Serum myeloperoxidase levels in predicting the severity of stroke and mortality in acute ischemic stroke patients

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Abstract. – **OBJECTIVE:** The aim of this study was to examine the level of myeloperoxidase (MPO) measured before specific treatment in patients presenting to the Emergency Department with acute ischemic stroke and its correlation to mortality and the severity of the stroke.

PATIENTS AND METHODS: The study was carried out on 55 patients with a confirmed diagnosis of ischemic stroke, and on 44 healthy control group. Before specific intervention, serum samples were taken to measure levels of MPO. The medical records, demographic, clinical, laboratory and neuro-imaging data were noted. The National Institutes of Health Stroke Scale was used to determine the severity of the stroke.

RESULTS: A total of 55 patients, of whom 32 (58.2%) were male, who had presented within 24 hours of the onset of symptoms of acute ischemic stroke were included in the study. Fifteen of these patients (27.2%), of whom five were women, died. There was a statistically significant difference in the serum MPO levels of patients who survived and those who died. When the patients were grouped as high or normal in terms of plasma MPO levels, a significant correlation was found between MPO level, cortical + subcortical stroke location and strokes with a lesion diameter of more than 4 cm. In the high MPO group, Troponin T and CRP levels were significantly higher than those of the normal MPO group.

CONCLUSIONS: The level of myeloperoxidase in the serum of acute ischemic stroke patients rises and there is a correlation between myeloperoxidase level and prognosis.

Key Words:
   Acute ischemic stroke, Myeloperoxidase, Inflammation, Mortality, Atherosclerosis.

Introduction

In recent years, neurobiochemical markers have gained importance in the determination of brain damage related to ischemic stroke. In ischemic stroke, many biochemical and immunological reactions occur secondary to the reduction in cerebral blood flow. Measurement of the level in the blood of various proteins and other materials released from neurons, glial cells, endothelium, leukocytes are used after cerebral hypoxia and ischemia in addition to neurological tests to establish brain damage[1]. The use of these materials provides an opportunity for speedy diagnosis and early treatment. Discussion is needed of the possibility of whether this kind of biochemical marker can show the severity of cerebral damage and whether it can indicate its localization and clinical importance[2].

Evidence is mounting of the role of the inflammatory process in atherogenesis and acute cerebral ischemia[3,4]. In ischemic stroke, many biochemical and immunological reactions occur secondary to the reduction in cerebral blood flow. Myeloperoxidase has a role in the oxidation of LDL. Macrophages have a role in the breakdown of the collagen layer in the atheroma plaque by secreting matrix metalloproteinases and metal-independent myeloperoxidase and bringing about acute coronary events by weakening the fibrous capsule[6,7].

Myeloperoxidase (MPO) is a lysosomal enzyme which is secreted by leukocytes in response to oxidative stress. It is a tetrameric, glycolised protein which contains a prosthetic heme group[8]. Oxidative stress and inflammation have a role in the pathogenesis of atherosclerosis. MPO, which is found in abundance in leukocytes and which forms reactive oxidant products, is found in atherosclerotic reactions, and shows catalytic activity. Advanced atheroma plaques contain high levels of MPO, which produces pro-oxidants such as hypochloric acid (HOCl)[9,10].

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Table I. Demographic characteristics of the patient and control groups.

	Patient group n = 55 (%)	Control group n = 40 (%)	p
Hypertension	25 45.5(%)	14 (35%)	0.399
Diabetes mellitus	16 (29.1%)	5 (12.5%)	0.079
Hypercholesterolemia	2 (3.6%)	5(12.5%)	0.128
Smoking	6 (10.9%)	6 (15%)	0.756
Ischemic heart disease	3 (5.5%)	2 (5%)	1.000
Hypertriglyceridemia	1 (1.8%)	2 (5%)	0.571
Thyroid function deficiency	3 (5.5%)	0 (0%)	0.261

The aim of this study was to investigate the correlation between the level of serum myeloperoxidase measured before specific treatment in patients presenting to the Emergency Department with acute ischemic stroke and mortality and stroke severity.

