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Association of paraoxonase 1 and oxidative stress with acute kidney injury in premature asphyxiated neonates☆

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ABSTRACT

Objectives: Acute kidney injury (AKI) is defined as a decrease in glomerular filtration rate with an increase in serum creatinine (sCr). Perinatal asphyxia (PNA) may be etiological factor for AKI with oxidative stress also implicated. Paraoxonase 1 (PON1) activity has been reported to be decreased in renal disease. The aim of our study was to evaluate paraoxonase 1 (PON1) activity and oxidative stress during the first hours and first days of life and to determine if these parameters could discriminate neonates having AKI from those who do not.

Methods: Serum samples at different time points after birth were obtained from 64 preterm newborns with PNA (45 defined as having AKI, 19 as non-AKI). Clinical markers, sCr, total oxidant status (TOS), total antioxidant status (TAS) and PON1 activity were measured.

Results: The AKI group had more newborns with hypoxic ischemic encephalopathy, significantly higher serum creatinine (sCr) at 3 and 7d, total antioxidant status (TAS) at 7d; decreased PON1 at 4h, 6h and 7d than the non-AKI group. Within the AKI group, significant positive correlations were found between PON1 activity at 2h and TAS at 2h, PON1 activity at 4h and base deficit (BD); whereas negative correlations between PON1 activity at 2h and ΔsCr (at 24h and at 3d), PON1 activity at 7d and ΔsCr (at 24h and 3d). Oxidative stress status parameters indicated excellent discriminative potential at 4h, 6h and 7d.

Conclusions: AKI neonates were characterised by a marked decrease in PON1 activity. PON1 activity may be an important factor for discrimination of newborns having AKI from those that do not.

1. Introduction

Acute kidney injury (AKI) is defined as a rapid decrease in glomerular filtration rate (GFR) [1] followed by an increase, usually reversible, of serum creatinine (sCr) [2]. There are different etiological factors for AKI: Perinatal asphyxia, ischemic renal failure, sepsis and hemolytic uremic syndrome [1]. SCr, although widely used, is not a reliable marker of renal damage [3] as SCr is a measure only of function rather than injury, its tubular secretion overestimates renal function and there is both muscle mass and age dependency.

There is substantial evidence suggesting that reactive oxygen species (ROS) are implicated in renal damage in AKI [2,4]. Renal tubules have a high density of mitochondria and are sites for significant xanthine and arachidonic acid metabolism which generate free radicals leading to structural and functional defects seen in AKI [5]. In addition, hypoxic preterm newborns are at increased risk of

Abbreviations: AKI, acute kidney injury; sCr, serum creatinine; PNA, perinatal asphyxia; TOS, total oxidant status; TAS, total antioxidant status; PON1, paraoxonase 1; BD, base deficit; GFR, glomerular filtration rate; ROS, reactive oxygen species; ΔsCr, difference in sCr between two time points; NICU, neonatal intensive care unit; AS, Apgar score; GA, gestational age; HIE, hypoxic ischemic encephalopathy; eCCl, estimated creatinine clearance; POase activity, paraoxonase 1 activity towards paraoxon; BW, birth weight; ICU LOS, intensive care unit length of stay; ROC curves, receiver operating characteristic; OR, odds ratio; AUC, area under the curve; HDL particle, high-density lipoprotein.

☆ Our work differs from the others in a way that it shows that AKI neonates were characterized by marked decreases in PON1 activity. It also shows an association between decrease of PON1 and progression in renal failure and reports that PON1 may be important in discrimination newborns having AKI from those not having it.

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oxidative stress in their first week of life [6].

Paraoxonase 1 (PON1) is an enzyme reported to have anti-inflammatory and antioxidative properties [7]. According to previous studies [8–10], PON1 activity decreased during chronic renal failure in adult patients and in low birth weight neonates [11]. Furthermore, oxidative stress can inactivate PON1 [12]. Newborns reach the level of PON1 normally seen in adults sometime between 6 and 24 months of age indicating a low level of PON1 at birth [13] and the likelyhood of an inadequate response to oxidative damage. Data regarding PON1 activity in neonates suffering from AKI, as well as the ability of PON1 and oxidative stress status to discriminate neonates with AKI from non-AKI newborns are lacking.

The aim of our study was to firstly evaluate how PON1 activity and oxidative stress markers change during the first hours and first days of life and secondly to correlate these with clinical markers of AKI to explore their discriminative potential.

2. Materials and methods

2.1. Study population

We recruited 64 preterm newborns with perinatal asphyxia (PNA) in a tertiary-level Neonatal Intensive Care Unit (NICU) at the Institute of Neonatology, Belgrade, Serbia. The indicator of PNA was an Apgar score (AS) $\leq$7 in the 5th minute of life and/or pH of blood $\leq$7.20 on admission to the NICU. A sCr-based definition and classification for AKI was used [14]. If the difference between sCr concentrations on the 1st and 3rd day of life was $\geq$26.5 μmol/L neonates were classified into the AKI group. Forty five out of 64 developed AKI and were placed in the AKI group. Nineteen neonates did not develop AKI (the non-AKI group).

