RESEARCH ARTICLE

Prognostic importance of paraoxonase, arylesterase and mean platelet volume efficiency in acute ischaemic stroke

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Abstract

Objective: To study the prognostic importance activity of paraoxonase and arylesterase, and the value of mean platelet volume in patients with acute ischaemic stroke.

Methods: This case-control study was conducted at Harran University Hospital, Sanliurfa, Turkey, from January to June 2014, and comprised patients with symptoms of acute ischaemic stroke who presented to the emergency department. Paraoxonase activity, expressed in units per litre, or U/L, of serum, was evaluated in the absence of basal activity, and arylesterase activity was defined as micromoles, of phenol generated/min, and was expressed as U/L of serum. Mean platelet volume was measured as a routine parameter. SPSS 20 was used for data analysis.

Results: Of the 94 participants, 48(51%) were patients with acute ischaemic stroke and 46(49%) were control subjects. Moreover, 27(56.3%) patients were females and 21(43.7%) were males. In the control group, 26(56.5%) were females and 20(43.5%) were males. The mean age of patients was 68.39±11.83 years compared to controls' 65±9.95 years. Decreased activity of prognostic importance and arylesterase were significant in patients than in the controls (p= 0.016 and p= 0.001, respectively). The median platelets of patients was significantly lower than that of the controls (p=0.004). However, the median mean platelet volume values were similar in the both groups (p=0.568). Binary logistic regression analyses showed that the paraoxonase and arylesterase were risk markers for the patients.

Conclusion: Decreased paraoxonase and arylesterase activity and decreased platelet counts were observed probably due to increased oxidative stress in acute ischaemic stroke patients.

Keywords: Acute ischemic stroke, Paraoxonase, Arylesterase, Mean platelet volume, Emergency department. (JPMA 67: 1679; 2017)

Introduction

Decreased blood flow in the brain's vessels causes decreased oxygen supply and brain tissue ischaemia, which is usually caused by a thrombus clogged vessel. Followed by neuronal death, acute brain ischaemia typically occurs if reduced intracerebral vasculature blood flow is not removed in a short time. Acute ischaemic stroke (AIS) is defined by a combined series of circumstances that develop over hours.[1-4] In this process, when the blood flow to the brain declines, the brain cannot meet to metabolic feeds and then brain ischaemia occurs.[1,2] Moreover, oxidative damage can result in secondary brain injury in ischaemic stroke patients. Studies have evaluated oxidative status by determining the paraoxonase (PON1) level and activity as an antioxidant enzyme in patients with stroke.[5] Serum PON1 is a high density lipoprotein (HDL)-associated enzyme and protects low density lipoprotein (LDL) from oxidation by hydrolysis of biologically active lipid peroxides.[6-8] Oxidised LDL critically has a role in the pathophysiology of atherosclerosis; thus, PON1 may play a preventive role against atherosclerosis.[9] However, a few studies have specifically examined the platelet (PLT) indices associated with PON1 and arylesterase (ARES) in acute ischaemic stroke patients.

Platelet volume is defined as a marker of platelet activation and function and is measured as mean platelet volume (MPV). Increased platelet volume is associated with increased platelet activity. Also, increased platelet volume has more active and higher thrombotic potency.[10]

The current study was planned to assess the levels of PON1, ARES and platelet counts in AIS patients who were admitted to the emergency department (ED) and compared to those of controls.

Patients and Methods

This case-control study was conducted at Harran University Hospital, Sanliurfa, Turkey, from January to June 2014, and comprised patients with symptoms of AIS who presented to the ED. The study protocol was approved by the ethics institutional committee.

Patients with acute AIS who suffered the condition for the

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first time were included. Healthy participants were included as controls. Patients who had a history of cerebrovascular attack were excluded. Vital functions of all patients were monitored. At the time of admission to ED, basic life support and advanced life support were provided to the patients, if necessary. Written permission from the legal guardians of all participants was obtained, and information was given concerning the study protocol. Inclusion criteria were as follows: age >40 years and presence of AIS. Controls were normal healthy individuals with no AIS. Patients with conditions that may potentially affect PON1 and ARES status, such as chronic medical disorders including malignancy, chronic renal disorder, chronic heart disease, and diabetes mellitus, were excluded from the study. Moreover, patients who suffered concomitant injuries due to trauma and patients taking diuretics, vitamins, antioxidants, and anabolic drugs, which are known to affect lipid and lipoprotein metabolism, were also excluded.

