ASPIRIN RESISTANCE AND OXIDATIVE STRESS IN PATIENTS WITH CORONARY ARTERY DISEASE

Zehra Serdar,[1] Elif Emre Doğruk,[1] Akın Serdar,[2] Şeyda Günay,[2] İsminur Saka Karagöz[1]

[1] Uludağ University, Faculty of Medicine, Biochemistry Department, Bursa
[2] Uludağ University, Faculty of Medicine, Cardiology Department, Bursa

ABSTRACT

Objective: *To examine the relationship between aspirin resistance and oxidative stress in patients with coronary artery disease.*

Material and Method: *A total of 100 patients (35 females, 65 males) with coronary artery disease were enrolled in the study. Platelet function was evaluated by a Multiple Platelet Function Analyzer according to impedance aggregometry method. Agregation Unite (AU) with a >706 AU*min despite regular aspirin therapy, was defined as Aspirin resistance. and aspirin semiresponders were defined as a 300-706 AU*min. Aspirin-sensitive patients were defined as a <300 AU*min. While serum malondialdehyde (MDA) and vitamin E concentrations were determined by the high-performance liquid chromatography, other oxidant and antioxidant parameters were measured spectrophotometrically.*

Results: *Of the patients studied, 7% were aspirin resistant, 15% were aspirin semiresponders and 78% were aspirin sensitive. While serum MDA, protein carbonyls and total sialic acid concentrations were significantly increased, serum antioxidant vitamins (vitamin E and total carotene) and enzymes (paraoxonase, arylesterase and catalase) were significantly decreased in patients with aspirin resistance.*

We found strong positive correlations (p<0.001) between aspirin resistance and oxidant parameters and weak negative correlations (p<0.05) between aspirin resistance and antioxidant vitamins and enzymes.

Conclusion: *Aspirin resistance is related to oxidant and antioxidant parameters in patients with coronary artery disease. However, there is still need for further studies to better elucidate the relationship between aspirin resistance and oxidative stress, which is now known to be a risk factor for cardiovascular events.*

Key Words: *Aspirin, drug resistance, coronary artery disease, oxidative stress, antioxidants* **Nobel Med 2013; 9(3): 74-81**

KORONER ARTER HASTALARINDA ASPİRİN DİRENCİ VE OKSİDATİF STRES

ÖZET

Amaç: Bu çalışmanın amacı, koroner arter hastalarında aspirin direnci ile oksidatif stres arasındaki ilişkiyi incelemektir.

Materyal ve Metod: Çalışmaya, koroner arter hastalığı olan total 100 hasta (35 kadın, 65 erkek) alındı. Trombosit fonksiyonu, trombosit fonksiyon analizörü ile impedans agregometri yöntemine göre değerlendirildi. Düzenli aspirin tedavisine rağmen agregasyon biriminin >706 AU*dak. olması aspirin direnci olarak, 300-706 AU*dak. arasında olması ise aspirine orta derecede duyarlılık olarak tanımlandı. Aspirine duyarlı hastalar ise <300 AU*dak. olarak tanımlandı. Serum malondialdehid (MDA) ve vitamin E konsantrasyonları yüksek performanslı sıvı kromatografisi ile belirlenirken, diğer oksidan ve antioksidan parametreler spektrofotometrik olarak ölçüldü.

Bulgular: Hastaların %7'si aspirine dirençli, %15'i aspirine orta derecede duyarlı, %78'i aspirine duyarlı bulundu. Aspirin direnci olan hastalarda serum MDA, protein karbonilleri ve total sialik asit konsantrasyonları anlamlı olarak artmış iken, serum antioksidan etkili vitaminler (vitamin E ve total karoten) ve enzimler (paraoksonaz, arilesteraz ve katalaz) ise anlamlı olarak azalmış bulundu. Aspirin direnci ile oksidan parametreler arasında güçlü pozitif korelasyonlar (p<0,001), antioksidan vitamin ve enzimlerle ise zayıf negatif korelasyonlar (p<0,05) bulundu.

Sonuç: Koroner arter hastalarında aspirin direnci oksidan ve antioksidan parametrelerle ilişkilidir. Ancak, kardiyovasküler olaylar için bir risk faktörü olduğu bilinen oksidatif stres ile aspirin direnci arasındaki ilişkiyi daha iyi açıklamak için ileri çalışmaların yapılmasına gereksinim vardır.

Anahtar Kelimeler: Aspirin, ilaç direnci, koroner arter hastalığı, oksidatif stres, antioksidanlar **Nobel Med 2013; 9(3): 74-81**

INTRODUCTION

Since aggregation of the platelets highly contributes to the development of cardiovascular events, inhibition of this process could play an important role in the prevention of cardiovascular disease. Aspirin (acetylsalicylic acid) is a powerful antiplatelet agent used in prevention of atherothrombotic vascular events. It exerts its antiplatelet effect by acetylation of the platelet cyclooxygenase, which results in an irreversible inhibition of the production of thromboxane A2 by platelets. Thromboxane A2 is a potent platelet activator that also causes vasoconstriction and smooth muscle proliferation. A decrease in thromboxane A2 leads to a reduced aggregation of platelets.[1] The clinical effectiveness of aspirin on the prevention of cardiovascular events has been well established. However, antiplatelet effect of aspirin is not uniform and some patients may not benefit from aspirin. These patients are clinically called as aspirin resistant or aspirin non-responders.[2,3] Mechanisms of aspirin resistance have not been elucidated yet even though a number of research has been carried out in this issue. Non-compliance, increased cyclooxygenase (COX)-2 gene expression and synthesis of thromboxane A2 bypassing the COX-1 enzyme, increased catecholamine release because of excessive exercise and mental stress, increased platelet aggregation triggered by cigarette smoking, erythrocyte-enhanced platelet reactivity, increased platelet sensitivity to collagen, genetic polymorphisms and oxidative stress, especially in recent years, are some of the suggested possible mechanisms of aspirin resistance.[4-7] Despite ongoing research, there is currently no standardized approach to the diagnosis and no proven effective treatment for aspirin resistance.

