of the activity of serum cardiac markers.

Patients with concomitant or recent acute or chronic illness were excluded. Patients with uncomplicated diabetes and those with properly controlled hypertension were included. In these AMI patients a single blood sample was drawn immediately after admission. All patients received routine emergency treatment and were transferred to the coronary care unit before initiating thrombolytic therapy. None of these patients had received thrombolytic treatment before admission to the hospital. The indices used to establish successful reperfusion; relief from chest pain, ECG-ST segment regression and reperfusion arrhythmias. Patients with AMI who were eligible for thrombolytic therapy received intravenous t-PA (tissue-type plasminogen activator) each. Following blood samples were collected 2 and 24 hours after initiation of →

thrombolysis with t-PA. Also, in AMI patients who did not receive thrombolytic therapy the following blood samples were collected 2 and 24 hours after admission. The control samples were taken from 45 apparently healthy age and sex-matched volunteers who had no history of any cardiac symptoms and who had a normal physical examination and normal electrocardiogram at rest. None of the subjects (cases or control) were taking antioxidant or vitamin supplements, and drugs known to affect plasma lipid values significantly. The following conventional cardiovascular risk factors were defined:[27] Arterial hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg on two or more occasions and/or use of anti-hypertensive drugs), hyperlipidemia [total cholesterol ≥5.2 mmol/L and/or low density lipoprotein cholesterol (LDL-C) ≥3.38 mmol/L and/or use of cholesterol lowering drugs], diabetes (fasting glucose ≥6.99 mmol/L and/or use of pharmacological treatment), family history of cardiovascular disease (symptomatic coronary artery disease occurring in first degree male relatives aged <55 years or first degree female relatives aged <65 years), obesity (body mass index ≥30 kg/m^2), and smoking (regular smoking or quitting <3 months ago).

This study was approved by the local ethics committee and written informed consent was obtained from all patients and control subjects. All reagents were purchased from Sigma (St. Louis, MO, USA), Riedel-de Haen (Seelze, Germany) and Merck (Darmstadt, Germany). Venous blood samples collected in heparin-coated and non-additive tubes (BD Vacutainer, Becton, Dickinson and Company, Plymouth, UK) were immediately separated by centrifugation at 3000 g for 10 minutes. Serum aliquots separated for MDA, protein carbonyls, total sialic acid, vitamin E, vitamin C, carotenoids, catalase, paraoxonase and arylesterase and plasma separated for measurement of GPBB were kept at -80°C until the analyses were performed. Tubes for vitamin E, vitamin C and carotenoid determination were protected against light exposure. Other biochemical parameters were measured on the day of blood collection.

Total cholesterol, HDL-cholesterol (HDL-C) and triacylglycerol concentrations were determined by enzymatic methods in the Aeroset automatic analyzer (Dallas, USA). LDL-C was calculated by the Friedewald formula.[28] Apolipoprotein AI, apolipoprotein B and lipoprotein (a) levels were analyzed by immunonephelometric assay (Dade Behring, Newark, USA). Serum total sialic acid determination was carried out according to the method of Sydow using Ehrlich reagent (intra-assay CV 3.8%, inter-assay CV 6.4%).[29] Serum MDA concentrations were determined by high-performance liquid chromatography (Shimadzu LC-10AT), using the technique of Young and Trimble (intra-assay CV 4.2%, inter-assay CV 6.8%).[30] Serum protein carbonyls, as an estimation of protein oxidation, were measured spectrophotometrically, using 2,4-dinitrophenyl-hydrazine (intra-assay CV 5.9%, inter-assay CV 8.7%).