After that, 5ml venous blood samples were drawn from patients and control participants, which was then transferred to heparinised tubes and stored at 4 degrees Celsius. Blood specimens were taken immediately after arrival to the emergency department, within 24 hours of injury. By centrifugation at 4,000 revolutions per minute (rpm) for 5 minutes, the serum was separated and plasma samples were stored at -80°C until analysis.

ARES and PON1 activities and value of MPV were the primary outcome variables measured in AIS patients, whereas other demographic and laboratory data was baseline outcome. Baseline factors including age, gender, heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), and history of diabetes mellitus (DM) were also measured.

PON1 activity, expressed in units per litre (U/L) of serum, was evaluated in the absence of basal activity. At an absorbance of 412 nm at 37°C the percentage of paraoxon hydrolysis (diethyl-p-nitrophenyl phosphate) was measured. The supply of generated p-nitrophenol was calculated from the molar absorptive at pH 8 (17,000 M-1 cm-1).[11] Phenylacetate was used as a substrate to evaluate ARES activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol (1,310 M-1 cm-1). ARES activity was defined as micromoles (μmol) of phenol generated/min was expressed as U/L of serum.[12]

MPV levels were measured in 48 patients with AIS and 46 age- and sex-matched healthy controls. MPV parameters were assessed using commercially available assay kits (Abbott Laboratories, Abbott Park, Illinois, United States) with an auto analyser (Aeroset, Abbott). Triglycerides (TG), total cholesterol (TC), HDL, LDL and troponin I levels were measured using commercially available assay kits (Abbott) with an auto analyser (Aeroset, Abbott).

SPSS 20 was used for data analysis. Statistics for continuous variables (e.g. ARE, PON1 activity and MPV and platelet levels) were measured as means and standard deviations (SD) or median (minimum-maximum range) values. Power analysis was performed to provide scientific justification of the sample size used in the study. On the basis of the PON1 and ARES values (taking alpha degree of freedom as 0.05), the two-tailed power was ≥80% in the study. Shapiro-Wilk test was performed for values of variables confirming normality or not in each group. The Mann-Whitney U-test was performed for numerical data that were not normally distributed while Student's t-test for numerical values with a normal distribution. Binary logistic analyses were used to assess the risk markers for the diagnosis of AIS. The probability of error less than 0.05 was used as a cut-off point for all statistically significant tests. Chi-square test or Fischer's exact test was performed for categorical variables. Pearson's correlation test was used to determine the relationship between continuous variables. $P<0.05$ was considered statistically significant.

Results

Of the 94 participants, 48(51%) were patients with AIS and 46(49%) were control subjects. Moreover, 27(56.3%) patients with AIS were females and 21(43.7%) were males; the mean age was 68.39±11.83 years. In the control group, 26(56.5%) were females and 20(43.5%) were males; the mean age was 65±9.95 years. Besides, 16(32%) of the AIS patients had diabetes compared to 15(31.25%) controls (p=0.682). The mean SBP was 124.68±13.46 mmHg among patients and 124.02±13.64 among controls (p=0.812). The mean DBP was 78.89±9.56 mmHg among patients and 79.71±10.00 among controls (p=0.685) (Table-1).

The median level of PLT was 251.8(79.1-630.8) × 103 L-1 in AIS patients and 318(152.1-525.8) × 103 L-1 in the controls (p=0.004). The median MPV was 8.00(5.6-16.3) fL in AIS patients and 7.80(5.6-15.7) fL in controls (p=0.111). PON1 activity was 110.97(61.97-190.86)U/dl and ARES activity was 99.63(36.65-155.20) U/dl in AIS patients and 132.21(73.97-210.36) U/dl and 111.12(61.28-164.02) in controls (p=0.016) (p=0.001), respectively (Table-2).

Of the AIS patients, 13(27%) died, and 35(73%) survived. At the time of admission to ED, Barthel index score and modified Rankin Scale (mRS) were significantly higher ($p < 0.001$ for both comparisons) in survivors compared with patients who died (Table-3).

