Akut Bronşiolitli Çocuklarda Human Paraoksonaz-1 Aktivitesi

Human Paraoxonase-1 Activity in Children with Acute Bronchiolitis

Ufuk Erenberk (0000-0002-5481-8356)[1], Feyza Ustabaş Kahraman (0000-0003-3842-7723)[1], Emin Özkaya (0000-0003-4553-819X)[1], Mustafa Atilla Nursoy (0000-0003-0520-1082)[1], Ayşegül Doğan Demir (0000-0002-2536-1422)[1], Özden Türel (0000-0002-6535-4147)[1]

1 Bezmialem Vakıf Üniversitesi Tıp Fakültesi, Pediatri Bölümü İstanbul, Türkiye

Sorumlu yazar yazışma adresi:

Feyza Ustabaş KAHRAMAN: Bezmialem Vakıf Üniversitesi Tıp Fakültesi, Pediatri Bölümü İstanbul, Türkiye

E-mail: feyzaustabas168@gmail.com

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ÖZ

GİRİŞ ve AMAÇ: Human paraoksonaz-1 (PON1), oksidatif strese karşı çalışan endojen antioksidan moleküllerden biridir. Bu çalışmada akut bronşiolitli çocuklarda serum PON1 aktivitesini araştırmayı amaçladık.

YÖNTEM ve GEREÇLER: Akut bronşiyolitli 3-21 aylık 29 çocuk ile yaş uyumlu 35 sağlıklı kontrol çalışmaya dahil edildi. Bronşiolit hastaları hafif (% 65) ve orta (% 35) olarak sınıflandırıldı. Akut bronşiolitli hastalar ile kontrol grubu arasında PON1'in paraoksonaz ve arilesteraz aktiviteleri karşılaştırıldı.

BULGULAR: Akut bronşiyolitli çocukların paraoksonaz aktivitesi sağlıklı kontrollere göre daha düşüktü, ancak fark anlamlı değildi (127.53 ± 64.17 U / L'ye karşı 153.95 ± 74.40 U / L) (p = 0.13). Hafif ve orta derecede bronşiyolitli çocuklarda arilesteraz aktivitesi kontrol grubuna göre anlamlı olarak daha azdı (142.43 ± 56.60 kU / L ve 103.05 ± 26.03 kU / L'ye karşılık 201.09 ± 57.26 kU / L) (p <0.001).

TARTIŞMA ve SONUÇ: Akut bronşiolitli çocuklarda serum PON1 aktivitesi kontrol grubuna göre daha düşüktü. Antioksidan kapasitenin arttırılması, viral kaynaklı akciğer hastalığında etkili bir tedavi aracı olabilir.

Anahtar Kelimeler: paraoksonaz, arilesteraz, oksidatif stres, bronşiolit, çocuk

ABSTRACT

INTRODUCTION: Human paraoxonase-1 (PON1) is one of the endogenous antioxidant molecules working against oxidative stress. We aimed to investigate serum PON1 activity in children with acute bronchiolitis.

MATERIALS and METHODS: A total of 29 children aged between 3-21 months with acute bronchiolitis and 35 age-matched healthy controls were included in the study. Bronchiolitis patients were further classified as mild (65%) and moderate (35%). Paraoxonase and arylesterase activities of PON1 were compared between patients with acute bronchiolitis and the control group.

RESULTS: Paraoxonase activity of children with acute bronchiolitis was lower than healthy controls but the difference was not significant (127.53 ± 64.17 U/L versus 153.95 ± 74.40 U/L) (p=0.13). Arylesterase activity was significantly decreased in children with mild and moderate bronchiolitis in comparison to the control group (142.43± 56.60 kU/L and 103.70± 26.03 kU/L versus 201.09± 57.26 kU/L) (p<0.001).

CONCLUSIONS: Serum PON1 activity was lower in children with acute bronchiolitis compared to controls. Increasing antioxidant capacity may be an effective means of therapy in viral induced lung disease.