GPBB: Glycogen phosphorylase isoenzyme BB, AMI: Acute myocardial infarction, a: Significantly different from admission (0 hour), b: Significantly different from 2 hour, †: p< 0.05, ‡: p< 0.01, #: p< 0.001

Figure 1. GPBB median levels in patients with AMI who received and did not received thrombolytic therapy at admission 0, 2 and 24 hours

Table 3: Oxidant parameters and total sialic acid levels in patients with AMI who received and did not receive thrombolytic therapy at admission 0, 2 and 24 hours

Time after emergency department admission	MDA	Protein Carbonyls	TSA
Thrombolysed AMI patients (n=50)			
0 h (admission)	0.74±0.24	0.68±0.16	1.42±0.15
2 h after thrombolytic therapy	1.25±0.25 a,#	1.16±0.23 a,#	1.74±0.20 a,#
24 h after thrombolytic therapy	0.96±0.25 a,†,b,#	0.83±0.13 a,‡,b,#	1.55±0.18 a,†,b,‡
Non-thrombolysed AMI patients (n=25)			
0 h (admission)	0.76±0.23	0.78±0.15	1.58±0.25
2 h after admission	0.82±0.18	0.84±0.17	1.65±0.31
24 h after admission	0.73±0.18	0.86±0.17	1.55±0.26

AMI: Acute Myocardial Infarction, MDA: Malondialdehyde, TSA: Total Sialic Acid, a: Significantly different from 0 h, b: Significantly different from 2 h, †: p< 0.05, ‡: p< 0.01, #: p< 0.001

Paraoxonase activity was determined as described by Eckerson et al. (intra-assay CV 3.7%, inter-assay CV 5.6%).[31,32] The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm at 25 °C. Paraoxonase activity was expressed as U/L serum. One unit of paraoxonase activity is defined as 1 µmol p-nitrophenol generated per minute under the above conditions. Arylesterase activity was determined by using phenylacetate as the substrate. One unit of arylesterase activity is defined as 1 mmol phenol generated per minute under the above conditions and expressed as kU/L serum (intra-assay CV 3.1%, inter-assay CV 4.9%).[33] Catalase activity was determined as described by Goth et al. (intra-assay CV 3.8%, inter-assay CV 4.5%). Vitamin E concentrations were quantified by HPLC (Shimadzu LC-10AT) using UV detection at 292 nm (intra-assay CV 4.3%, inter-assay CV 6.5%).[34,35] Plasma vitamin C (intra-assay CV 6.5%, inter-assay CV 8.7%) and carotenoid (intra-assay CV 5.4%, inter-assay CV 8.9%) levels were measured using a spectrophotometric method.[36,37] →

Table 4: Vitamin levels in patients with AMI who received and did not receive thrombolytic therapy at admission 0, 2 and 24 hours

Time after emergency department admission	Vitamin E	Vitamin C	Total Carotenoids
Thrombolysed AMI patients (n=50)			
0 h (admission)	32.20±8.42	35.20±9.60	2.33 ± 0.64
2 h after thrombolytic therapy	27.56±4.73 a‡	24.41±7.38 a#	2.19 ± 0.57 a‡
24 h after thrombolytic therapy	26.24±5.84 a#	21.00 ±6.81 a#b†	1.77 ± 0.56 a#b‡
Non-thrombolysed AMI patients (n=25)			
0 h (admission)	30.51±6.86	33.50±9.62	2.39±0.67
2 h after admission	29.44±7.67	30.10±9.60	2.33±0.72
24 h after admission	29.12±7.24	22.70±7.38 a#b#	2.16±0.71 a†b‡

AMI: Acute Myocardial Infarction, a: Significantly different from 0 h, b: Significantly different from 2 h, †: p< 0.05, ‡: p< 0.01, #: p< 0.001

Determination of GPBB concentrations in plasma was performed batch wise using a commercially available ELISA kit (intra-assay CV 3.2%, inter-assay CV 6.04%, Diacordon®; Diagenics, Woburn, MA, USA). This test is performed as a single-stage immunoenzymometric assay.

Statistical Analysis

Average values and rates for risk factors were calculated for patients and the control group. Data were expressed as mean ± standard deviation (x±SD), median (range) and percentage. Homogeneity of variances was tested with Levene test. Depending on the normality of distribution of variables, the unpaired Student's *t*-test or Mann-Whitney *U*-test were used for between-groups comparison and the paired *t*-test or Wilcoxon test were used within-groups comparison. The significance of any difference in proportions was tested by using the chi-square test. Because of the highly skewed distribution of serum lipoproten (a) and GPBB concentrations, all values are given in median and range. P value below 0.05 was considered as statistically significant. All analyses were conducted by using a statistical software (Statistical Package for the Social Sciences for Windows, version 10.0, Chicago, IL).

