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Paraoxonase-1 and Arylesterase Activities in Children with Acute Bronchiolitis

Akut Bronşiyolitli Çocuklarda Paraoksonaz-1 ve Arilesteraz Aktiviteleri

Fatih Akın[1], Abdullah Yazar[1], Esra Türe[2], Ümit Gültekin[3], Ahmet Osman Kılıç[1], Cemile Topcu[4], Dursun Odabaş[5], Alaaddin Yorulmaz[6]

[1]Necmettin Erbakan University Meram School of Medicine Department of Pediatrics, Konya, Turkey
[2]Karadeniz Technical University School of Medicine Department of Pediatrics, Division of Pediatric Emergency, Trabzon, Turkey
[3]University of Health Sciences Siirt Training and Research Hospital Department of Pediatrics, Siirt, Turkey
[4]Necmettin Erbakan University Meram School of Medicine Department of Biochemistry, Konya, Turkey
[5]Karamanoglu Mehmet Bey University School of Medicine Department of Pediatrics, Karaman, Turkey
[6]Selçuk University School of Medicine Department of Pediatrics, Konya, Turkey

Abstract

Aim: Acute bronchiolitis is a disease frequently seen in children under two years of age. Paraoxonase 1 (PON1) and arylesterase (ARES) are enzymes in the esterase group which are encoded by the same gene and whose active centers are similar. The common characteristics of PON1 and ARES are their ability to hydrolyze organophosphates, aryl and alkyl halides. PON1 enzyme functions as an antioxidant by inhibiting the oxidation of low density lipoprotein (LDL) and neutralizing free radicals such as hydrogen peroxide. ARES is accepted as an indicator of the main protein, which is not affected by changes in PON1. The aim of this study is to investigate whether PON1 and ARES activity levels can be used as an indicator of the disease in children with acute bronchiolitis.

Material and Method: Ninety one patients with acute bronchiolitis who admitted to pediatric emergency unit and 39 age- and sex-matched healthy children were included in the study. Patients were divided into 3 groups as mild, moderate and severe bronchiolitis according to Wang scoring system.

Results: The mean serum PON1 activity levels of all patients with bronchiolitis were 188.05±101.94 U/L, and the mean of the control group was 302.87±170.52 U/L. The mean serum ARES activity levels of all patients with bronchiolitis were 408.44±109.95 kU/L, and the mean of the control group was 785.45±168.45 kU/L. Mean serum PON1 and ARES activity levels were found to be statistically significantly lower in patients with acute bronchiolitis. No statistically significant difference was found among groups according to disease severity.

Conclusion: We found that PON1 and ARES activity levels were lower in patients with acute bronchiolitis than in controls. The results of our study show that low PON 1 and ARES activity levels may play a role in the pathogenesis of acute bronchiolitis and that oxidative stress may have an effect on the development of bronchiolitis.

Keywords: acute bronchiolitis, arylesterase (ARES), child, oxidative stress, paraoxonase 1 (PON1)

Öz

Amaç: Akut bronşiolit iki yaş altı çocuklarda sık görülen bir hastalıktır. Paraoksanaz-1 (PON1) ve arilesteraz (ARES) aynı gen tarafından kodlanan ve aktif merkezleri benzer olan esteraz grubunda yer alan enzimlerdir. PON1 ve ARES'in ortak özellikleri, organofosfatları, aril ve alkil halojenürleri hidrolize edebilmeleridir. PON1 enzimi, düşük yoğunluklu lipoproteinin (LDL) oksidasyonunu inhibe ederek ve hidrojen peroksit gibi serbest radikalleri nötralize ederek bir antioksidan görevi görür. ARES, PON1'deki değişikliklerden etkilenmeyen ana proteinin bir göstergesi olarak kabul edilir. Bu çalışmanın amacı, akut bronşiyolitli çocuklarda PON1 ve ARES aktivite düzeylerinin hastalık göstergesi olarak kullanılıp kullanılamayacağını araştırmaktır.

