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Effects of paraoxonase, arylesterase, ceruloplasmin, catalase, and myeloperoxidase activities on prognosis in pediatric patients with sepsis

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ABSTRACT

Background: The present study aimed to investigate the levels of paraoxonase (PON), stimulated paraoxonase (SPON), arylesterase (ARE), ceruloplasmin (CLP), myeloperoxidase (MPO), and catalase (CAT) in pediatric sepsis and to explore their effects on the prognosis of sepsis.

Methods: Patients diagnosed with sepsis ($n = 33$) and healthy controls ($n = 30$) were included. PON, SPON, ARE, CLP, MPO, and CAT activities were measured in the sepsis and control groups. Additionally, the parameters were compared between survivors and non-survivors in the sepsis group. The levels of hemoglobin, white blood cell, platelet, lactate, and C-reactive protein were measured in the blood samples drawn from the patients with sepsis at diagnosis, at the 48th hour, and on day 7. The pediatric risk of mortality and pediatric logistic organ dysfunction scores of the patients were used for the estimation of severity of disease.

Results: Lower ARE (153.24 vs. 264.32 U/L; $p < 0.001$), lower CLP (80.58 vs. 97.98 U/L; $p = 0.032$), lower MPO (91.24 vs. 116.55 U/L; $p = 0.023$), and higher CAT levels (256.5 vs.145.5 kU/L; $p = 0.003$) were determined in the sepsis group as compared to the control group. There was no difference between the groups in terms of PON or SPON levels. No difference was determined between the survivors and non-survivors in terms of any of the parameters.

Conclusions: The present study determined that ARE, CLP, CAT, and MPO levels are different between the pediatric patients with sepsis and healthy controls. ARE level can be a potent biomarker for sepsis in critical patients in intensive care units. Further studies with larger samples are required to demonstrate the value of these parameters as prognostic biomarkers in pediatric sepsis.

1. Introduction

Sepsis is a major cause of morbidity and mortality among infants and children [1]. Mortality rate in pediatric sepsis ranges between 5% and 40% despite all efforts to improve early diagnosis and treatment [2]. Therefore, early diagnosis and intervention measures should be taken promptly to protect children from devastating effects of sepsis. Availability of various biomarkers, which play a role in the pathogenesis of sepsis, in enabling early diagnosis and in predicting prognosis in sepsis has been a research topic for many years. These biomarkers include C-reactive protein (CRP), procalcitonin, lactate, white blood cell (WBC) count, erythrocyte sedimentation rate, interleukin (IL)-6, IL-8, cluster of differentiation 64 (CD64), the soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) [3]. Oxidative stress and antioxidant defense systems are also among the factors that play a role in the pathogenesis of sepsis. Studies have reported an increase in the amount of reactive oxygen species resulting from oxidative stress in patients diagnosed with sepsis [4,5]. Additionally, it has been shown that the antioxidant defense in these patients is depleted and becomes ineffective [5]. It has been reported that antioxidant potential might be low in sepsis patients at the beginning of the disease as compared to healthy subjects, but then begin to increase up to normal levels and above. The oxidative stress biomarkers may lead to successful interventions and this will improve outcome in critically ill patients [6]. The role of antioxidant supplementation in pediatric sepsis is also a matter of debate [6,7].

Many components of the antioxidant defense system have been described to date including enzymatic (glutathione peroxidase, glutathione reductase, catalase (CAT), superoxide dismutase, peroxiredoxin) and non-enzymatic antioxidants (thiol antioxidants, melatonin, coenzyme Q, and metal chelating proteins). Moreover, some vitamins and trace elements also play a role as antioxidants [8,9]. The present study aimed to investigate the levels of certain biomarkers, which play a role in anti-inflammatory and antioxidant processes, in pediatric patients

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with sepsis and to explore their effects on the prognosis of sepsis. For this purpose, paraoxonase (PON), stimulated PON (SPON), arylesterase (ARE), ceruloplasmin (CLP), myeloperoxidase (MPO), and CAT activities were measured in pediatric sepsis patients.

2. Methods

2.1. Study population

The present study included children between the ages of 4 months and 18 years old who developed sepsis while being followed in the pediatric intensive care unit (PICU) from March 2015 through December 2015, prospectively. The PICU is a 22-bed unit of a tertiary referral center with all pediatric specialties, in which nearly 900 patients are followed-up in a year. Patients who were considered having "high probable sepsis" according to the criteria defined by Gitto et al. [10] based on their clinical and/or laboratory findings were included in the present study. These criteria includes the followings: 1) having at least 3 sepsis-related clinical signs (temperature instability, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distention), 2) having a CRP level of >1 mg/dL, and 3) having at least 2 other altered serum parameters (WBC count, platelet count) in addition to CRP, 4) blood culture being positive or clinical sepsis (when blood culture was negative). Patients having chronic diseases such as diabetes mellitus, chronic renal failure, and rheumatologic diseases and oncologic patients were excluded. Accordingly, the sepsis group included 33 patients and a control group was formed from 30 healthy children (patients who had no co-morbid or chronical diseases) who were admitted to the pediatric clinic of the same hospital for routine follow up. The study was approved by the Local Ethics Committee and informed consent of the patients was obtained from the parents/caregivers of the patients.

2.2. Procedures

The levels of hemoglobin, WBC, platelet, lactate, and CRP were measured in the blood samples drawn from the patients with sepsis at diagnosis, at the 48th hour, and on Day 7.