Patients and Methods

Study Population

This study was performed between June and December 2010 on 55 patients with focal neurological deficit and a diagnosis of acute ischemic stroke, who had presented to the Emergency Service of our Hospital within 24 hours of the onset of symptoms. Approval was first obtained from the Faculty Ethics Committee. The patients were evaluated prospectively. A control group was formed from 40 healthy individuals, matched for age and sex. Patients were included who had presented within 24 hours of the onset of symptoms, were aged 40 years or older, who had not previously suffered stroke, and whose diagnosis of ischemic stroke was confirmed by brain computed tomography (CT) or magnetic resonance imaging (MRI). Excluded were patients with an infection, those with renal illness or hepatic deficiency, those with a malign illness, immunodeficiency, or a history of peripheral vascular disease, those with a diagnosis of arrhythmia such as atrial fibrillation, those who had previously suffered from a stroke, those who reported more than 24 hours after suffering the stroke, those with prosthetic heart valves, those with advanced cardiac deficiency, those who did not show lesions in magnetic resonance imaging (MRI), those with hemorrhagic stroke confirmed by imaging techniques, and those with ischemic stroke showing hemorrhagic transformation. During the study period, 16 patients who did not meet the criteria presented, and were excluded from the study.

Blood samples were taken over five days from each patient while under care but before specific treatment. Cerebral BT or MRI scans were performed on all patients in order to exclude hemorrhagic stroke and other intracerebral diseases and to categorise the stroke subtype. A detailed history was taken from each patient with regard to vascular risk factors. In order to determine the potential mechanisms of cerebral infarct, all patients were given the following tests: electrocardiography, echocardiography and carotid and vertebral artery Doppler ultrasonography, PA pneumography, blood glucose, BUN, creatinine, hepatic function test and hemogram.

Patients were divided into three groups according to the severity of neurological deficit of each patient measured according to the NIHSS (NIH Stroke Scale) scale. Patients who scored 1-7 on the scale were defined as having slight neurological deficit, those scoring 8-14 as having medium deficit, and those with an NIHSS score of > 15 as having severe neurological deficit. In addition, patients were scored for disability on the first and fifth days according to the Barthel index. Then a correlation was sought between these groupings according to neurological deficit levels and serum MPO (myeloperoxidase) levels.

Methods

Serum samples were separated from blood taken from patients and controls by centrifuging at 3500 rpm for five minutes. Samples were stored at –80°C until all samples had been collected. After thawing, MPO levels were measured using human-specific R&D Systems Quantikine® (Minneapolis, MN, USA) kit, lot no: DMYE00, and the solid phase Enzyme Linked Immuno Assay (ELISA) method. Wells in the

Table II. Myeloperoxidase (MPO) levels of patient and control groups by follow-up period.

	Patient	Control	*p* value
Arrival MPO level[*] (mean+SD; pg/mL)	1272.13 + 1019.81	522.5 + 380.6	< 0.0001
5th day MPO level[**] (mean+SD; pg/mL)	700.59 + 423.20	522.5 + 380.6	0.038

[*]Patient group = n 55; control group = n 40; [**]Patient group = n 55; control group = n 40.

plate were covered with antibodies specific to MPO. After standards, controls and samples had been added to the wells they were incubated and MPO antigens bound to the immobilised antibodies. Extra antibodies were washed out of the medium, and then enzyme-linked polyclonal antibodies specific to MPO were added. The colour absorbance so formed was in direct proportion to the concentration of MPO in the sample. Results in ng/ml were calculated from concentration-absorbance curves formed from standards.