Gereç ve Yöntem: Çalışmaya çocuk acil servisine akut bronşiolit nedeniyle başvuran 91 hasta ile yaş ve cinsiyet olarak eşleştirilmiş 39 sağlıklı çocuk dahil edildi. Hastalar Wang skorlama sistemine göre hafif, orta ve şiddetli bronşiolit olarak 3 gruba ayrıldı.

Bulgular: Tüm bronşiyolitli hastaların ortalama serum PON1 aktivite düzeyleri 188,05±101,94 U/L, kontrol grubunun ortalaması 302,87±170,52 U/L idi. Tüm bronşiyolitli hastaların ortalama serum ARES aktivite düzeyleri 408,44±109,95 kU/L, kontrol grubunun ortalaması 785,45±168,45 kU/L idi. Akut bronşiyolitli hastalarda ortalama serum PON1 ve ARES aktivite düzeyleri istatistiksel olarak anlamlı derecede düşük bulundu. Hastalık şiddetine göre gruplar arasında istatistiksel olarak anlamlı fark bulunmadı.

Sonuç: Akut bronşiyolitli hastalarda PON1 ve ARES aktivite düzeylerinin kontrollere göre daha düşük olduğunu bulduk. Çalışmamızın sonuçları, düşük PON 1 ve ARES aktivite düzeylerinin, akut bronşiyolit patogenezinde rol oynayabileceğini ve oksidatif stresin bronşiolit gelişimi üzerine etkisi olabileceğini göstermektedir.

Anahtar Kelimeler: akut bronşiolit, arilesteraz(ARES), çocuk, oksidatif stress, paraoxonaz 1 (PON1)

Corresponding *(İletişim)*: Abdullah YAZAR, Necmettin Erbakan University Meram School of Medicine, Department of Pediatrics, Konya, Turkey
E-mail *(E-posta)*: drabdullahyazar@gmail.com
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INTRODUCTION

Acute bronchiolitis is a disease characterized by inflammatory obstruction of small airways, characterized by tachypnea, retractions and wheezing. Viruses often play a role in the etiology of the disease. Most cases are under two years old.[1,2] Respiratory syncytial virus (RSV) is responsible for almost half of the cases. While it is accepted that bacteria do not cause disease, bacterial superinfection can be observed with bronchiolitis.[1] Frequency of bronchiolitis varies seasonally. It is more dominant in the winter months. Every year, hundreds of thousands of children under one year old in the world are hospitalized for acute bronchiolitis, and each passing year, the number of cases increases. Oxidative stress is known to contribute to the pathogenesis of acute and chronic lung inflammatory diseases.

Paraoxonase 1 (PON1) and arylesterase (ARES) are enzymes in the esterase group which are encoded by the same gene and whose active centers are similar. Despite PON1 is known to show polymorphic changes, the ARES enzyme does not show a genetic polymorphic change. Although the natural substrates of the two enzymes are different, the PON1 enzyme has the ability to hydrolyze the phenyl acetate, the natural substrate of ARES. The common characteristics of PON1 and ARES are their ability to hydrolyze organophosphates, aryl and alkyl halides. PON1 enzyme functions as an antioxidant by inhibiting the oxidation of low density lipoprotein (LDL) and neutralizing free radicals such as hydrogen peroxide. ARES is accepted as an indicator of the main protein, which is not affected by changes in PON1.[3,4]

Dundaroz et al.[5] reported that total oxidative status was higher and total antioxidant capacity was lower in patients with acute bronchiolitis compared to the control group, and this situation was effective in the pathogenesis of the disease. There are studies reporting that PON 1 and ARES activity levels contribute to the pathogenesis of diseases including asthma, pulmonary tuberculosis, allergic rhinitis, chronic obstructive pulmonary disease and beta-thalassaemia major.[6-11]

The aim of this study is to determine whether PON1 and ARES activity levels can be used as an indicator of the disease in patients with acute bronchiolitis and to investigate the association of the activities of these enzymes with the severity of the disease.