RESULTS

The clinical baseline characteristics and lipid profiles of the AMI and control groups are summarized in Table 1. Although the presentation of conventional risk factors was higher in the patient group, significant differences were observed only in hypertensive, hyperlipidemic and smoking subjects. There were no significant differences in age, body mass index, diabetes and family history between the groups. As expected, patients with AMI had higher LDL-C, triacylglycerol, apolipoprotein B, lipoprotein (a) and lower HDL-C and apolipoprotein AI values as compared to the control group. All these differences were statistically significant (Table 1).

In 45 patients with AMI, oxidant parameters, total sialic acid and GPBB levels were increased compared with a healthy control group. However, antioxidant parameters were decreased (Table 2).

In the patients with AMI receiving thrombolytic therapy, evident increases in serum MDA, protein carbonyls, total sialic acid and GPBB concentrations were noted especially 2 hours after such therapy. However, no such spike was seen in the group that did not undergo thrombolysis (Table 3, Figure 1).

Table 4 and 5 show the effect of t-PA infusion on serum vitamins and enzymes. Vitamin C showed a significant drop at both 2 and 24 hours after t-PA infusion. However, total carotenoids and vitamin E were significantly decreased especially in the 24 hours after t-PA infusion.

While catalase activity showed a significant drop at 2 hours after t-PA infusion, decrease in the paraoxonase and arylesterase activities reached statistical significance only 24 hours after t-PA infusion.

DISCUSSION

Biochemical cardiac markers now play an important role in the detection of disease, risk stratification and the monitoring of therapy, and these markers may gain increasing importance for the noninvasive monitoring of the effectiveness of thrombolytic treatment.[38] In our study, we found significant myocardial release of GPBB into the circulation after reperfusion. Myocardial injury caused by reperfusion may lead to efflux of GPBB into the extracellular fluid.[39] Similar to known biochemical cardiac markers, Rabitzsch et al. suggested that GPBB levels in AMI patients are markedly influenced by early reperfusion of the infarct-related coronary artery.[40] The so-called "wash-out" phenomenon and accelerated GPBB release from cardiomyocytes after successful thrombolysis leads to earlier and usually higher peak serum GPBB values. Therefore, GPBB may be useful, along with other biochemical cardiac markers, for assessing the effectiveness of thrombolytic therapy non-invasively.

In the AMI patients, because of ischemia, the ATP is drastically reduced and is converted to hypoxanthine and then to uric acid by xanthine oxidase upon reperfusion. During this process, enormous amounts of superoxide anions formed which can stimulate Haber-Weiss reaction for further generation of reactive oxygen species, initiating lipid and protein oxidation.[3] Numerous studies in a variety of models have established that oxidative modifications of proteins render them more susceptible to proteolytic attack.[41,42] We observed increased concentrations of MDA and protein carbonyls in the circulation of AMI patients →

compared with control group (Table 2). Increased lipid and protein oxidation are thought to be a consequence of oxidative stress which occurs when the dynamic balance between prooxidant and antioxidant mechanism is impaired in the AMI patients.[43] In our study, 2 hours after thrombolytic therapy there was a significant elevation in both MDA and protein carbonyls generation upon successful thrombolysis (Table 3). Therefore, the present study confirms the intense generation of lipid peroxides and protein carbonyls during reperfusion. Also, some authors reported that myocardial proteins are oxidized during cardiac reperfusion and might contribute to ischemia-reperfusion injury.[44,45]

In the present study, we have shown that serum total sialic acid is elevated in patients with AMI. Furthermore, we have also found that total sialic acid levels increased significantly 2 hours after reperfusion compared with baseline values before reperfusion, which indicated a very rapid release from the myocardium with the onset of reperfusion. Similarly, it was demonstrated that total sialic acid levels increased significantly within 10 min of reperfusion in patients undergoing cardiac surgery.[46] These findings indicate that serum total sialic acid levels may be useful in noninvasive assessment of thrombolytic therapy. An elevated concentration of serum total sialic acid in the blood might result either from the shedding or secreting of sialic acid from the cell membrane surface, or release of cellular sialic acid from the cell into the bloodstream because of cell damage after myocardial injury. Furthermore, increased sialidase activity or removal of sialic acid from the LDL by reactive oxygen species, probably via damage to the oligosaccharide structures, may be responsible for increased serum total sialic acid concentrations.[47,48] One of the possible sources of an increased serum total sialic acid concentration in AMI may be an increased output of serum proteins by the liver due to some type of acute phase reaction because many of the acute phase proteins are glycoproteins with sialic acid residues at the terminal position of their oligosaccharide side chains.[49]