Statistical Analysis

Statistical analysis of the data was performed by use of the program SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Results were given as mean ± sd. In the univariance statistical analysis the chi-square test was used for categoric variables and the Student-t test was used for continuous variables. In order to determine the effect of mortality on independent risk factors Step-Wise Logistic regression analysis was performed in multivariance analysis for variables which were significant in univariance analysis. $p < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 55 acute ischemic stroke patients (58.2% male) presented. The average age of patients was 65.69 ± 11.47 years. The control group was composed of 24 (60%) males and 16 (40%) females, with an average age of 62.28 ± 10.23 years. Table I shows the clinical and demographic characteristics of the patient and control groups.

Serum MPO Levels

When the first and fifth day MPO levels of the patient and control groups were compared, it was found that first day MPO levels of the patient group were significantly higher ($p < 0.0001$), but that the fifth day levels of the two groups were close to each other ($p = 0.038$) (Table II).

Mortality and Serum MPO Levels

Fifteen (27.2%) of the patients (five female) in the study died. When the relation between NIHSS scores of those who died and those who survived and mortality was compared, a significant relationship was found between survival and an NIHSS score of 1-7 ($p < 0.0001$), while an NIHSS score of 8-14 had no relation to mortality ($p = 0.768$). The relationship of an NIHSS score of 15 and over to mortality was again significant ($p < 0.0001$).

The relationship between survival and dying and stroke localisation and mortality was investigated, and a significant relationship was found between subcortical lesions and survival ($p = 0.004$), while concomitant cortical and subcortical lesions were significantly related to mortality ($p = 0.001$) (Table III).

MPO levels of patients who survived and those who died were compared, and it was found that the MPO levels of those who died was significantly higher than that of those who survived ($p < 0.0001$). Table IV shows a comparison of biochemical parameters and other numerical variables of surviving and dying patients.

Serum levels of MPO in atherosclerotic stroke patients at the time of arrival was significantly higher than those of the control group. Moreover, there was a significant difference in MPO levels between dying and surviving patients: that of patients who died was significantly higher than that of patients who survived ($p < 0.001$). However, the factors which were determined as a result of the univariance analysis to be factors for mortality, namely a lesion diameter of > 4 cm, a cortical + subcortical localization and an NIHHS score on arrival of > 15, and serum troponin T, CRP and MPO levels, and the factors determined by multivariance analysis using the Step-Wise Lo-

Table III. Relationship between stroke localisation and lesion diameter of surviving and non-surviving patients.

	Survivors n = 40 (%)	Non-survivors n = 15 (%)	*p* value
Stroke localization			
Cortical	3 (7.5%)	–	0.554
Subcortical	20 (50%)	1 (6.7%)	0.004
Cortical+subcortical	17 (42.5%)	14 (93.3%)	0.001
NIH score			
1-7	20 (50%)	–	< 0.0001
8-14	19 (47.5%)	8 (53.3%)	0.768
> 15	1 (2.5%)	7 (46.7%)	< 0.0001
Lesion diameter			
< 2 cm	16 (40%)	–	0.003
2-4 cm	13 (32.5%)	2 (13.3%)	0.192
> 4 cm	11 (27.5%)	13 (86.7%)	< 0.0001

gistic Regression Model, arrival NIHSS of > 15 (Odds Ratio [OR] = 0.007, 95% safety margin [95% GA] = 0.000-0.144, *p* = 0.001) and serum CRP level (OR = 5.586, 95% GA = 2.034-15.342, *p* = 0.001), were found to be independent predictors of mortality.

Discussion

We confirmed that serum MPO levels in acute ischemic stroke patients were higher than in healthy individuals. There have been many studies of the use of biomarkers in the prognosis of acute ischemic stroke. Recently, particular attention has been paid to the diagnosis of ischemic stroke, its treatment, and the use of inflammatory markers in its prognosis.

The commonest cause of ischemic stroke is atherosclerosis[11,12]. Atherosclerosis is a condition characterized by thickening of blood vessel walls as a result of pathological changes, a narrowing of the blood vessel lumen, and a loss of elasticity[13,14]. The inflammatory process plays a basic role in the pathophysiology of cerebral ischemia and the etiology of cerebrovascular disease[15]. It has recently

Table IV. Comparison of biochemical parameters and other numerical variables of surviving and non-surviving patients.