The pediatric risk of mortality (PRISM III) and pediatric logistic organ dysfunction (PELOD) scores of the patients were used for the estimation of severity of disease.

Fasting blood samples were obtained from the patient and control groups and collected into plain tubes to measure antioxidants. Serum samples were separated by centrifugation at 1600*g* for 10 min and stored at −80 °C until the time of analysis. The type and manufacturers of the specimen tubes used for collection of samples are plain tubes, BD Vacutainer ®, SST II Advance, REF 367955, UK.

2.3. Measurements

Activities of PON and ARE were measured using commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey). Measurements of PON activity were performed in the absence (basal activity) and presence of NaCl (salt-stimulated activity-SPON). The increase of absorbance at 412 nm at 37 °C was recorded as the activity of paraoxon hydrolysis (diethyl-p-nitrophenyl phosphate). The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient (18,290 M^{-1} cm^{-1}) at a pH of 8.5 [11]. PON activity was expressed as U/L serum. Measurement of ARE activity was performed using phenylacetate as the substrate. Enzymatic activity was calculated from the molar absorptivity coefficient (1310 M^{-1} cm^{-1}) of the produced phenol. One unit of ARE activity was defined as 1 μmol phenol generated/min under the above-defined assay conditions and expressed as kU/L serum [12].

Measurements of CLP levels were performed by the method described by Erel [13]. This is an automated and colorimetric method, which is based on the enzymatic oxidation of ferrous ion to ferric ion. The results were expressed in U/L serum.

Activity of MPO was measured by a modified O-dianisidine method, a kinetic measurement, in which the rate of yellowish orange product formation from the oxidation of O-dianisidine with MPO in the presence of hydrogen peroxide (H_2O_2) was measured at 460 nm [14]. One unit of MPO was defined as the degradation 1 μmol of H_2O_2 min^{-1} at 25 °C. A molar extinction coefficient of 1.13×10^4 of oxidized O-dianisidine was used for the calculation. MPO activity was expressed in U/L serum.

Activity of CAT was measured by Goth's method [15]. Sample (0.2 mL) was incubatedin 1.0 mL substrate (65 μmol per H_2O_2 in 60 mmol/L sodium–potassium phosphate buffer, pH 7.4) at 37 °C for 60 s. The enzymatic reaction was stopped with 1.0 mL of ammonium molybdate (32.4 mM) and the yellow complex of molybdate and H_2O_2 were measured at 405 nm and the activity of CAT was expressed as kU/L. All parameters were measured in the automatic analysis of roche-hitachi cobas c501 (Mannheim Germany).

2.4. Statistical analyses

The statistical analyses were performed using the Predictive Analytics Software (PASW) Statistics for Windows (SPSS Inc., Chicago, IL, USA) version 18.0. Descriptive statistics were expressed as numbers and percentages for categorical variables and as mean, standard deviation, median, 25th percentile (Q1), 75th percentile (Q3) for numerical variables. Normal distribution of the data was tested using visual (histograms and probability plots) and analytical methods

Table 1
Distribution of age and gender among sepsis and control groups.

	Sepsis Group ($n = 33$)	Control Group ($n = 30$)	p
Gender, n (%)			
Girls	17 (51.5)	15 (50.0)	0.904
Boys	16 (48.5)	15 (50.0)	
Age, years, median (Q1–Q3)	4 (1 − 12)	5 (2 − 11)	0.715

Table 2
Values of certain critical parameters at diagnosis, at 48 h, and on day 7 in patients with sepsis.

	Diagnosis Median (Q1-Q3)	48 h Median (Q1–Q3)	Day 7 Median (Q1–Q3)	p
WBC, $\times 10^3$/μL	12.2 (5.1–21.8)[b]	10.7 (4.9–13.9)[a]	10.15 (7.3–16.2)	0.018
Hemoglobin, g/dL	10.9 (9.1–13)	10.5 (9.5–11.7)	10.7 (9.7–12.1)	0.767
Platelet, $\times 10^3$/μL	112 (36–288)	111.5 (42–193)	133.5 (47–333)	0.291
Lactate, mmol/L	2.4 (1.6–4.3)	2.2 (1.4–6.8)	2.3 (1.5–3.5)	0.744
CRP, mg/dL	5.1 (1.5–19.0)	6.3 (2.8–23.3)	5.7 (1.9–20.5)	0.611

WBC, white blood cell; CRP, C-reactive protein.
[a] Significantly different from the value at the diagnosis.
[b] Significantly different from the value at 48 h. (Wilcoxon Signed Rank Test with Bonferroni correction was used, $p < 0.017$).

Table 3
Antioxidant levels in the sepsis and control groups.

	Sepsis group Median (Q1–Q3)	Control group Median (Q1–Q3)	p
Paraoxonase, U/L	88.05 (63.9–142.4)	95.55 (72–232.3)	0.315
Stimulated paraoxonase, U/L	469.31 (293.7–537.41)	325.55 (234–808.3)	0.720
Arylesterase, kU/L	153.24 (84.64–208.14)	264.32 (219.04–306)	<0.001
Ceruloplasmin, U/L	80.58 (61.61–106.79)	97.98 (81.88–117.78)	0.032
Catalase, kU/L	256.5 (128.0–320.7)	145.5 (91.9–224.0)	0.003
Myeloperoxidase, U/L	91.24 (53.05–127.05)	116.55 (100–148.53)	0.023

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