Various studies have reported the beneficial effects of antioxidants as these agents render resistance to the heart against the ischemia reperfusion injury.[50-52] However, other investigators have failed to observe such results.[53,54] We have observed decreased activity of catalase, paraoxonase and arylesterase in the AMI patients compared to control group. However, only catalase activity was decreased significantly in thrombolysed AMI patients at 2 h compared to before thrombolytic therapy. Decrease in the activity of catalase could be due to inactivation of the enzyme by cross-linking or enzyme protein oxidation. Furthermore, catalase may act as a first line of cellular defense against oxidative stress during ischemia reperfusion.[55] Our results reveal significant decrease in serum paraoxonase and arylesterase activity only 24 h in the patients with AMI receiving thrombolytic therapy compared to before thrombolytic therapy. However, in AMI patients we found significantly lower levels of vitamins C, E and carotenoids compared with controls. Similarly, Levy Y et al. found significantly lower levels of these vitamin levels in AMI patients.[56] Furthermore, in our study, there was a significant drop at 2 and 24 hours in serum vitamins C, vitamin E and carotenoids in the patients with AMI receiving thrombolytic therapy. The decrease in the antioxidant vitamin levels, in our study, could be due to increased utilization in scavenging the reactive oxygen species generated by reperfusion injury. Similarly, Dhalla et al. reported that endogenous antioxidants, including enzymatic free radical scavengers and nonenzymatic antioxidants, are significantly reduced after ischemia and reperfusion.[55] On the other hand, some authors suggest that vitamin deficiency may be a risk factor of AMI and these patients may benefit by administration of these antioxidant vitamins for prevention of coronary artery disease.[57,58] Also, Gasparetto et al. demonstrated that vitamin treatment improves the antioxidant system and reduces oxidative stress after the AMI.[59] Moreover, protective effects of various antioxidants in ischemia-reperfusion systems have been reported.[60,61] The dietary supplementation of vitamin E and alpha-lipoic acid has been reported to decrease lipid peroxidation during *in vivo* ischemia-reperfusion injury in young adult rats.[62]

Table 5: Enzyme activities in patients with AMI who received and did not received thrombolytic therapy at admission 0, 2 and 24 hours

Time after emergency department admission	PON	AE	Catalase
Thrombolysed AMI patients (n=50)			
0 h (admission)	208±67	100±13	34.6±11.6
2 h after thrombolytic therapy	195±56	94±10	25.0±9.34 a ‡
24 h after thrombolytic therapy	187±64 a †	86±14 a ‡	27.4±8.08
Non-thrombolysed AMI patients (n=25)			
0 h (admission)	195±58	97±14	35.9±13.8
2 h after admission	180±47	89±14	32.2±13.3
24 h after admission	174±56	85±13 a ‡	30.6±15.4

AMI: Acute Myocardial Infarction, PON: Paraoxonase, AE: Arylesterase, a: Significantly different from 0 h, b: Significantly different from 2 h, †: $p<0.05$, ‡: $p<0.01$, #: $p<0.001$

CONCLUSION

Both reduction in antioxidants and elevations in total sialic acid, protein carbonyls and lipid peroxide generation indicate the significance of free radical generating processes in reperfusion injury in AMI patients, and suggest the future introduction of antioxidants to the management of patients with AMI treated by thrombolysis. Furthermore, GPBB can be used for monitoring the effectiveness of reperfusion therapy. But, GPBB will need further investigation →

before its potential utility as an indicator of the effectiveness of thrombolytic therapy in AMI patients can be established.

Moreover, further clinical studies are needed to examine closely the relation between free radical generation and various components of myocardial reperfusion injury.

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