Parameter (mean+SD)	Survivors n = 40	Non-survivors n = 15	*p* value
Age (years)	64.30 + 11.14	69.40 + 11.89	0.163
NIHSS at presentation	8.38 + 4.98	12.4 + 3.5	0.002
GCS at presentation	14.08 + 2.04	9.53 + 3.13	< 0.0001
CNS at presentation	8.31 + 4.15	3.76 + 1.65	< 0.0001
BI at presentation	59.13 + 28.05	22 + 14.49	< 0.0001
RS at presentation	2.68 + 1.45	4.27 + 0.45	< 0.0001
CKMB (U/mL)	80.23 + 73.1	113.6 + 72.64	0.142
Troponin T (pg/mL)	406.5 + 323.7	694 + 313.7	0.006
Troponin I (ng/mL)	0.36 + 0.94	0.47 + 0.81	0.682
CRP (mg/L)	1.15 + 0.71	3.64 + 1.03	< 0.0001
Cholesterol (mg/dL)	199.7 + 45.69	182.73 + 38.74	0.180
HDL (mg/dL)	43.78 + 11.72	39.27 + 7.43	0.099
Triglyceride (mg/dL)	173.63 + 110.09	111.33 + 45.3	0.004
LDL (mg/dL)	121.78 + 41.56	124.82 + 42.59	0.814
MPO (ng /mL)	775.93 + 449.23	2595.33 + 932.68	< 0.0001

NIHSS: NIH Stroke Scale; GCS: Glasgow Coma Scale score; CNS: Canadian Neurological Scale; BI: Barthel Index; RS: Rankin Scale; CKMB: Creatinine phosphokinase-MB; CRP: C-reactive protein; HDL: High density lipoprotein; LDL: Low density lipoprotein; MPO: myeloperoxidase.

been shown that inflammation has a role in the formation of atheroschlerotic plaques, and this has become a topic for discussion[16]. MPO is one of the enzymes which is involved in this inflammatory process. Myeloperoxidase contributes to atherosclerosis through mechanisms related to its role in nitric oxide consumption, which is the cause of inflammation, LDL oxidation and endothelial dysfunction[17-26]. The enzyme MPO triggers atherogenesis, increasing the production of oxidized LDL cholesterol, and upsets the stability of plaques. Upsetting plaque stability sets off the process which causes acute ischemic stroke.

Zhang et al[27] showed that the prevalence of coronary artery disease rose in those whose MPO enzyme level was raised, and that MPO levels were raised even in coronary artery disease patients with no known risk factors. With regard to acute ischemic stroke, Cojocaru et al[28] found a significantly high level of MPO in the first 24 hours, which could be used in the diagnosis of acute ischemic stroke. This is the first and only study on this topic. It has been observed that serum levels of MPO examined in the first 24 hours were significantly higher in patients than in controls, but that they had returned to normal levels by the fifth day. This observation is in accordance with data obtained by Biasucci et al[29], who showed that MPO returned to starting levels within a week, even in patients with myocardial infarction. Also, when taken along with the finding in our study that CRP levels are a significant independent predictor of mortality, these data show that CRP and MPO can complement each other and can provide information in different areas: CRP is an indicator of disease activity and vascular inflammation, and is useful for long-term risk deposition, while MPO is an indicator of plaque instability and neutrophil activation, and may be related to short-term deposition particularly in patients with negative levels of troponin.

Conclusions

We have shown that serum MPO level measured before specific treatment is given can be used as a marker of the severity of ischemic stroke and to predict its outcome. Serum levels of MPO are raised in acute ischemic stroke patients, and MPO levels, along with CRP, can be an indicator of inflammation and a guide to stroke severity.

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