Key words: paraoxonase, arylesterase, oxidative stress, bronchiolitis, child

INTRODUCTION

Acute bronchiolitis is one of the most common infections in infants [1]. It is characterized by extensive inflammation of the airways, necrosis of small airway epithelial cells, increased mucus production and bronchospasm [2]. Overproduction of reactive oxygen species (ROS) through direct injurious effects or by involvement in the molecular mechanisms is believed to play a central role in the pathogenesis of several lung diseases [3-8]. To protect against oxidants, the lungs have a well-developed antioxidant system and when the balance between oxidant and antioxidant systems is disturbed oxidative stress develops [9]. The paraoxonases (PONs) comprise a family of closely related enzymes that includes PON1, PON2 and PON3. All PON proteins share considerable structural homology and have the capacity to protect cells from oxidative stress; therefore, they have been implicated in the pathogenesis of several inflammatory diseases, particularly atherosclerosis [10]. In human serum PON1 activity predominates [11]. PON1 is also detected in many tissues including liver, kidney, brain, and in cells of bronchiolar epithelium and type 1 pneumocytes of lung [12].

PON1 activity was studied in various acute and chronic inflammatory conditions [13-17]. Nevertheless, knowledge about activity of PON1 in patients with bronchiolitis is lacking. Since there is not an acknowledged biological substrate for PON1, its activity is measured through its degrading function towards artificial substrates paraoxon and phenylacetate (e.g. paraoxonase and arylesterase activities). In the present study we aimed to investigate arylesterase and paraoxonase activities of PON1 in children with acute bronchiolitis in comparison to healthy controls and to assess whether these enzyme activities changed in relation to the severity of disease.

MATERIALS and METHODS:

A total of 29 children aged 3-21 months treated with a diagnosis of acute bronchiolitis at Bezmialem Vakıf University Pediatric Emergency Department during January 2012 and April 2012 were included in this study. Acute bronchiolitis was defined as presence of fine inspiratory crackles and/or high-pitched expiratory wheeze in a child presenting with fever, nasal discharge and dry wheezy cough [18]. Patients were excluded from the study if they had: 1) an underlying disease that might affect the cardiopulmonary status (e.g. bronchopulmonary dysplasia, prematurity, assisted ventilation during the neonatal period, congenital heart disease or immunodeficiency); 2) asthma diagnosed by a physician, 3) wheezing or cough that had previously been treated with bronchodilators or corticosteroids within the preceding 2 weeks, 4) recurrent wheezing or history of chronic lung disease. Assessment of severity was done according to the algorithm for the management of children with breathing difficulty (Table 1). The control group consisted of 35 age matched healthy children who were recruited during routine yearly examination at pediatric out-

patient clinics who had no signs of a previous or ongoing illness. Male to female ratio was 13/16 in children with acute bronchiolitis and 18/17 in control group.

Table 1. Clinical scoring parameters in children with acute bronchiolitis [18]

Variable	**Severity**		
	Mild	**Moderate**	**Severe**
Oxygen saturation	>95%	92-95%	<92%
Chest wall in-drawing	None/mild	Moderate	Severe
Nasal flaring	Absent	May be present	Present
Grunting	Absent	Absent	Entire expiration or audible on expiration without stethoscope
Apnea/pausing	None	Absent	Trachea-sternal recession
Feeding history	Normal	½ of normal intake	Less than ½ of normal intake
Behavior	Normal	Irritable	Lethargic Unresponsive Flaccid Decreased level of consciousness Inconsolable

Sample collection and measurement of paraoxonase and arylesterase activity: Serum samples were immediately separated from cells by centrifugation at 3000 x g for 10 min, and then stored at –80°C until further analysis of paraoxonase and arylesterase activities. Serum paraoxonase and arylesterase activities of PON1 were measured spectrophotometrically using commercially available kits (relassay, Gaziantep, Turkey) [19]. Rate of paraoxon hydrolysis was measured by the increase of absorbance at 412 nm at 25.8°C. Amount of generated *p*-nitrophenol was calculated from molar absorptivity coefficient at pH 8, which was 17,000 M^{-1} cm^{-1}. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure arylesterase activity. Reaction was initiated by addition of serum and increase in absorbance was read at 270 nm. Blanks were included to correct spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated from molar absorptivity coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 µmol phenol generated/min under the above conditions and expressed as kU/L serum.

