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Pentraxin 3 in neonates with and without diagnosis of pulmonary hypertension

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ABSTRACT

Objectives: Pentraxin 3 is a novel biomarker produced by vascular endothelial cells and macrophages. In recent studies involving adults, pentraxin 3 has been introduced as a reliable biomarker in the evaluation of cardiovascular disease and pulmonary hypertension. This study was conducted with an aim to measure the level of pentraxin 3 in neonates with pulmonary hypertension and comparing with normal healthy controls.

Design and methods: In a case-control study, plasma pentraxin 3 levels were evaluated in 3 groups of neonates including neonates with pulmonary arterial hypertension (PAH), neonates with congenital heart disease without pulmonary arterial hypertension (CHD-PAH) and normal healthy neonates.

Results: Plasma pentraxin 3 levels in 72 neonates (21 in PAH, 19 in CHD-PAH, and 32 in control group) were measured. Demographic characteristics had no significant statistical difference among the 3 groups. Pentraxin 3 levels in PAH group was significantly higher than CHD-PAH and control groups (2.12 ± 2.32 vs. 0.58 ± 0.57 and 1.03 ± 1.38 ng/mL, $P = 0.008$, respectively). No significant correlation was found between concentrations of pentraxin 3 and cardiac ejection fractions between PAH and CHD-PAH ($r = 0.009$, $P = 0.97$). However, significant positive correlation was detected between PTX3 concentrations and pulmonary pressures between these two groups ($r = 0.499$, $P = 0.001$).

Conclusions: Results from our study showed that pentraxin 3 levels were increased in newborn infants with pulmonary hypertension. Plasma pentraxin 3 could be considered as a novel adjunct diagnostic tool in the evaluation of pulmonary hypertension in combination with echocardiography or as a diagnostic tool when echocardiography is not readily available for confirmation of increased pulmonary pressure.

1. Introduction

Pulmonary arterial hypertension (PAH) is one of the most important causes of respiratory impairment in neonates. PAH could be a complication during the course of over 10% of all newborns who have respiratory failure, and is a significant cause of morbidity and mortality in this population [1]. PAH during the neonatal period could be primary or secondary to various causes such as congenital heart disease, meconium aspiration, and bronchopulmonary dysplasia [1–3]. Early diagnosis of PAH is important due to its association with adverse cardiorespiratory outcome [4]. Although cardiac catheterization is the gold standard for diagnosis of PAH, it is invasive and rarely applied as the initial diagnostic tool. Thus echocardiography is the preferred screening test of choice for detection of PAH in the neonatal population. PAH can be diagnosed by the presence of at least one of the following echocardiographic findings: right to left shunting, right ventricular hypertrophy, interventricular septal flattening or elevated tricuspid regurgitation jet velocity in the absence of pulmonary stenosis [4]. However echocardiography may not always be readily available in all neonatal intensive care units [4]. So measurement of specific biomarkers may be useful in screening of newborns with PAH [4].

Pentraxin 3 (PTX3) is a novel biomarker and its measurement has been found effective for the evaluation of patients with PAH in adults [5]. PTX3 is a biomarker like C-reactive protein (CRP) which belongs to pentraxin superfamily. CRP is a short pentraxin originated from liver, whereas PTX3 is a long pentraxin that is highly expressed in the heart and synthesized by vascular endothelial cells and macrophages [5–8]. PTX3 has a well-established role in the innate immune response to infection [9,10]. Serum level of PTX3 has a correlation with severity of infectious disease and can be used as an independent prognostic biomarker in bacteriemia and fatal diseases [11,12]. Many studies showed that PTX3 is a widely used biomarker in clinical situations [12,13].

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Recently, the role of PTX3 in cardiovascular diseases has been demonstrated in several studies [13,14]. It has been shown that the level of PTX3 elevated in the acute myocardial infarction and congestive heart failure [7,8]. The role of PTX3 as a specific biomarker for the diagnosis of PAH has also been recently demonstrated [5].

Most PTX3-related cardiovascular disease studies are limited to adult patients; only a few studies have been carried out on clinical utility of PTX3 as a biomarker in neonatal morbidities. To the best of our knowledge, PTX3 has not been fully evaluated in newborn infants with PAH. Therefore we examined the role of plasma PTX3 in neonates with a diagnosis of pulmonary arterial hypertension.

2. Materials & methods

This case-control study was conducted in the NICU and neonatal ward of Bu-Ali Sina Teaching Hospital (Sari, Iran) between October 2013 and March 2015. Study population included 72 newborn infants >34 weeks of gestation and were categorized into three groups: 21 with PAH, which was primary or secondary to congenital heart disease, 19 with congenital heart disease without pulmonary arterial hypertension (CHD without PAH), and 32 healthy normal controls. The control group was selected from healthy neonates who were referred to the hospital for evaluation of neonatal jaundice and to monitor serum bilirubin and had a normal cardiovascular assessment on clinical evaluation by an experienced neonatologist. They were age, sex and gestation matched with neonates of other two groups. Diagnosis of congenital heart disease and pulmonary hypertension was made according to echocardiographic findings by pediatric cardiologist using echocardiography machine (Sonosite®, USA, probe 5–8 Hz). Neonates meeting any of the following criteria were excluded from the study: diagnosis of suspected or proven sepsis by elevated CRP >10 mg/dL and/or positive blood culture, disseminated intravascular coagulation, severe hypoxic respiratory failure (PaO_2 < 50 mm Hg despite adequate invasive mechanical ventilation), low Apgar score, non-congenital heart diseases (e.g., endocarditis) or maternal history of chorioamnionitis. This study was approved by the Research Ethic Committee of Mazandaran University of Medical sciences, Sari, Iran. Parental written informed consent was obtained from all study participants. Demographic and clinical data such as age, sex, birth weight, and gestational age were documented in all groups and type of congenital heart disease and ejection fraction ratio were recorded in groups of PAH and CHD without PAH.