Table-1: Baseline demographic and laboratory characteristics of patients with AIS and control subjects.

Parameters	Patients with AIS [48;N or Mean±SD]	Control subjects [46;N or Mean±SD]	*P Value
Male/Female	21 /27	20/26	0,651
Age, years	67.38±10,84	66±9,94	0.397
Diabetes mellitus	16 (32%)	15 (31.25%)	0.682
SBP, mmHg	124.68± 13.46	124.02 ± 13.64	0.812
DBP, mmHg	78.89± 9.56	79.71± 10.00	0.685
HR, times/min	72.68 ± 5.29	71.74 ± 7.20	0.719
BMI, kg/m^2	29.14± 7.31	28,22±6.21	0.877
Glucose,mg/dl	153,56±71,10	126,61±56,17	0.044
LDL-C, mg/dL	115,86±32,74	127,57±27,71	0.065
TG, mg/dL	153,05 ±87,79	152,59± 56,80	0.977
APTT, seconds	25,33± 6,04	28,48±3,86	0.004
CK-MB, ng/ml	2,41±,99	2,06±,91	0.065

* Student t test and Chi-square test.
SBP: Systolic blood pressure,
DBP: Diastolic blood pressure
HR: Heart rate
BMI: Body mass index
LDL: Light density lipoprotein
TG: Triglycerides
APTT: Activated partial thromboplastin time
CK-MB: Creatine kinase MB
AIS: Acute ischaemic stroke
SD: Standard deviation.

Table-2: Platelet incidence, PON1, ARES levels and Laboratory characteristics in patients with AIS and healthy controls.

Parameters	Patients with AIS[N=48] (Median, Min-Max)	Control subjects[N=46] (Median, Min-Max)	*P value
PLT, 10^3. Lμ1	251.8(79.1-630.8)	318(152.1-525.8)	0.004
MPV, fL	8.00(5.6-16.3)	7.80(5.6-15.7)	0.111
PON1, U/dL	110.97(61.97-190.86)	132.21(73.97-210.36)	0.016
ARE, U/dL	99.63(36.65-155.20)	111.12(61.28-164.02)	0.001
Troponin t, ng/mL	22.3(6.1-92.7)	16.0(7.0-132.0)	0.009
WBC, mcL	8.8(4.8-20.8)	7.7(4.8-13.8)	0.026

* Mann Whitney U Test
PLT: Platelets
MPV: Mean platelet volume
PON1: Paraoxonase 1
ARE: Arylesterase
WBC: White blood cell
AIS: Acute ischaemic stroke.

Pearson correlation test for ARE, PON1, MPV and study variables were performed. ARES was significantly and positively correlated with age (r=0.259; p=0.011) and gender (0.211; p=0.035). In addition, PON1 showed positive correlation with TG (r=0.221; p=0.038). Nevertheless, no correlation was detected between PON1, ARE and MPV levels. Binary logistic regression analyses of PON1and ARES as risk markers for patients with AIS showed that odds ratio was significantly higher in PON (OR =1.01, confidence interval (CI) 95% =1.00 - 1.04, p=0.013) and ARES (OR =1.03, CI 95% =1.01-1.06, p=0.003) than MPV (Table-4).

Table-3: Baseline demographic characteristics of died and survived patients in study group.

Patients Parameters	Survivor (N=33)	Died (N=15)	*P value
Barthel index score	36.5 ± 29.4	19.7 ± 5.1	<0.001
Modified Rankin Scale	2.9± 0.4	4.9 ± 0.6	<0.001
Length of stay (days)	21.7 ± 15.7	23.9± 16.9	0.09

* p value is significant at the level <0.05.

Table-4: Binary Logistic regression analysis of the associations between MPV, PON1 and ARE in patients with AIS and controls.

Explanatory variables	Odds ratio 95%	Confident Interval	p value
MPV, fL	0.87	0.67-1.35	0.319
PON1, U/dL	1.01	1.00- 1.04	0.013
ARE, U/dL	1.03	1.01-1.06	0.003

*p value is significant at the level <0.05.
MPV: Mean platelet volume
PON1: Paraoxonase 1
ARE: Arylesterase.