Statistical analysis: All statistics were performed using the program SPSS 15.0 for Windows. Pearson qui square test was used to compare gender of children with acute bronchiolitis and control group. Student *t* test was used for comparing mean values of age, paraoxonase, and arylesterase among bronchiolitis and control groups. Comparison of mean values of paraoxonase and arylesterase were performed by one-way ANOVA test among mild bronchiolitis, moderate bronchiolitis and control subjects. Multiple comparisons were analyzed by Posthoc Tukey HSD test.

RESULTS:

Male to female ratio was 13/16 in children with acute bronchiolitis and 18/17 in control group (p=0.59). Mean age of patients with acute bronchiolitis and control group were similar (9.44±3.60 months versus 9.91±4.62 months) (p=0.66). Paraoxonase activity of patients with acute bronchiolitis was lower than control group but the difference was not significant (127.53 ± 64.17 U/L versus 153.95 ± 74.40 U/L (p=0.13). Arylesterase activity was significantly decreased in children with acute bronchiolitis than control group (129.08 ± 51.26 kU/L versus 201.09 ± 57.26 kU/L (p<0.001). Among bronchiolitis patients, 19 (65%) were classified as mild and 10 (35%) as moderate. Age and gender of patients with mild bronchiolitis, moderate bronchiolitis and control group was similar (Table 2).

Table 2. Age and Gender Analysis of Patient and Control Groups

	Control group (*n*=35)	**Mild bronchiolitis (*n*=19)**	**Moderate bronchiolitis (*n*=10)**	***p***
Gender (M/F)	18/17	8/11	5/5	0.80
Age (months)	9.91±4.62	9.68±3.65	9.00±3.65	0.83

Paraoxonase activity of patients with moderate bronchiolitis was decreased in comparison to patients with mild bronchiolitis and control group but the difference was not statistically significant (Table 3). Arylesterase activity was significantly decreased in children with mild and moderate bronchiolitis than the control group (p<0.001).

Table 3. Paraoxonase and arylesterase activities in study groups and p- values.

Variables	Control group (2)	Mild bronchiolitis (1)	Moderate bronchiolitis (3)	p	Tukey
Paraoxonase U/l *	153.94 ±74.40	131.01 ±74.95	120.92 ±38.75	0.31	NS
Arylesterase U/I*	201.09 ±57.26	142.43 ±56.60	103.70 ±26.03	<0.001	1, 3 vs 2 (**)

**mean±SD ** Arylesterase activities of patients with mild bronchiolitis and moderate bronchiolitis were significantly lower than control group*

DISCUSSION

An increasing rate of hospitalization among healthy children with bronchiolitis is reported during the recent years [1]. Supportive care is the mainstay of treatment. Debate on the use of pharmacological agents is ongoing but there is still no common consensus. PON1 is an antioxidant enzyme which prevents oxidative stress by inhibiting oxidation of cell membrane lipids induced by ROS, reduces lipid hydro peroxidases to hydroxides, and degrades hydrogen peroxide under oxidative stress [10, 20, 21]. There is growing evidence from experimental, clinical and epidemiological studies that underscores the role of PON1 in protection against atherosclerosis [21]. Determination of PON1 status in bronchiolitis may aid in understanding the disease as well as development of new therapeutic modalities. Plasma PON1 was shown to decrease in respiratory tract infections due to influenza A and *Chlamydia pneumonia* in mice [22, 23]. To our knowledge this is the first study evaluating PON1 activity in children with acute bronchiolitis.