Events leading to hyperactivity of human blood platelets are accompanied by an enhanced risk of atherosclerosis and arterial thrombosis. Oxidative stress is an important mediator of both atherosclerotic cardiyovascular diseases and abnormal platelet function. Oxidative stress has clearly been shown to enhance platelet aggregation and thrombosis.[8,9] Oxidative modification of lipoproteins, especially that of oxidized low density lipoprotein (Ox-LDL) stimulates platelets and causes them to aggregate, eventually leading to stroke and myocardial infarction.[10] Furthermore, reactive oxygen species (ROS) derived from both platelets and other vascular sources have been shown to alter platelet responses. For example, superoxide radicals produced by platelets is known to augment platelet aggregation responses. The production or release of ROS by the platelets can evoke an oxidative stress that supports lipid and protein oxidation, induces cellular activation, and promotes vascular dysfunction.[9,11]

Table 1: Clinical characteristics and risk factors of the study groups

	Aspirin sensitive (n=78)	Aspirin semiresponders (n=15)	Aspirin resistant (n=7)
Age (years)	66.3±9.6	65.9±13.7	72.3±7.2
Female / Male	24 / 54	7 / 8	4 / 3
BMI (kg/m^2)	27.8±5.4	31.1±6.4 a†	31.3±4.3
Systolic blood pressure (mmHg)	115±19	110±13	131±12$^{a‡, b†}$
Diastolic blood pressure (mmHg)	70±10	67±8	85±9$^{a\#, b\#}$
Ejection fraction (%)	42±13	32±9^{a‡}	27±8^{a‡}
AU (Agregation Unite)	169±72	416±74$^{a\#}$	852±73$^{a\#, b\#}$
Involved coronary vessel, n (%)			
One-vessel	38 (49%)	7 (47%)	3 (43%)
Multiple-vessels	40 (51%)	8 (53%)	4 (57%)
Risk factors, n (%) *			
Diabetes mellitus	35 (45%)	6 (40%)	5 (71%)$^{a‡, b\#}$
Hypertension	36 (46%)	8 (53%)	5 (71%)$^{a‡, b†}$
Hyperlipidemia	34 (44%)	7 (47%)	4 (57%)^{a†}
Smoking	36 (46%)	8 (53%)	5 (71%)$^{a‡, b†}$
Family history	35 (45%)	5 (33%)	4 (57%)^{b†}

BMI: Body Mass Index *: chi-square test, a: Significantly different from aspirin sensitive group, b: Significantly different from aspirin semiresponders group, †: $p< 0.05$, ‡: $p< 0.01$, #: $p< 0.001$

Under oxidative stress, proteins as well as lipids are the major targets of ROS. Reactive oxygen species oxidize protein side chains, and the oxidized protein functional groups can lead to alterations in protein structure and function.[12] Moreover, oxidative modification of proteins by ROS can cause loss of catalytic activity of the proteins and marks the protein for subsequent proteolytic degradation. Damage to protein components of cells can lead to substantial decreases in the amount of important enzymes and the accumulation of the damaged protein. Such damaged protein can seriously compromise cellular integrity.[13] Although the association between protein oxidation and coronary artery disease has been well documented, the relationship between aspirin resistance and protein oxidation is still not very clear.

Sialic acids, a family of acetylated derivatives of neuraminic acid, are located at the terminal ends of many carbohydrate chains of glycolipids and glycoproteins.[14] It was reported that non-reducing terminal sialic acid residues may be a target molecule of ROS and ROS specifically cleave and liberate the sialic acid residues. Elevated serum total sialic acid concentration has been shown to be a cardiovascular risk factor.[15] No study published in the current literature investigated the relationship between the aspirin resistance and sialic acid levels in patients with coronary artery disease.

The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants. Antioxidants may inhibit atherogenesis and improve vascular function by enzymatic and nonenzymatic →

Table 2: Biochemical and hematological parameters of the study groups*

	Aspirin sensitive (n=78)	Aspirin semiresponders (n=15)	Aspirin resistant (n=7)
Glucose (mg/dl)	121±47	134±42	132±51
Creatinine (mg/dl)	1.06±0.23	1.02±0.27	1.03±0.24
AST (IU/L)	23±7	27±5	24±9
ALT (IU/L)	22±12	25±10	22±8
Total Cholesterol (mg/dl)	164±42	166±33	190±48
HDL- Cholesterol (mg/dl)	37±12	41±13	43±8
LDL- Cholesterol (mg/dl)	102±32	97±23	121±43
Triacylglycerol (mg/dl	127±65	134±57	127±39
Erythrocyte (M/μl)	4.4±0.7	4.5±0.8	4.8±0.6
Hemoglobin (g/dl)	12.4±1.7	12.6±2.1	12.7±2.4
Hematocrit (%)	37±5	36±5	36±5
Platelet (K/μl)	239±75	250±69	253±43