2.1. Assessment of plasma levels of pentraxin 3

Venous blood samples were drawn from neonates in each group immediately after diagnosis of CHD or PAH by echocardiography and just before beginning of either drugs or interventions. Plasma was separated from EDTA-anticoagulated blood samples by centrifuging in 2700 *g* for 10 min and stored at −80 °C until analyzed. PTX3 was determined by enzyme-linked immunosorbent assay (ELISA) kits (Adipo Bioscience®, CA, USA). Sample preparation and assay procedure were followed according to the manufacturer's recommendation. Briefly, plasma samples were placed into pre-coated wells with monoclonal antibody specific for PTX3. After washing, a biotinylated polyclonal antibody specific for PTX3 was added and afterwards horseradish peroxidase (HRP) link streptavidin was added. During the next phase the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3.3′,5,5′-tetramethylbenzidine (TMB). A reference concentration of PTX3 was used to prepare assay calibration. The absorption was determined with an ELISA reader (Biotek ELX800, USA) at 450 nm. The concentrations were interpolated from standard curves expressed in ng/mL. Inter-and intra-assay coefficients of variation were below 10%. All samples were run in duplicate with the appropriate standards on 96-well micro plates. To avoid any bias, all samples were blindly analyzed to clinical status.

2.2. Statistical analysis

For comparing the categorical data chi-squared test was applied. ANOVA test (Analysis of Variance) was used for comparison of continuous variables which are expressed as mean ± SD. Continuous variables were tested for normality by the Kolmogorov–Smirnov test. Bonferroni post hoc test was used for detection of differences between groups. Receiver operating characteristic (ROC) curve was constructed to determine optimal threshold values for plasma PTX3. Area under the curve (AUC) and 95% confidence interval were calculated to evaluate the effectiveness of PTX3 as a biomarker for PAH. The statistical analysis was performed with SPSS Statistics Version 20 (SPSS Inc., Chicago, IL, USA). The results were evaluated within a 95% confidence interval (CI). Value of <0.05 was considered as statistically significant.

3. Results

Baseline characteristics of 72 neonates including 21 PAH, 19 CHD without PAH, and 32 healthy controls are shown in Table 1. The mean age of study population was 7.4. ± 3.76 and 38 (52.8%) were females. As it was shown in Table 1, there is no significant difference in age, sex, gestational age, and birth weight between three study groups ($P = 0.33, 0.87, 0.13, 0.73$, respectively).

Mean pulmonary arterial pressure in PAH patients was 58.8 ± 12.9 mm Hg compared with 27.63 ± 5.1 mm Hg in CHD without PAH patients ($P < 0.0001$). In PAH group 61% and in CHD without PAH group 49% of patients required mechanical ventilation ($P = 0.81$). 3 out of 21 PAH patients had primary pulmonary hypertension of newborn (PPHN). As Table 2 shows, patent ductus arteriosus (PDA) was the most frequent primary congenital heart diseases in both patient groups. In addition, although atrial septal defect (ASD) was more prevalent primary congenital heart diseases in PAH group compared to CHD without PAH, but this difference met no significant difference (57.1% vs. 31.6%, $P = 0.09$).

Plasma concentrations of PTX3 by Kolmogorov–Smirnov test between the 3 groups of study revealed a normal distribution pattern. Plasma PTX3 concentration in PAH group is significantly higher than CHD without PAH and control groups (2.12 ± 2.32, 0.58 ± 0.57, and 1.03 ± 1.38 ng/mL, respectively, $P = 0.008$). Bonferroni post hoc test was used to compare plasma levels of PTX3 among the three study groups. Our results showed a statistically significant differences between PAH and CHD without PAH groups ($P = 0.009$) and also between PAH patients and healthy controls ($P = 0.049$) (Fig. 1).

Ejection fraction ratio in PAH patients was not significantly differed with CHD without PAH patients (65.09 ± 10.03% vs. 69.15 ± 4.75%, $P = 0.11$). On the other hand, there was no significant correlation between PTX3 concentrations and ejection fractions ($r = 0.009$, $P = 0.97$) (Fig. 2), meanwhile a significant correlation was observed between PTX3 concentrations and pulmonary pressures ($r = 0.499$, $P = 0.001$) (Fig. 3).

The area under the ROC curve was 0.683 (95% confidence interval: 0.545–0.820). Plasma PTX3 > 2.47 ng/mL yielded 90.5% sensitivity, 33.3% specificity, likelihood ratio positive 1.36 and likelihood ratio negative 0.56 for diagnosing of PAH. When we used a PTX3 concentration > 0.7 ng/mL as the cut-off value, specificity was 64.7%, but the sensitivity decreased to 66.7% (likelihood ratio positive 1.89 and likelihood ratio negative 0.52) (Fig. 4).

4. Discussion

Inflammatory signals can produce PTX3 from innate immune and vascular cells via Toll-like receptor (TLR) recruitment. PTX3 takes part in complement activation, pathogen recognition and antibody generation following its interaction with ligands [15]. Therefore PTX3 plays an important role in innate immunity and inflammation. The presence of PTX3 may represent infection and inflammation [16]. The elevated