We determined that paraoxonase activity of PON1 was lower in children with bronchiolitis than control subjects although the difference was not significant. Arylesterase activity was significantly lower in children with acute bronchiolitis compared to controls. Paraoxonase and arylesterase activities of PON1 are considered the major determinants of the antioxidant action of HDL. Recent studies indicate that arylesterase activity best reflects the antioxidant activity of PON1, although it is not directly responsible for it [24]. PON1 also expresses a lactonase activity which allows neutralization of homoserine lactones (HSL) [25]. Lactonases can act as quorum quenching agents to limit bacterial virulence. It has been clearly demonstrated that the paraoxonases, by hydrolysis of HSL, limit infection and biofilm formation by *Pseudomonas aeruginosa* [26].

Serum PON1 expression is downregulated by oxidative stress [27]. Our results support the hypothesis that during acute bronchiolitis oxidative stress is increased and antioxidants are reduced. Increased oxidative stress has been involved in the pathogenesis of various lung diseases [3-8]. Enzymic and non-enzymic antioxidant activities were shown to decrease significantly in children with acute pneumonia [6]. Hosakote et al reported that expression and/or activity of superoxide dismutase (SOD), catalase, glutathione S-tranferase, and glutathione peroxidase were decreased in the nasopharyngeal secretions of children with severe RSV bronchiolitis [7].

Changes in lipid and peptide composition of HDL caused by inflammation influence PON1 activity. HDLs, not only provide a serum transport vector for PON1 but also contribute to enzyme activity, stability and, function [28]. Plasma lipids were reported to decrease in patients with lower respiratory infections.[29]. Misbalance between synthesis and utilization of plasma lipids, usage of lipids to restore damaged cell membranes and interaction of cytokines and bacterial toxins with lipids have been discussed as causes of hypocholesteraemia in severe illness. Novak et al reported that PON1 activity showed good correlation with decreased HDL cholesterol in critically ill patients with sepsis [30]. They supposed that increased binding of free radicals to PON1 accounted for the decrease in PON1 activity in the circulation. Future studies investigating plasma lipids and PON1 status concurrently in children with acute bronchiolitis will help to interpret our results.

In our study, paraoxonase and arylesterase activities of PON1 were lower in children with moderate bronchiolitis compared to mild cases but the difference was not statistically significant. Gornicka et al found lower serum PON1 activity in children with exacerbated bronchial asthma. There was an inverse correlation between the severity of asthmatic symptoms and serum PON1 activity. Serum PON1 activities increased parallel with improving asthma symptoms [16]. Koc et al reported that paraoxonase and arylesterase activities were significantly higher in patients operated for chronic adenotonsillitis as compared to control group [17]. It was supposed that in chronic diseases the body tries to cope with oxidative stress by increasing the antioxidants again.

Polymorphisms in the coding region of the *PON1* gene have also been shown to impact PON activity [31]. Other factors that may influence PON1 activity have been recently reviewed and include age, gender, and drugs [32]. Studies have shown that PON1 activity is preserved by dietary antioxidants [33]. Dietary modulators of PON1 status include fat and fatty acids, antioxidant vitamins (e.g. ascorbic acid, tocopherol), polyphenols and polyphenol-rich foods [34]. Vitamin C was shown to attenuate hypochlorite-mediated loss of PON1 activity in vitro and may, therefore, preserve cardioprotective properties of HDL during inflammation [33]. Bose et al showed that 60 days of tomato supplementation in patients with coronary heart disease resulted in a significant improvement in the levels of serum enzymes with antioxidant activities and decreased lipid peroxidation rate [34].

Conclusion*:* Defects in the antioxidant system capacity and altered PON1 activity may be involved in the pathogenesis of acute bronchiolitis. Increasing antioxidant level of body may be a therapeutic approach in virus induced lung infection.

Conflict of Interest: We declare that there is no conflict of interest.

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