MATERIAL AND METHOD

This study was conducted with the approval of Necmettin Erbakan University Clinical Research Ethics Committee (Date: 24.02.2017, Decision No: 2017-825). All procedures were carried out in accordance with the Declaration of Helsinki.

Study Population

Patients who admitted to pediatric emergency department of Necmettin Erbakan University Meram Medical Faculty in Konya region of Turkey between the period September 2018 to May 2019, and diagnosed as acute bronchiolitis were included in the study. Informed consent was obtained from the families of the patients. Children who had a history of congenital heart disease, bronchopulmonary dysplasia, chronic lung disease, prematurity, assisted ventilation during the neonatal period, immunodeficiency, asthma and who received bronchodilator or corticosteroid treatment in the previous two weeks were not included in the study. The clinical, laboratory and demographic features of the patients were recorded in a standardized form. Serum samples that remained from the blood examination routinely taken for analysis from patients who were followed up with the diagnosis of acute bronchiolitis were used to evaluate PON1 and ARES activity levels. The blood samples were collected immediately after admitting the emergency department, before receiving any medication. No extra blood was collected from the patients for these tests. Patients were excluded from the study when the serum sample left was insufficient for evaluation.

The control group was consisted of age and gender matched healthy children who did not have any signs of septicemia, pulmonary, metabolic and rheumatological diseases, but tested for a routine check up. Remaining serum samples were used to evaluate PON1 and ARES activities.

Wang scoring system was used to evaluate the severity of acute bronchiolitis.[12] Patients were divided into 3 groups as mild, moderate and severe bronchiolitis according to this scoring. A Wang score of 1-3 points considered as mild disease, 4-8 points as moderate disease, and 9-12 points as severe disease.

Laboratory testing

Blood samples were centrifuged at 3000 rpm for 15 minutes for serum separation, and then stored at -80 ° C until analysis. When all samples were completed, frozen serums were melted at room temperature and studied on the same day.

While ARES activity was measured by using an ARES assay kit and PON1 activity by a full automatic PON activity measurement kit (Assay Rel Diagnostics, Gaziantep, Turkey).

Paraoxonase activity measurement: The method consists of two different sequential reagents. The first reagent is an appropriate Tris buffer and it also contains calcium ion, which is a cofactor of PON1 enzyme. The second reagent is a new developed stabile substrate solution. The sample is mixed with the Reagent 1 and the substrate solution is added. Linear increase of the absorbance of p-nitrophenol, produced from paraoxon, is followed at kinetic measurement mode. Nonenzymatic hydrolysis of paraoxon was substracted from the total rate of hydrolysis. The molar absorptivity of p-nitrophenol is 18,290 M^{-1} cm^{-1} and one unit of paraoxonase activity is equal to 1 mol of paraoxon hydrolyzed per liter per minute at 37°C

Arylesterase activity measurement: The assay PON1, present in the sample, hydrolyses phenyl acetate to its products which are phenol and acetic acid. The produced phenol is colorimetrically

measured via oxidative coupling with 4-aminoantipyrine and potassium ferricyanide. Nonenzymatic hydrolysis of phenyl acetate was subtracted from the total rate of hydrolysis. The molar absorptivity of colored complex is 4000 M^{-1} cm^{-1} and one unit of arylesterase activity is equal to 1 mmol of phenyl acetate hydrolyzed per liter per minute at 37°C.

Statistical Analysis

Statistical analysis of the study was done using the SPSS (Statistical Package for the Social Sciences for Windows version. 20.0) package program. Descriptive analyzes were used in the distribution and frequency analysis of the data, and chi-square tests were used in frequency data to compare two independent groups. Independent t-test was used to compare the mean of two independent groups. One-way analysis of variance was used to compare the mean of more than two independent groups. Normality analysis was performed on continuous variables. Pearson correlation analysis was used for data that distributed normal, and Spearman correlation analysis was used for data that did not match normal distribution. The significance level was accepted as <0.05 in all statistical analyzes.

