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Original Article

Lipoprotein-associated phospholipase-A_2 activity and its diagnostic potential in patients with acute coronary syndrome and acute ischemic stroke

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ABSTRACT

Background: The study examined the Lp-PLA_2 activity at the patients presented to the emergency department with acute coronary syndrome (ACS) or acute ischemic stroke (AIS), as well as its diagnostic value.

Methods: The prospective study included consecutive male and female patients aged >18 years that presented to the our emergency department with ACS or AIS between November 2009 and January 2010. Blood samples were obtained immediately following diagnosis in the ACS and AIS groups. The diagnostic value of Lp-PLA_2 was determined based on receiver operating characteristic curves, sensitivity, specificity, predictive values, likelihood ratios and accuracy rates.

Results: In all, 34 ACS and 32 AIS patients were included in the study, and the control group included 35 patients. Lp-PLA_2 enzyme activity was significantly lower in the ACS and AIS groups than in the control group (26.7 ± 13.8, 31.4 ± 13.6, and 41.4 ± 8.1 nmol $min^{-1} \cdot mL^{-1}$, respectively; $p < 0.0001$, $p = 0.022$). In the ACS group the area under the curve (AUC) was 0.825 (95%CI: 0.722–0.929), sensitivity was 71% for an optimal Lp-PLA_2 cut-off value of 31.4 nmol $min^{-1} \cdot mL^{-1}$, and specificity was 91%, whereas in the AIS group the AUC was 0.768 (95%CI: 0.652–0.884), sensitivity was 75% for an optimal Lp-PLA_2 cut-off value of 38.1 nmol $min^{-1} \cdot mL^{-1}$, and specificity was 74%.

Conclusions: Lp-PLA_2 enzyme activity was significantly lower during the early stage of both ACS and AIS. The obtained statistic data suggest that low Lp-PLA_2 enzyme activity can be used for diagnostic purposes.

1. Introduction

The lipoprotein-associated phospholipase-A_2 (Lp-PLA_2), which is also known as platelet-activating factor acetyl hydrolase (PAF-AH). Lp-PLA_2 is a member of the phospholipase A_2 super family. It is stated that Lp-PLA_2 is produced by myeloid-originated inflammatory cells, is associated with atherogenic lipoproteins in the circulation, and is highly expressed in lesion regions.[1]

Lp-PLA_2 hydrolyzes oxidized phospholipids, which form pro-inflammatory products that contribute to endothelial dysfunction, plaque inflammation and formation of the necrotic core in the plaque. Moreover, it is thought that Lp-PLA_2 constitutes a link between oxidative modification of LDL (Low Density Lipoprotein) and increased inflammatory response in the arterial intima.[2] Additionally; Lp-PLA_2 exhibits pro-inflammatory characteristics. It hydrolyzes oxidized phospholipids into lysophosphatidylcholine and free fatty acids. The atherogenic potential of LDL is related to this

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high lysophosphatidylcholine content.[3]

In the last decade, many studies have examined the effects of Lp-PLA_2 on atherosclerosis. The purpose of these studies was to determine the utility of Lp-PLA_2 in risk prediction or risk classification of cardiovascular diseases (CVDs).[4] To the best of our knowledge the literature **contains few studies** on the diagnostic use of the Lp-PLA_2 level in patients with acute coronary syndrome (ACS) or acute ischemic stroke (AIS).[5,6] As such, the present study aimed to examine the Lp-PLA_2 activity and its diagnostic potential in early period ACS and AIS patients.

2. Methods

2.1. Patients and process

The prospective study included consecutive male and female patients aged >18 years that presented to our emergency department with ACS or AIS between November 2009 and January 2010.The control group included consecutively patients without a history of or current thromboembolic events that presented to the emergency department with other complaints.The number of patients in each group was determined as about 30 considering the financial support of the study. The patients under 18 years were not included in the study in accordance with ethical rules. The patients who had the following were also excluded from the study due to their potential to affect lipoprotein metabolism, acute inflammatory response, cardiac troponin and CK-MB (Kreatin Kinaz−MB) levels and thereby the results of the study [7–10]: accompanying serious trauma, acute or chronic liver or renal failure, traumatic venous occlusion, hemorrhagic diathesis or coagulation disorders, hematologic malignancies, acute or chronic inflammatory disease, use of anti-aggregates, anticoagulants, anti-hyperlipidemia, or anti-inflammatory agents, and pregnancy. All ACS patients were examined by a cardiologist, whereas all AIS patients were examined by a neurologist and a neuroradiologist. In the diagnosis of ACS, the criteria which were published currently in guidelines were considered.[11]

For AIS group, all patients who had acute focal or systemic stroke signs (eg. alteration in consciousness, weakness of extremities, etc.) were determined with their first evaluation and blood sampling was taken. After the diagnosis of AIS was confirmed with computed tomography (CT) and/or diffusion weighted magnetic resonance imaging (DWI), those patients were included into AIS group.