*No significant differences were observed among the groups. HDL:High Density Lipoprotein, LDL:Low Density Lipoprotein

mechanisms. Enzymatic protection against ROS is provided by superoxide dismutase, glutathione peroxidase, catalase and paraoxonase. Nonenzymatic detoxification is provided by carotenoids, vitamin E and C.[16] Furthermore, antioxidants may indirectly inhibit platelets through scavenging of ROS, many of which alter platelet function and show prothrombotic effects. Decreased human platelet antioxidant content is associated with enhanced platelet activation responses.[9] Especially in recent years, some studies have been made to examine the relationship between the aspirin resistance and oxidative stress in patients with coronary artery disease. However, the number of these studies are limited and conflicting. While some authors reported that oxidative stress may involve in the process of aspirin resistance, others reported that it may not.[17,18] Therefore, the relationship between oxidative stress and aspirin resistance is still unknown and the reasons of these contradictory findings are not clear.

Our aim was to investigate the changes caused by aspirin in oxidant and antioxidant parameters and to examine the relationship between the aspirin resistance and oxidative stress in patients with coronary artery disease.

MATERIAL and METHOD

Patients

This study was approved by the local ethics committee and written informed consent was obtained from all patients. A total of 100 patients (35 females, 65 males; mean age 67±10 years) with coronary artery disease were enrolled in the study.

Patients were on regular aspirin therapy (100-150 mg/day) for at least 1 month. Platelet function assays were performed at least 1 month after the cessation of drugs that might affect in vitro platelet function tests (eg. non-steroidal anti-inflammatory drugs, dipyridamole, heparin, low-molecular weight heparins, clopidogrel, ticlopidine, warfarin and glycoprotein antagonists). Exclusion criteria were thrombocytopenia (<100,000/mm^3) or thrombocytosis (>400,000/mm^3), anemia (hemoglobin <10 g/dl), polycythemia (hematocrit >50%), end-stage renal disease, hematologic diseases and malignancies.

None of the participating subjects were taking vitamins, dietary supplements, or drugs with known antioxidant activity during the study.

Diagnostic coronary arteriography was carried out using the Judkins technique and all the coronary angiograms were determined visually by two different cardiologists. 50% or greater luminal obstruction in one or more of the three major coronary arteries on angiography was accepted as coronary artery disease.

The following conventional cardiovascular risk factors were defined:[19] 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 ≥200 mg/dl and/or low density lipoprotein cholesterol (LDL-C) ≥130 mg/dl and/or use of cholesterol lowering drugs], diabetes mellitus (fasting glucose ≥126 mg/dl 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).

Blood Samples

Three samples of whole blood were obtained from patients via superficial veins of the arm. One sample was collected in hirudin (Dynabyte GmbH- Munich, Germany) for platelet function test; 1 sample was collected in ethylenediaminetetraacetic acid (EDTA) for the complete blood count and the other blood sample was collected in non-additive tube (BD Vacutainer, Becton Dickinson, Plymouth, UK) and also centrifuged at 2,000 g for 10 minutes. Serum aliquots were separated for malondialdehyde (MDA), protein carbonyls, total sialic acid, vitamin E, vitamin C, carotenoids, catalase, paraoxonase and arylesterase analyses. These serum aliquots were kept at -80°C until the analyses were performed. Tubes for vitamin E, vitamin C and carotenoid determination were protected against light exposure. Platelet function assay and complete blood count were processed within 1 hour of blood collection. →

Platelet Function Tests

Platelet function was evaluated with the Multiplate Platelet Function Analyzer on a new generation impedance aggregometer (Multiplate Analyzer, Dynabyte Medical, Munich, Germany). Impedance aggregometry measures the change in electrical impedance between two electrodes when platelets are aggregated by an agonist. We used hirudin as anticoagulant, which is recommended by the manufacturer and used arachidonic acid as agonist. The increase in electrical impedance was recorded continuously for 6 min. The mean values of the 2 independent determinations are expressed as the area under the curve (AUC) of the aggregation tracing. The Multiple Electrode Aggregometry instrument allows two ways to express the AUC: as AU*min (arbitrary aggregation units) or as U (units). We use AU*min system for expressing the AUC. Aspirin resistance despite regular aspirin therapy was defined as a >706 AU*min, and aspirin semiresponders were defined as a 300-706 AU*min. Aspirin-sensitive patients were defined as a <300 AU*min.

Hematological and Biochemical Analysis

Complete blood count were processed in a Cell-Dyn 3700 Hematology Analyzer (Abbott Diagnostics, USA). Other biochemical parameters were processed in the Aeroset automatic analyzer (Dallas, USA).

Analysis of oxidant and antioxidant parameters

Serum MDA concentrations were determined by high-performance liquid chromatography (HPLC) (Shimadzu LC-10AT), using the technique of Young and Trimble (intra-assay CV 4.2%, inter-assay CV 6.8%).[20] 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%).[21] 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%).[22]

Paraoxonase activity was determined as described by Eckerson et al. (intra-assay CV 3.7%, inter-assay CV 5.6%).[23] 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.[24] One unit of arylesterase activity is defined as 1 µmol phenol generated per minute under the above conditions and expressed as kU/L serum (intra-assay CV 3.1%, inter-assay CV 4.9%).