RESULTS

91 patients diagnosed with bronchiolitis and 39 age- and sex-matched healthy children were included in the study. Of 91 patients diagnosed with bronchiolitis, 57 (62.6%) were boy and 34 (37.4%) were girl. The mean age of all patients was found to be 12.34±4.84 months (boys: 9.25±3.27; girls: 17.52±6.84). The control group consisted of 39 healthy children.

Twenty-two (24.2%) patients were diagnosed as mild, 44 (48.4%) moderate and 25 (27.5%) severe bronchiolitis. The analyse of hospitalization duration revealed that, 48 (52.7%) patients stayed in the hospital most frequently for less than 24 hours, followed by 33 (25.4%) hospital stays between 24-48 hours. When the number of bronchiolitis attacks were examined, it was seen that 50 (54.9%) patients had their first attack, and only 12 (13.2%) patients had more than 3 attacks. The mean attack score of all patients was 5.38±2.61, that of boys was 5.45±2.62 and girls 5.26±2.63. 84 (92.3%) patients were treated with inhaled bronchodilator, 43 (47.3%) patients with inhaled corticosteroid, 10 (11%) patients with inhaled ipratropium bromide, 13 (14.3%) patients with inhaled magnesium, and 23 patients received oseltamivir. Demographic characteristics of patients and the control group is given in **Table 1**.

Rhinovirus in 14 (15.4%) patients, Respiratory Syncytial Virus (RSV) type A in 25 (27.5%) patients, RSV type B in 24 (26.4%) patients, Human bocavirus in one (1.1%) patient, Adenovirus in five (5.5%) patients, Human metapneumovirus in six (6.6%) patients, Influenza A virus in four (4.4%) patients, Coronovirus 229E in three (3.3%) patients, Enterovirus in one (1.1%) patient, Human paraechovirus was detected in one patient (1.1%) by nasal swab test.

Table 1. Demographic and Clinical Characteristics of the Patients and the Control Group

		Bronchiolitis Group		Control Group		p
		n	%	n	%	
Gender	Male	57	62,6	23	58,9	0,594
	Female	34	37,4	16	41,1	
Disease Severity	Mild	22	24,2			
	Moderate	44	48,4			
	Severe	25	27,4			
Duration of Hospitalization (hour)	<24 hours	48	52,7			
	24-48 hours	33	36,3			
	48-72 hours	5	5,5			
	>72 hours	5	5,5			
Corticosteroid Need	Yes	39	42,9			
	No	52	57,1			
Inhaled Serum Sale Need	Yes	7	7,7			
	No	84	92,3			
Family history of atopy	No	54	59,3			
	Yes	37	40,7			
Exclusive intake of breast milk in the first 6 months of life	Yes	14	15,4			
	No	77	84,6			
Smoke Exposure	Yes	35	38,5			
	No	56	61,5			
Oxygen supplementation method	No oxygen	51	56,0			
	Simple oxygen mask	15	16,5			
	HFNC	6	6,6			
	CPAP	9	9,9			
	Hood	6	6,6			
	BIPAP	4	4,4			
		Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Age (Month)		11,96±11,34	8,64 (2,40-72,00)	12,01±6,72	9,24 (2,0-84,0)	0,788

HFNC: High Frequency Nasal Cannule Oxygen, CPAP: Continuous Positive Airway Pressure, BIPAP: Bilevel Positive Airway Pressure

It was observed that 77 (84.6%) of the patients were exclusively breastfed for the first six months of life. It was determined that 45 (49.5%) of the patients lived in a house heated by a stove, and 56 (61.5%) had contact with cigarette smoke. It was observed that 37 (40.7%) patients had a family history of asthma and/or atopy. Of 22 patients whose serum levels of IgE were measured, 18 (81.8%) were found to be above the normal range for age.

