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The activities of serum paraoxonase and arylesterase and lipid profile in acute myeloid leukemia: preliminary results

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Abstract. – OBJECTIVE: To investigate the activities of serum paraoxonase-1 (PON1) and arylesterase (ARE), and the lipid profile in newly diagnosed acute myeloid leukemia (AML) patients.

PATIENTS AND METHODS: Thirty-two persons (16 of AML and 16 of healthy control) were included to the study. PON1 and ARE activities were measured as spectrophotometrically in serum samples. High density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG) were analyzed in autoanalyzer.

RESULTS: PON1 activities were respectively 16.04 U/L and 18.6 U/L in AML and healthy controls. There was no statistical significance between groups ($p > 0.05$). The mean ARE activities were respectively 0.21 U/L and 0.36 U/L in AML and healthy controls. Serum ARE activity significantly decreased in AML group ($p < 0.001$). Serum HDL values were significantly decreased (181.8 ± 76.2 mg/dl; $p = 0.002$) in AML. There was no difference in total cholesterol, LDL and triglyceride values (respectively; 181.8 ± 76.2 mg/dl, 120.6 ± 64.6 mg/dl, 157.3 ± 87.2 mg/dl; $p > 0.05$) between AML and controls.

CONCLUSIONS: This is the first documented study about serum PON1 activity in AML patients. Although serum PON1 activities were not changed in both groups, our data suggest that the decreased serum ARE activity and HDL levels may be related the pathogenesis of AML.

Key Words:
Paraoxonase, Arylesterase, Acute myeloid leukemia, Lipid profile.

Introduction

Acute myeloid leukemia is a hematopoietic stem cell disorders, characterized by over proliferation and accumulation of abnormal white blood cell in bone marrow[1]. Prevalence of acute myeloid leukemia's (AMLs) is 5-8 cases per 100.000 and increases with age[2,3]. Chemotherapeutic agents, such as alkylating agents and topoisomerase II inhibitors, chronic exposure to certain chemicals, benzene, embalming fluids, ethylene oxides, and herbicides, smoking etc. have been reported to increase the incidence of AML[4-7].

Serum paraoxonase-1 (PON1) is a 45 kDa glycoprotein[8]. It is also calcium-dependent esterase that is known to catalyze hydrolysis of organophosphates, and is widely distributed among tissues such as liver, kidney, intestine, and also serum, where it is associated with HDL. PON1 which is the lipophilic antioxidant component of HDL cholesterol has been found to decrease the susceptibility of LDL to lipid peroxidation with its antioxidant activity that protects lipoproteins against oxidation[9-12]. It has three activities which are paraoxonase, arylesterase and dyazoxonase[13].

Reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl anion ($OH^\cdot$) are scavenged by the antioxidant defense system components. These radical species can subsequently peroxidize unsaturated bonds of membrane lipids, denature proteins and attack nucleic acids[14]. This situation may constitute the molecular basis of many diseases including the inflammation process and cardiovascular alterations and also contribute to carcinogenesis[15].

In the present study, we aimed to measure the activities of PON1 and ARE, and lipid profile in patients with AML.

Patients and Methods

Patients

The Study group consisted of 16 newly diagnosed acute myeloid leukemia patients (8 males and 8 females). The diagnosis was made by

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means of cytochemical stains and bone marrow smears. All the patients were enrolled in the study before receiving the first course of chemotherapy. The patients were obtained from Medical Faculty of Hematology Department of Yuzuncu Yil University, Turkey. The control group consisted of 16 healthy subjects (8 males and 8 females). Both two groups were of the similar socioeconomic status and similar food habits.

The study subjects were briefed about the purpose of the study, and written consent was taken from each of them. Ethical approval was obtained from the local Ethics Committee. The selection criteria for the patients and controls were the lack of recent blood transfusion history and taking any medication with mineral supplement. The control subjects were selected from healthy individuals. All subjects had to go through clinical examination to determine existence of other diseases such as liver disease that might alter the activities of PON1 and ARE, and lipid profile. The samples were drawn before induction of chemotherapy.

Blood Samples

Blood samples were obtained following an overnight fasting state. They were collected into empty tubes and immediately stored on ice at 4°C. The serum samples were then separated from the cells by centrifugation at 3000 rpm for 10 min, lipid parameters were measured freshly. Remaining sera were stored at –20°C.

Measurement of Paraoxonase and Arylesterase Activities

PON1 and ARE activities were measured using commercially available kits (Relassay, Turkey). PON1 activity measurements were performed both in the absence and presence of NaCl (salt-stimulated activity). The rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) was measured by monitoring the increase of absorption at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290 M^{-1} cm^{-1} [16]. PON1 activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the ARE activity. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of ARE activity was defined as 1 μmol phenol generated per minute under the above conditions and expressed as U/L[17].