Table 3: Oxidant parameters and total sialic acid levels of the study groups

	Aspirin sensitive (n=78)	Aspirin semiresponders (n=15)	Aspirin resistant (n=7)
Malondialdehyde (nmol/ml)	0.85±0.29	1.17±0.33[a#]	1.56±0.29 [a #, b ‡]
Protein Carbonyls (nmol/mg protein)	2.41±0.81	2.89±0.74[a†]	3.73±0.63 [a ‡, b ‡]
Total Sialic Acid (mg/dl)	47.3±9.9	54.6±9.2[a‡]	63.4±5.0 [a #, b †]

a: Significantly different from aspirin sensitive group, b: Significantly different from aspirin semiresponders group, †: p<0.05, ‡: p<0.01, #: p<0.001

Table 4: Antioxidant parameters of the study groups

	Aspirin sensitive (n=78)	Aspirin semiresponders (n=15)	Aspirin resistant (n=7)
Paraoxonase (U/L)	165±45	132±40[a†]	110±39[a‡]
Arylesterase (kU/L)	113±23	100±22[a†]	90±17[a†]
Catalase (kU/L)	49.5±18	36.5±13[a†]	35.3±8[a†]
Vitamin C (mg/dl)	0.53±0.18	0.51±0.13	0.46±0.10
Vitamin E (µg/ml)	17.1±4.32	14.1±3.08[a†]	13.9±3.22[a†]
Total Carotenoids (µg/ml)	1.29±0.38	1.04±0.34[a†]	0.95±0.39[a†]

a: Significantly different from aspirin sensitive group, †: p<0.05, ‡: p<0.01

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%).[25] Serum 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.[26,27]

Catalase activity was determined as described by Goth et al. (intra-assay CV 3.8%, inter-assay CV 4.5 %).[28]

Statistical Analysis

All variables were tested for homogeneity of variance and normal distribution, before any statistical analysis was applied. The results of quantitative variables are presented as mean±1SD and the results of qualitative variables as percentages. Chi-square and Fisher's exact tests were used to compare qualitative variables. For the quantitative variables, analysis of variance (ANOVA) techniques or Kruskal-Wallis tests (if not normally distributed) were used to compare the three groups.

The association of aspirin resistance with oxidant and antioxidant parameters was assessed with the Pearson's correlation coefficient. A p value <0.05 was regarded as statistically significant. Statistical analysis was performed using the SPSS library (SPSS, Chicago, IL). →

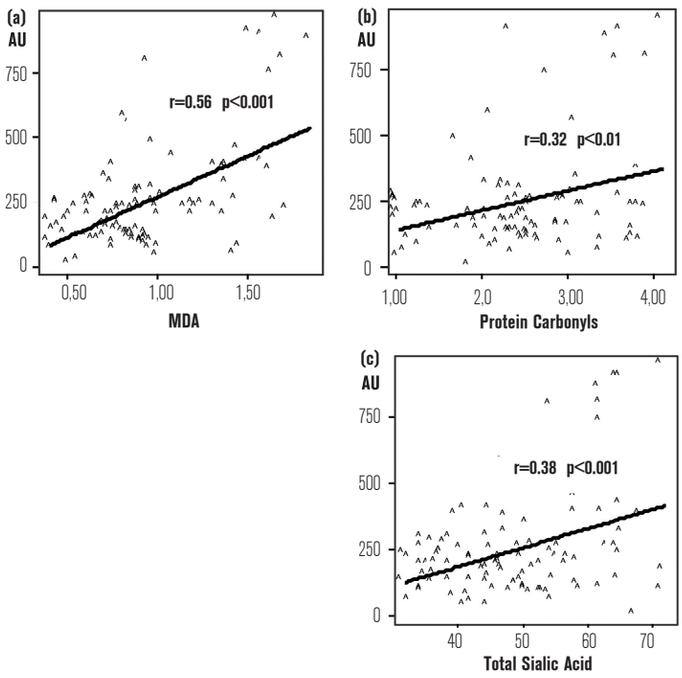

Figure 1: Correlation between aspirin resistance and oxidative parameters in patients with coronary artery disease. Aspirin resistance positively correlated with (a) MDA (r=0.56, p<0.001); (b) protein carbonyls (r=0.32, p<0.01); (c) total sialic acid (r=0.38, p<0.001). AU: Agregation Unite, MDA: Malondialdehyde.

RESULTS

Patient demographics, comparing aspirin resistant, aspirin semiresponders, and aspirin sensitive patients by impedance aggregometry testing are provided in Table 1. One hundred patients with coronary artery disease were recruited in the study. Of the patients 7% were aspirin resistant, 15% aspirin semiresponders and 78% were aspirin sensitive.

There were no significant differences between the aspirin-sensitive and aspirin semiresponder groups with regard to age, systolic and diastolic blood pressure, involved coronary vessels and classic atherosclerotic risk factors, while there were significant differences in terms of body mass index, ejection fraction and agregation unite (AU).

Compared with aspirin-sensitive and aspirin-semiresponder patients, the aspirin resistant patient group had higher number of smokers, diabetics, hypertensives and hyperlipidemics and they had lower left ventricular ejection fraction. Systolic and diastolic blood pressures were significantly higher in aspirin resistant patients compared to other groups and, the frequency of multiple-vessel disease was insignificantly higher in aspirin resistant patients compared to other groups (Table 1).

No significant differences were observed in biochemical and hematological parameters among the groups (Table 2).