When bronchiolitis severity was compared according to gender, it was found that 12 (54.5%) of 22 patients with mild attack were boy and 10 (45.5%) were girl; 29 (65.9%) of 44 patients with medium attack were boy, 15 (34.1%) were girl; 16 (64%) of 25 patients with severe attack were boy, and 9 (36%) were girl. There was no statistically significant difference between gender and bronchiolitis severity (p> 0.05).

When the drugs used by the patients were compared according to the severity of bronchiolitis attack, 6 (60%) of 10 (11%) patients who received ipratropium bromide treatment were found to be in the severe attack group with statistical significance (p:0.019). Eight (61.5%) of 13 (14.3%) patients who received inhaled magnesium were also in the severe attack group with statistical significance (p:0.011). It was seen that 12 (52.2%) of 23 patients who received oseltamavir treatment were in moderate group, 11 (47.8%) were in severe attack group, and no patient in mild attack group was given oseltamavir treatment (p: 0.002). 5 (38.5%) of 13 patients (14.3%) who were given inhaled magnesium therapy had more than three bronchiolitis attacks, which was statistically significant (p: 0.015).

Nasal swab samples of the patients were compared with the grades of bronchiolitis. The results revealed that 8 (47.1%) of 17 (18.7%) patients with RSVA+RSVB had a statistically significant moderate bronchiolitis attack (p:0.041). 9 (52.9%) of 17 (18.7%) patients with RSVA+RSVB had experienced a mild attack. It was determined that 5 (83.3%) of 6 (6.6%) patients with metapneumovirus were found to be in severe bronchiolitis attack group with statistical significance (p: 0.023).

The mean serum PON1 activity levels of all patients with bronchiolitis were 188.05±101.94 U/L, and the mean of the control group was 302.87±170.52 U/L. The mean of those with mild, moderate and severe bronchiolitis were 199.23±133.84, 190.33±100.88, 174.20±68.80 U/L, respectively. The mean serum ARES activity levels of all patients with bronchiolitis were 408.44±109.95 kU/L, and the mean of the control group was 785.45±168.45 kU/L. The mean of the mild, moderate and severe bronchiolitis groups were 431.93±107.83, 405.65±122.03, 392.69±87.74 kU/L respectively.

The mean of serum PON1 activity levels in all bronchiolitis patients was statistically significantly lower than the mean of the control group (p: 0.001). Similar to PON1, the mean of serum ARES activity levels in all bronchiolitis patients was found statistically significantly lower than the mean of the control group (p: 0.001) (**Table 2**, **Figure 1**).

The mean of serum PON1 and ARES activity levels of the four groups; the control group, mild, moderate and severe bronchiolitis groups, were compared. No statistically significant difference was found among groups according to disease severity. PON1 and ARES activity levels according to patient characteristics are given in **Table 3**.

Table 2. Patient Characteristics According to Gender

	Boy		Girl		Total		p
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Age (months)	9,69±8,23	7,44 (2,4-49,2)	15,75±14,56	11,64 (2,4-72)	11,96±11,34	8,64 (2,4-72)	0,013
Duration of hospitalization (date)	2,20±1,49	1,00 (2-6)	1,77±0,92	2,0 (1-4)	2,07±1,35	2 (1-6)	0,499
Corticosteroid dose number	1,82±1,42	1,0 (1-6)	1,37±0,83	1,0 (1-4)	1,65±1,25	1,0 (1-6)	0,290
WANG Score	5,46±2,63	4 (1-12)	5,26±2,63	5 (1-10)	5,38±2,62	5 (1-12)	0,835
Glucose (mg/dl)	97,61±18,66	91 (71-164)	98,09±16,17	97 (52-137)	97,79±17,68	92 (52-164)	0,325
White blood cell count (/mm3)	10846,67±5033,62	10000 (0-26000)	11525,29±6016,14	10500 (0-33900)	11100,22±5398,66	10200 (0-33900)	0,631
C-reactive protein (mg/dl)	13,11±20,82	4,8 (0,1-96,7)	16,02±21,03	7,5 (0-95,1)	14,23±20,83	5,4 (0-96,7)	0,548
Sedimentation (mm/h)	16,73±13,57	13,5 (4-73)	18,85±14,18	15 (4-59)	17,54±13,75	14 (4-73)	0,533
Immunglobuline E (IU/ml))	40,32±64,54	18,5 (6-302)	54,4±54,61	32,55 (18,51-134)	42,67±62,12	18,51 (6-302)	0,103
Total eosinophil count (/ mm3)	109,35±153,09	36,5 (0,29-564)	173,21±255,77	77 (1-974)	133,4±198,91	41 (0,29-974)	0,397
ARES Activity (kU/L)	420,15±123,09	414,84 (212,32-853,16)	388,82±81,38	381,42 (207,83-552,46)	408,44±109,95	407,85 (207,83-853,16)	0,301
PON1 Activity (U/L)	181,34±97,49	177,29 (34,89-494,69)	199,30±109,55	161,00 (59,34-514,47)	188,05±101,94	166,73 (34,89-514,47)	0,431