Discussion

PON1 and ARES activity levels were significantly lower in patients with AIS than in control subjects. Additionally, the median PLT was significantly lower in patients with AIS. In our study, at the time of admission to ED, Barthes index score and mRS were significantly higher in survivors compared with patients who died. Binary logistic regression analyses showed that the PON1 and ARES were risk markers for patients with AIS in the study.

Free radicals play a critical role in the pathophysiology of brain ischaemia and neuronal diseases. Therefore, elucidating oxidative stress and lipid peroxidation excitotoxicity mechanisms may lead to the clinical management of stroke patients. Moreover, the brain is more sensitive to excitotoxicity and oxidative damage by free radicals than other tissues due to its high degree of oxidative processes and intense synaptic activity.[5,7] As an enzyme with antioxidant activity, PON1 plays several crucial roles, including protection of HDL by inhibiting the oxidation of LDL and HDL, reduction of the effect of oxidised LDL by decreasing the oscillation of cellular cholesterol from macrophages, and reduction of lipid peroxide levels in atherosclerotic lesions.[13] Strokes are

associated with changes in serum PON1 activity.[13,14] Kim et al. reported that reduced PON1 activity was a risk factor for ischaemic stroke in Korean patients.[15] Demirdögen et al. demonstrated that reduced PON1 activity in ischaemic stroke patients was accompanied by PON1 polymorphisms, which are considered a major risk factor for stroke.[16] Moreover, Kirbaslar et al. reported significantly lower serum malondialdehyde levels in stroke patients.[17] Similarly, we reported significant reductions in PON1 and in ARE activity in patients with AIS compared with controls.

Platelets may play a role in the advancement of focal cerebral ischaemia by participating in thromboembolism and in various other ways. Platelet activation may become abnormal in patients with stroke; plasma levels of alpha granular contents (platelet factor 4 [PF4] and β-TG) released with platelet activation may be increased.[18]

Previous studies have found that MPV was significantly increased in AIS patients, accompanied by a decrease in platelet count. In contrast, a study by Ntaios et al. demonstrated that there was no significant increase in MPV.[19] Similarly, a study by Du et al. found significantly lower platelet counts in AIS patients who died.[20] An increase in the volume of platelets was shown in cases of acute myocardial infarction, acute cerebral ischaemia, and transient ischaemic attack, and an increase in MPV is an independent risk factor for recurrent vascular events and death.[21-23]

MPV is a crucial haematological parameter that has haemostatic importance since it is a determinant of platelet function.[24] Larger platelets have specific characteristics compared with smaller platelets, including higher activity, greater production of prothrombotic factors, and the ability to cluster more easily.[21,23] In addition, they contain denser granules and release more serotonin and β-thromboglobulin. Greiseneg et al. examined the association between stroke severity and MPV levels in a group of 776 AIS and transient ischaemic attack patients;[25] severe stroke patients had MPV levels in the highest quintile. These findings are supported by two other studies that demonstrated higher MPV values in stroke patients compared with control groups.[20,25] In a previous study, O'Malley et al. demonstrated that platelet count and MPV values in AIS patients were higher than those in controls at baseline; however, measurements taken after 6 months showed decreased platelet counts in AIS patients compared with controls.[26] In our study, PLT counts significantly decreased in AIS patients. Our results suggest that AIS patients may have had decreased PLT counts prior to stroke onset, reflecting increased platelet activity. Despite the increase in AIS patients, this increase was not significant compared to the MPV level in control group. This happened probably due to a small number of AIS patients in our study.

We did not detect an association among PON1, ARES and MPV, may be due to the small sample size of this study. However, ARES and PON1 activity and PLT count were significantly lower in AIS patients, while MPV values increased.

This study is not without limitations. It was performed in a single department and we were only able to utilise data from one time point for each patient. Moreover, only a small number of AIS patients were enrolled.

Conclusion

Patients with AIS had decreased PON1 and ARES activities and PLT count compared to healthy subjects. This suggests that in addition to their antioxidant function, platelets may play an underlying role in AIS aetiology such that patients subjected to oxidative stress also experience increased platelet indices. Decreased activities of PON1 and ARES and PLT count in serum might be considered as prospective prognostic markers of the progress of acute ischaemia. Nevertheless, further studies are warranted to confirm this relationship.

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