Among the oxidant parameters, MDA and protein carbonyl levels were significantly higher in aspirin resistant patients compared to aspirin-sensitive and aspirin semiresponder patients. Similarly, total sialic acid levels were significantly higher in aspirin resistant patients (Table 3).

Among the antioxidant parameters: paraoxonase, arylesterase, catalase, vitamin E and total carotenoid levels were significantly lower in aspirin resistant and aspirin semiresponder patients compared to aspirin-sensitive patients (Table 4).

In order to clarify whether oxidative stress was correlated with aspirin resistance, we determined the relationship between levels of MDA, protein carbonyls, total sialic acid, paraoxonase, arylesterase catalase, vitamin E, vitamin C, total carotenoids and aspirin resistance (Figure 1, 2 and 3). When all patients with coronary artery disease (n=100) were considered together, there were significant positive correlations between aspirin resistance and MDA (r=0.56, p<0.001) and protein carbonyls (r=0.32, p<0.01) and total sialic acid (r=0.38, p<0.001), (Figure 1 a,b,c). There were significant negative correlations between aspirin resistance and paraoxonase (r=-0.30, p<0.01) and arylesterase (r=-0.20, p<0.05) and catalase (r=-0.23, p<0.05), (Figure 2 a,b,c) and vitamin E (r=-0.24, p<0.05) and total carotenoids (r=-0.25, p<0.05), (Figure 3 a,b)

DISCUSSION

In this study, we investigated whether the levels of some oxidant and antioxidant parameters are correlated with aspirin resistance in patients with coronary heart disease. And as a result, we demonstrated aspirin resistance to be positively associated with oxidative stress parameters and negatively associated with antioxidant parameters in patients with coronary artery disease.

Patients with coronary artery disease have an excessive increase in lipid peroxidation and lipid peroxidation generates prothrombotic mediators that play a crucial role in cardiovascular diseases.[29,30] MDA, a stable metabolite of the free radical-mediated lipid peroxidation cascade, is widely used as a marker of oxidative stress.[31] As a prothrombotic mediator, an increase in the MDA level may affect platelet functions and may contribute to aspirin resistance in patients with coronary artery disease.[9] Marwali et al. showed that aspirin reduced ROS released by activated platelets was measured as MDA release.[32] →

Steer et al. reported that aspirin, both in vivo and in vitro, protects low-density lipoprotein (LDL) against subsequent oxidative modification, providing an additional mechanism whereby aspirin may protect against atherosclerosis.[33] Moreover, Ashidate et al. demonstrated that gentisic acid, an aspirin metabolite, has an antioxidant effect and inhibits oxidation of LDL.[34]

In this study, we found that MDA levels were significantly higher in aspirin resistant patients and also, there was significant positive correlation between aspirin resistance and MDA levels. These results showed us that aspirin reduced the production of ROS and that there was a relationship between the aspirin resistance and oxidative stress in patients with coronary artery disease.

Oxidant stress leads to covalent oxidative modification of several plasma proteins. Among plasma proteins, Shacter and colleagues showed that fibrinogen underwent oxidative modification to form carbonyl groups.[35] In another study, Upchurch et al. showed that oxidized fibrinogen by ROS manifests prothrombotic effects and that acetylation of fibrinogen with aspirin can inhibit these prothrombotic effects.[36] Moreover, it is reported that salicylate, an aspirin metabolite, directly scavenges hydroxyl radicals, inhibits protein oxidation and attenuates low density lipoprotein oxidative modification and thus may halt progression of atherosclerosis.[1]

Since we found significant positive correlation between protein carbonyls and aspirin resistance, we can also add that there was a relationship between protein oxidation and the aspirin resistance in patients with coronary artery disease. However there are not enough studies in this topic.

In the literature, we have found no study investigating the effect of aspirin treatment on sialic acid levels in humans. In our study, we found that total sialic acid levels were significantly higher in aspirin resistant patients compared to aspirin-sensitive and aspirin-semiresponder patients. Increased sialidase activity or secretion of sialic acid from the cell or cell membrane surface or removal of sialic acid from the LDL by ROS, probably via damage to the oligosaccharide structures, may be responsible for increased serum total sialic acid concentrations in patients with coronary artery disease.[37,38]

The beneficial cardiovascular effects of aspirin are generally attributed to its immediate platelet inhibitory function. However, accumulating evidence suggests that aspirin may have additional biological