The levels of TG, TC, HDL and LDL were determined by using commercially available assay kits (Abbott®) with an autoanalyzer (Aeroset®, Abbott®, Wiesbaden-Delkenheim, Germany).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Non-parametric Mann Whitney U test was done on all the study group. The comparisons of parameters were performed using ANOVA test. A p value < 0.05 was accepted as significant. Data were analyzed using the SPSS® for Windows computing program (Version 13.0) (SPSS Inc., Chicago, IL, USA).

Table I. General parameters of patients and controls.

	AML group (n=16)	Control group (n=16)	p value
Age	34.6 ± 17	40.2 ± 13.9	0.32
TC (mg/dl)	181.8 ± 76.2	163 ± 28.7	0.37
HDL (mg/dl)	31.5 ± 10.2	43.7 ± 10	0.002*
LDL (mg/dl)	120.6 ± 64.6	92 ± 28.7	0.12
TG (mg/dl)	157.3 ± 87.2	136.2 ± 70.5	0.46

*p < 0.05 vs. controls. AML, acute myeloid leukemia; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Values are mean ± SD.

Results

The demographic characteristics of the groups are listed in Table 1. The mean age, gender, were similar in both groups. There was no difference in total cholesterol, LDL and triglyceride values (respectively; 181.8 ± 76.2 mg/dl, 120.6 ± 64.6 mg/dl, 157.3 ± 87.2 mg/dl; $p > 0.05$) between patients and controls whereas HDL values were different (181.8 ± 76.2 mg/dl; $p = 0.002$).

The median, minimum and maximum values of AML and control groups are listed in Table 2. The mean PON1 activities were respectively 16.04 U/L and 18.6 U/L in AML and control groups. There was no statistical significance between groups ($p > 0.05$). The mean ARE activities in AML were statistically lower than in the control group, at 0.21 U/L versus 0.36 U/L, respectively ($p < 0.001$).

Discussion

In this study, we evaluated that the activities of serum PON1 and ARE, and lipid profile in AML patient versus healthy controls. This is the

Table II. Paraoxonase and arylesterase activities of AML and control groups.

	AML group (n=16)			Control group (n=16)			p value
	Median	Maximum	Minimum	Median	Maximum	Minimum	
PON1	11.46	50.70	0.72	14.79	57.32	6.38	0.381
ARE	0.18	0.46	0.11	0.34	0.56	0.22	0.001

AML, acute myeloid leukemia; PON1, paraoxonase-1; ARE, arylesterase.

first paper reporting the PON1 and ARE activities in AML patients. A search of PUBMED (October 2012; search terms: "paraoxonase" and "arylesterase" and "AML") revealed no other cases. We found that the activity of ARE in AML patients was lower than that of the control group whereas the activity of PON1 was not change between the groups. However, the HDL levels in AML group was significantly lower than that of the healthy controls. We know that PON1 is a HDL associated enzyme with three activities which are paraoxonase, arylesterase and dyazoxonase[13]. Because of the relatively limited number of the patients, we could not evaluate the regression analysis between the clinical data and the enzyme activities in AML groups. In a previous study, the lipid profile in 530 patients newly diagnosed with cancer (of whom 97 had hematological malignancies) were analyzed and total cholesterol, HDL and LDL levels were found significantly lower and triglyceride concentration was higher[18]. However, some Authors[19] reported a decrease in the total cholesterol and HDL levels in 48 patients with newly diagnosed hematological malignancies. In another study[20] which supports to our results, in the active disease period the lowest HDL concentration was found for the AML patients, than for those with non-Hodgkin lymphoma, Hodgkin's disease, multiple myeloma, and myeloproliferative syndrome. Baroni et al[21] studied the serum lipid and lipoprotein changes before and after induction treatment in 25 acute nonlymphocytic leukemia (ANLL) and 18 acute lymphocytic leukemia (ALL) patients in order to investigate their relationship with disease activity and their prognostic relevance. They found a close relationship between the serum lipids and acute leukemia. Experimental studies[22] have proved that leukemic cells secrete a leukemia inhibitory factor (hLIF), which reduces the plasma cholesterol level through up-regulation of the LDL receptors on liver cells.

Camuzcuoglu et al[23] found that the activities of serum PON1 and ARE were significantly lower in patients with ovarian cancer compared to controls.

In some previous studies[24-31], it was reported that serum PON1 and ARE activities were significantly lower in some diseases such as insomnia, lung and breast cancer patients compared to healthy subjects. However, conflict to the other studies, we found no significant decrease in the activity of serum PON1 in AML patients. The limited number in patient group could be the reason of this contrary to the other studies. Otherwise, the activity of serum ARE was significantly lower in patient group shows similarity to the previous studies. Goncalves et al[32] investigated the PON1 polymorphism in a series of Brazilian children with an emphasis on early infancy. They found PON1 rs854560 (L55M) was associated with an increased risk of developing childhood leukemia.

Conclusions

The decrease of the serum ARE activity and HDL levels and the unchanged serum PON1 activity in patients may be related with the pathologic basis of the AML disease. Further studies are needed to clarify the mechanisms underlying the decreased enzyme activities and the pathogenesis of AML in a large population.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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