Gestational age (GA) was determined by calculation from the last menstrual period and/or antenatal ultrasonography and was confirmed by the Ballard score in the postnatal period [15]. Hypoxic ischemic encephalopathy (HIE) was defined according to Sarnat and Sarnat [16].

Neonates with one or more of the following findings were excluded from the study: Known renal or any other congenital anomalies, sepsis or metabolic disease. Newborns whose mother had chronic kidney disease, hypertension, severe systemic disease, diabetes mellitus or some other type of chronic disease were also excluded from the research. Data on the health of mothers were obtained through retrospective reviewing of medical records belonging to maternity hospitals.

The study was planned according to ethical guidelines following the declaration of Helsinki and was approved by the Ethics Committee of the School of Medicine, University of Belgrade (reference number 440/III-6). Written consent was obtained from parents of the examined newborns.

2.2. Sample collection

Serum samples corresponding to 2h, 4h, 6h, 3d and 7d after birth were obtained after admission to the NICU. All blood samples were immediately centrifuged (3000 rpm, 5 min) to obtain serum. The samples were then stored at −80 °C and thawed immediately before analyses.

2.3. Biochemical parameters

SCr was measured using a modified Jaffe's method on a Dimension Auto-analyser (Siemens Healthcare GmbH, Germany). Estimated creatinine clearance (eCCl) was assessed at an appropriate time point according to the formula [17]; eCCl (ml/min/1.73 m^2) = K x body length (BL)/sCr, where BL is expressed in cm, sCr in μmol/L and coefficient K is 29.2 for neonates born before 37 weeks of gestation [18].

Total anti-oxidant status (TAS) was determined in serum according to a previously published method [19]. Antioxidants present in serum led to decolouration of the 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) radical cation. Change in colour intensity of ABTS from an intense green (when no serum was added and with no antioxidants) to a less intense green (when serum was added with antioxidants present) was measured using an ILAB 300 + analyser (Instrumentation Laboratory, Milan, Italy). The reaction was calibrated with Trolox (a water-soluble analogue of vitamin E, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). TAS values were expressed as μmol Trolox equivalent/L. The intra-assay and inter-assay coefficients of variance were 4.3% and 8.8%, respectively.

Total oxidant status (TOS) was determined according to a previously published method [20]. The presence of various oxidising substances in serum led to oxidation of the ferrous ion-o-dianisidine complex to ferric ion. Ferric ion was measured using xylenol orange. The assay was performed on an ILAB 300 + analyser using hydrogen peroxide (H_2O_2) as a calibrator. The results were expressed as micromolar H_2O_2 equivalent per litre (μmol H_2O_2 equivalent/L). The intra-assay and inter-assay coefficients of variance were 5.6% and 9.5%, respectively.

Paraoxonase 1 (PON1) activity was assessed using paraoxon as a substrate (POase activity) using an ILAB 300 + analyser according to a previously published method [21].

2.4. Statistical analysis

Characteristics of the study populations are presented as means ± standard deviation for normally-distributed data, as geometric means and 95% confidence intervals for log-normally distributed data and as absolute frequencies for categorical variables. Comparisons of continuous variables were performed using the Student's *t*-test. Differences between categorical variables were examined with the Chi-square test for contingency tables. Spearman's correlation analysis was employed to estimate possible associations of PON1 with oxidative stress status and clinical markers of AKI. Binary logistic regression analysis was performed to assess the ability of parameters to predict AKI. Neonates without AKI were designated as 0 and neonates with AKI were designated as 1. Logistic models taking into account TAS, TOS and PON1 for each time point (one model consisted of TAS, TOS and PON1 from an appropriate time point) were created and the odds ratio (OR) was determined for each parameter. Receiver operating characteristic (ROC) curve analysis for each time point compared the ROC curve generated from a logistic model (consisting of TAS, TOS and PON1) and the ROC curve of sCr. This analysis was used to assess the ability of oxidative stress status, PON1 and sCr to discriminate subjects in the AKI and non-AKI groups. ORs obtained from logistic analysis indicated the relative weight of each variable in the model and contribution to the ROC curve. Using the Hosmer-Lemeshow rule for logistic models, discriminative abilities were classified according to their areas under the curve (AUC) as poor ($0.5 \leq AUC < 0.7$), acceptable ($0.7 \leq AUC < 0.8$), excellent ($0.8 \leq AUC < 0.9$) or outstanding ($AUC \geq 0.9$) [22]. The studied parameters were analysed in combination as models of continuous variables. All statistical analyses were performed using MS Excel, PASW Statistics Version 18.0 and MedCalc version 11.4. All statistical tests were considered significant at the 0.05 probability level.

3. Results

General information about the neonates is summarised in