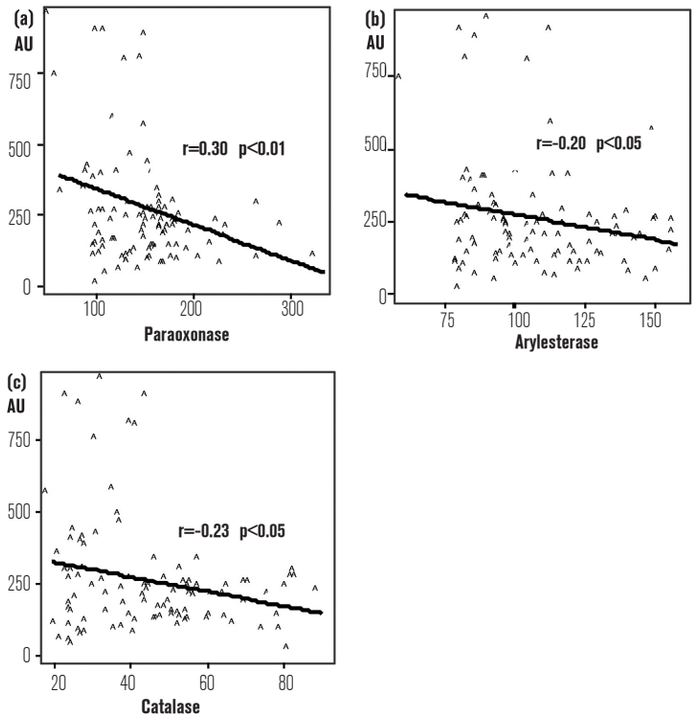

Figure 2: Correlation between aspirin resistance and antioxidant enzymes in patients with coronary artery disease. Aspirin resistance negatively correlated with (a): paraoxonase (r=-0.30, p<0.01); (b): arylesterase (r=-0.20, p<0.05); (c): catalase (r=-0.23, p<0.05). AU:Agregation Unite

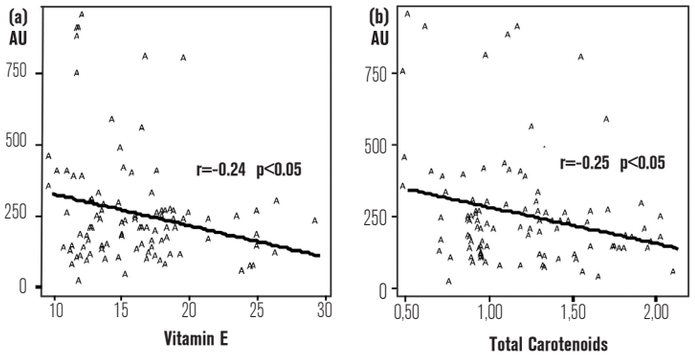

Figure 3: Correlation between aspirin resistance and antioxidant vitamins in patients with coronary artery disease. Aspirin resistance negatively correlated with (a): vitamin E, (r=-0.24, p<0.05); (b): total carotenoids (r=-0.25, p<0.05). AU: Agregation Unite

properties on the vascular endothelium.[39] Podhaisky et al. reported that aspirin possess antioxidant and radical scavenging properties which may explain the aspirin-induced endothelial protection against oxidative injury.[17] Antioxidant effects of aspirin leading to the suppression of lipid peroxidation have also been demonstrated in vivo in experimental animals and humans.[33,40]

Aspirin predominantly exists in the form of salicylic acid which is an antioxidant, in the plasma. This suggests that the antioxidant effects of salicylic acid might play an additional role in its anti-atherosclerotic effects.[41] Many studies have demonstrated that aspirin metabolite →

salicylate had free radical-scavenging properties and exerted an inhibitory effect on LDL oxidation.[42,43]

Because aspirin may possess antioxidant properties that could influence serum oxidant and antioxidant balance we have determined serum paraoxonase, arylesterase and catalase activities and vitamin C, vitamin E and carotenoid levels.

In our study, we demonstrated that antioxidant enzymes and vitamins were significantly lower in aspirin resistant patients and there were significant negative correlations between aspirin resistance and antioxidant enzymes and vitamins (except vitamin C).

Paraoxonase is an antioxidant enzyme that is synthesized in the liver. It has been shown to protect the serum lipids from oxidation and attenuates atherosclerosis development.[44] Paraoxonase catalyses the hydrolysis of various substrates such as aryl esters, phosphate esters, lactones, and reduces lipid peroxides to hydroxides. Aspirin is also an aryl ester and it would be effectively hydrolyzed by paraoxonase.[45] Recent studies indicate that aspirin users have increased paraoxonase activity in the plasma.[46] Consistent with these results, we found that aspirin sensitive patients had higher paraoxonase levels. Jaichander et al. have shown that mice treated with aspirin showed a 2-fold increase in plasma paraoxonase activity.[47] They have suggested that the antiatherosclerotic effects of aspirin might be mediated by its hydrolytic product salicylate and that the induction of paraoxonase and apoA-I might be important in the cardioprotective effects of aspirin.

Antioxidant vitamins protect cell membranes against free radical-mediated lipid and protein oxidation and play pivotal role in maintaining normal endothelial function.[48]

Vitamin E is the principal lipid-soluble antioxidant in human plasma and lipoproteins. Some studies indicate that vitamin E inhibited platelet aggregation and the combination of acetylsalicylic acid with vitamin E modified interactions between platelets and the vascular wall.[49,17] Furthermore, several investigators demonstrated that vitamin E potentiates the antiplatelet effect of aspirin (in vitro) by inhibiting the platelet activation pathways.[50,51]

Catalase, an important peroxidase located in the peroxisomes, catalyzes hydrogen peroxide to water and oxygen.[52] Also, it is reported that catalase reduces hydrogen peroxide production by activated platelets. Catalase appears to protect against oxidative stress in atherosclerosis.[53] But, there is a limited number of studies published in the current literature investigating the relationship between the aspirin resistance and catalase activity in the coronary artery disease.

An in vitro study demonstrated that catalase inhibits all the biochemical pathways leading to collagen-induced platelet aggregation by quenching platelet production of hydrogen peroxide.[54] Durak et al. demonstrated that in human erythrocytes, catalase activities were increased after aspirin treatment.[55] In our study, catalase activity was significantly decreased in aspirin resistant patients and there was significant negative correlation between aspirin resistance and catalase activity in patients with coronary artery disease.