Patients were enrolled in the study following verification of their diagnosis via appropriate diagnostic tests and provision of written informed consent. Blood samples were obtained from these patients for Lp-pLA2, Crp (C reaktif protein), Tn-I (Troponin I) and CK-MB analyses, and the Lp-pLA2 levels were compared to the other parameters. The sample drawn period was defined as the time from the onset of symptoms to blood drawn and it was categorized as $\leq$6 h, 6<−<12 h, and $\geq$12 h in the ACS and AIS groups. A possible difference between the sample drawn periods as well as Lp-PLA2 levels both within and between the ACS and AIS groups was investigated. The study protocol adhered to the Helsinki Declaration and was approved by the Ethics Committee of Selcuk University Meram Medicine School. The study was supported by the Scientific Research Projects Coordination Office of Selcuk University.

2.2. Sample drawn

Each patient provided 10 cc of venous blood, which was collected into test tubes containing EDTA (Etilendiamin tetraasetik asit). After plasma was separated from the obtained samples via cold centrifugation at 4 °C and 700−1000 g for 10 min, the plasma samples were placed in Eppendorf tubes and preserved at −80 °C until used.

2.3. Biochemical method

A commercially available colorimetric assay (PAF Acetyl hydrolase Assay Kit, Catalogue no. 760901, Cayman Chemical Company, USA) was used to measure the plasma PAF-AH. This method uses 2-thio PAF to serve as a substrate for all PAF-AHs. Free thiols emerging from hydrolysis of the acetyl thioester bond in the *sn*-2 position are identified using 5,5′ -dithio-*bis*-(2-nitrobenzoic acid) (DTNB; Ellman's reagent). All laboratory personnel were blinded to the study and control group patients' clinical data and outcomes. Electrochemiluminesans assay (Roche Hitachi, Cobas e 411, Roche Diagnostic Turkey Company) was used to measure CK-MB and Troponın I. Nephalometrically assay (BN2 SIEMENS) was used to measure CRP.

2.4. Statistical analysis

Study data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows v.19.0. Parametric testing was used for data with normal distribution (according to the Lilliefors test) and non-parametric testing was used for data not normally distributed. The chi-square test was used to evaluate the distribution of gender, hypertension (HT), diabetes mellitus (DM), coronary heart disease (CHD), hyperlipidemia (HL), and smoking, according to group. One-way analysis of variance (ANOVA) was used to compare patient age and Lp-PLA_2 activity between groups. When ANOVA test results were significant Tukey's HSD post hoc test was used. Time is used as covariant in order to control the effect.

The relationship between the sample drawn period and LP-PLA_2 activity within groups was assessed using the Kruskal-Wallis test and between groups using Pearson's correlation analysis. The correlation of level of Lp-PLA_2 and other biochemical parameters between groups were performed by Spearman's Test and its' accuracy was tested by Scatter-Dot Graphs. The level of statistical significance was set at $p < 0.05$. ROC (Receiver Operating Characteristic) curves were used to determine the diagnostic properties of specific Lp-PLA_2 activity values in the ACS and AIS groups, as compared to the control group. In addition to the area under the curve (AUC), the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios (LR), accuracy rate (AR), odd ratio and confidence interval (CI) were determined. In addition to cut-off values in which sensitivity and specificity were the highest in total, calculations also were made for probable cut-off values in which sensitivity or specificity would be higher. In ACS group, the second ROC curve was performed with reference to Tn-I and the diagnostic potential was calculated.

3. Results

In all, 34 ACS and 32 AIS patients, and 35 control patients were enrolled in the study. Mean age of the ACS patients was 62.2 ± 12.0 years (range 23−83), 28 (82.4%) of them were male; mean age of the AIS patients was 64.7 ± 13.9 years (range 40−91), 18 (56.3%) of them were male, and mean age of the control patients was 51.5 ± 17.2 years (range 20−90), 17 (48.6%) of them were male (Table 1). Mean age in the AIS group was significantly higher than that in the control group ($p = 0.007$). Similarly, male patients were significantly more prevalent than that in the other groups ($p = 0.003$). A correlation was not detected between Lp-PLA_2 levels and age, gender of the groups ($p = 0.72$ ve $p = 0.91$ respectively). Mean sample drawn period in the ACS group was 11.2 ± 17.0 h,

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