In conclusion; our research demonstrated that oxidative stress parameters were correlated with aspirin resistance in patients with coronary heart disease. Therefore, it is reasonable to suspect that oxidative stress contributes to aspirin resistance.

Moreover, we found that antioxidant enzymes and vitamins were significantly lower in aspirin resistant patients and there were significant negative correlations between aspirin resistance and antioxidant enzymes and vitamins. Hence, treatment with additional antioxidant agents may be useful for patients who fail to respond to aspirin therapy. However, further prospective studies are necessary to elucidate the exact mechanisms underlying aspirin resistance and to assess the most appropriate management of these patients.

CORRESPONDING AUTHOR: Zehra Serdar Uludağ Üniversitesi, Tıp Fakültesi, Biyokimya AD. 16059, Bursa, Turkey zserdar@uludag.edu.tr

DELIVERING DATE: 06 / 09 / 2012 • **ACCEPTED DATE:** 09 / 04 / 2013

REFERENCES

1. Maree AO, Fitzgerald DJ. Aspirin and coronary artery disease. Thromb Haemost 2004; 92: 1175-1181.
2. Gabriel SA, Beteli CB, Taniguchi RS, et al. Aspirin resistance and atherothrombosis. Braz J Cardiovasc Surg 2007; 22: 96-103.
3. Mason PJ, Jacobs AK, Freedman JE. Aspirin resistance and atherothrombotic disease. Am Coll Cardiol 2005; 46: 986-993.
4. Hovens MC, Snoep JD, Eikenboom CJ, et al. Prevalence of persistent platelet reactivity despite use of aspirin: A systematic review. Am Heart J 2007; 153: 175-181.
5. Aktürk E, Topal E, Aksoy Y. Aspirin direnci. Türk Kardiyol Dern Arş 2005; 33: 480-487.
6. Pamukçu B, Oflaz H, Nişancı Y. Aterotrombotik hastalıklarda güncel bir sorun-aspirin direnci: Tanımı, oluşum mekanizmaları, laboratuvar yöntemleri ile belirlenmesi ve klinik sonuçları. Anadolu Kardiyol Derg

2007; 7: 20-26.
7. Kranzhofer R, Ruef J. Aspirin resistance in coronary artery disease is correlated to elevated markers for oxidative stress but not to the expression of cyclooxygenase (COX) 1/2, a novel COX-1 polymorphism or the PlA(1/2) polymorphism. Platelets 2006; 17: 163-169.
8. Relou IA, Hackeng CM, Akkerman JW, Malle E. Low density lipoprotein and its effect on human blood platelets. Cell Mol Life Sci 2003; 60: 961-971.
9. Freedman J.E. Oxidative stress and platelets. Arterioscler Thromb Vasc Biol 2008; 8: 11-16.
10. Verhoye E, Langlois MR, Rietzchel E, et al. Asklepios Investigators. Circulating oxidized low-density lipoprotein: a biomarker of atherosclerosis and cardiovascular risk. Clin Chem Lab Med 2009; 47: 128-137.
11. Wachowicz B, Olas B, Zbikowska HM, Buczyñski A. Generation of reactive oxygen species in blood platelets. Platelets 2002; 13: 175-182.
12. Stadtman ER, Levine RL. Protein oxidation. Ann N Y Acad Sci 2000; 899: 191-208.
13. Hawkins CL, Davies MJ. Generation and propagation of radical reactions on proteins. Biochim Biophys Acta 2001; 1504: 196-219.
14. Millar JS. The sialylation of plasma lipoproteins. Atherosclerosis 2001; 154: 1-13.
15. Lindberg G, Eklund GA, Gullberg B, Rastam L. Serum sialic acid concentration and cardiovascular mortality. BMJ 1991; 302: 533-534.
16. Schwenke DC. Antioxidants and atherogenesis. J Nutr Biochem 1998; 9: 424-445.
17. Podhaisky HP, Abate A, Polte T, Oberle S, Schröder H. Aspirin protects endothelial cells from oxidative stress--possible synergism with vitamin E. FEBS Lett 1997; 417: 349-351.
18. Cheng G, Shan J, Xu G, et al. Relationship between endothelial dysfunction, oxidant stress and aspirin resistance in patients with stable coronary heart disease. J Clin Pharm Ther 2007; 32: 287-292.
19. Greenland P, Smith SC, Grundy SM. Improving coronary heart disease assessment in asymptomatic people. Role of traditional risk factors and noninvasive cardiovascular tests. Circulation 2001; 104: 1863-1867.
20. Young IS, Trimble ER. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. Ann Clin Biochem 1991; 28: 504-508.
21. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 1994; 233: 357-363.
22. Sydow G. A simplified quick method for determination of sialic acid in serum. Biomed Biochem Acta 1985; 44: 1721.
23. Eckerson HW, Wyte MC, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet 1983; 35: 1126-1138.
24. Haagen L, Brock A. A new automated method for phenotyping arylesterase (E.C.3.1.1.2.) based upon inhibition of enzymatic hydrolysis of 4-nitrophenyl acetate by phenyl acetate. Eur J Clin Chem Clin Biochem 1992; 30: 391-395.
25. Teissier E, Walters-Laporte E, Duhem C, et al. Rapid quantification of a-tocopherol in plasma and low- and high-density lipoproteins. Clin Chem 1996; 42: 430-435.
26. McCormick DB, Greene HL. Vitamins, Burtis CA, Ashwood ER.(eds.) Tietz Textbook of Clinical Chemistry. W.B. Saunders Co. Philadelphia, 2nd ed, 1994: 313-314.
27. Neeld JB, Pearson WN. Macro-and micromethods for determination of serum vitamin A using trifluoroacetic acid. J Nutr 1963; 79: 454-462.
28. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991; 196: 143-151.
29. Demircan G, Dıraman E, Demircan S. Kalp hastalıklarında oksidatif stresin rolü. Türk Kardiyol Dern Arş 2005; 33: 488-492.
30. Siess W. Platelet interaction with bioactive lipids formed by mild oxidation of low-density lipoprotein. Pathophysiol Haemost Thromb 2006; 35: 292-304.
31. Steinberg D, Witztum JL. Lipoproteins and atherogenesis. Jama 1990; 264: 3047-3052.
32. Marwali MR, Hu CP, Mohandas B, et al. Modulation of ADP-induced platelet activation by aspirin and pravastatin: role of lectin-like oxidized low-density lipoprotein receptor-1, nitric oxide, oxidative stress, and inside-out integrin signaling. J Pharmacol Exp Ther 2007; 322: 1324-1332.
33. Steer KA, Wallace TM, Bolton CH, Hartog M. Aspirin protects low density lipoprotein from oxidative modification. Heart 1997; 77: 333-337.
34. Ashidate K, Kawamura M. Mimura, et al. Gentisic acid, an aspirin metabolite, inhibits oxidation of low-density lipoprotein and the formation of cholesterol ester hydroperoxides in human plasma. Eur J Pharmacol 2005; 513:173-179.
35. Shacter E, Williams JA, Lim M, Levine L. Differential susceptibility of plasma proteins to oxidative modification: Examination by western blot immunoassay. Free Radic Biol Med 1994; 7: 429-437.
36. Upchurch Jr GR, Ramdev N, Walsh MT, Loscalzo J. Prothrombotic consequences of the oxidation of fibrinogen and their inhibition by aspirin. J Thromb Thrombolysis 1998; 5: 9-14.
37. Gökmen SS, Özçelik F, Birsin A, Gülen Ş. Miyokard infarktüslü hastalarda total sialik asidin diagnostik önemi. Türk Biyokimya Dergisi 2000; 25: 59-65.
38. Tertov VV, Kaplun VV, Sobenin IA, et al. Human plasma trans-sialidase causes atherogenic modification of low density lipoprotein. Atherosclerosis 2001; 159: 103-115.
39. Mehta JL. Salutary effects of aspirin in coronary artery disease are not limited to its platelet inhibitory effects. Clin Cardiol 1998; 21: 879-884.
40. Wu R, Lamontagne D, de Champlain J. Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. Circulation 2002; 105: 387-392.
41. Kurban S, Mehmetoglu I. Effects of acetylsalicylic acid on serum paraoxonase activity, Ox-LDL, coenzyme Q10 and other oxidative stress markers in healthy volunteers. Clin Biochem 2010; 43: 287-290.
42. Hermann M, Kapiotis S, Hofbauer R, et al. Salicylate inhibits LDL oxidation initiated by superoxide/nitric oxide radicals. FEBS Lett 1999; 445: 212-214.
43. Lapenna D, Ciofani G, Pierdomenico SD, et al. Inhibitory activity of salicylic acid on lipoxygenase-dependent lipid peroxidation. Biochim Biophys Acta 2009; 1790: 25-30.
44. Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radic Biol Med 2004; 37: 1304-1316.
45. Santanam N, Parthasarathy S. Aspirin is a substrate for paraoxonase-like activity: implications in atherosclerosis. Atherosclerosis 2007; 191: 272-275.
46. Blatter-Garin MC, Kalix B, De Pree S, James RW. Aspirin use is associated with higher serum concentrations of the anti-oxidant enzyme paraoxonase-1. Diabetologia 2003; 46: 593-594.
47. Jaichander P, Selvarajan K, Garelnabi M, Parthasarathy S. Induction of paraoxonase 1 and apolipoprotein A-I gene expression by aspirin. J Lipid Res 2008; 49: 2142-2148.
48. Sies H, Stahl W. Vitamins E and C, b-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 1995; 62: 1315-1322.
49. Freedman JE, Keaney JF Jr. Vitamin E inhibition of platelet aggregation is independent of antioxidant activity. J Nutr 2001; 131: 374-377.
50. Celestini A, Pulcinelli FM, Pignatelli P, et al. Vitamin E potentiates the antiplatelet activity of aspirin in collagen-stimulated platelets. Haematologica 2002; 87: 420-426.
51. González-Correa JA, Arrebola MM, Guerrero A, et al. Influence of vitamin E on the antiplatelet effect of acetylsalicylic acid in human blood. Platelets 2005; 16: 171-179.
52. Chelikani P, Fita I, Loewen P. C. Diversity of structures and properties among catalases. Cell Mol Life Sci 2004; 61: 192-208.
53. Park JG, Oh GT. The role of peroxidases in the pathogenesis of atherosclerosis. BMB Rep 2011; 44: 497-505.
54. Pignatelli P, Pulcinelli FM, Lenti L, Gazzaniga PP, Violi F. Hydrogen peroxide is involved in collagen-induced platelet activation. Blood 1998; 91: 484-490.
55. Durak I, Karaayvaz M, Çimen MY, et al. Aspirin impairs antioxidant system and causes peroxidation in human erythrocytes and guinea pig myocardial tissue. Hum Exp Toxicol 2001; 20: 34-37.