Table 3. PON1 and ARES Activity Levels According to Patient Characteristics

		ARES			PON1		
		Mean±SD	Median (Min-max)	p	Mean±SD	Median (Min-max)	p
Study Group	Patients	408,45±109,95	407,86 (207,83 - 853,16)	<0,001	188,06±101,94	166,73 (34,89 - 514,47)	<0,001
	Controls	785,46±168,05	814,2 (394,33 - 1091,67)		302,88±170,53	281,86 (110,36 - 682,08)	
Gender	Boy	420,15±123,09	414,84 (212,32-853,16)	0,301	181,34±97,49	177,29 (34,89-494,69)	0,431
	Girl	388,82±81,38	381,42 (207,83-552,46)		199,30±109,55	161,00 (59,34-514,47)	
Disease Severity	Mild	431,93±107,83[a]	430,32 (243 - 639,07)	<0,001	199,23±133,85[a]	163,83 (34,89 - 514,47)	<0,001
	Moderate	405,66±122,04[a]	403,84 (207,83 - 853,16)		190,34±100,88[a]	167,85 (48,49 - 457,65)	
	Severe	392,7±87,74[a]	384,18 (212,32 - 575,25)		174,21±68,81[a]	182,3 (68,86 - 281,06)	
	Controls	785.45±168.05[b]	814,2 (394,33 - 1091,67)		302,88±170,53[b]	281,86 (110,36 - 682,08)	
Viral Agent	Not detected	438,83±140,61	432,03 (227,88-775,43)	0,292	216,60±132,66	186,21 (62,32-494,69)	0,445
	Detected	397,02±93,70	394,39 (207,83-639,07)		171,88±78,46	164,87 (34,89-303,42)	
Oxygen Need	No	406,06±107,33	401,33 (212,32-853,16)	0,248	189,17±98,93	171,34 (48,49-514,47)	0,347
	Yes	415,93±120,12	422,66 (207,83-636,63)		184,55±113,25	153,86 (34,89-494,69)	
Duration of Hospitalization (hour)	<24	387,71±87,82	393,61 (207,83 - 554,96)	0,503	178,4±89,3	193,35 (34,89 - 349,38)	0,450
	24-48	431,32±119,69	430,16 (243 - 853,16)		191,76±124,98	160,93 (62,32 - 514,47)	
	48-72	435,61±103,67	429,06 (307,56 - 596,86)		234,44±54,16	219,98 (171,34 - 301,63)	
	>72	429,46±212,35	387,45 (212,32 - 775,43)		209,89±87,7	200,05 (115,57 - 344,47)	
Exclusive intake of breast milk in the first 6 months of life	No	433,62±124,5	435,79 (225,17 - 639,07)	0,248	193,66±65,14	191,69 (96,83 - 297,17)	0,422
	Yes	403,87±107,36	401,34 (207,83 - 853,16)		187,04±107,58	164,35 (34,89 - 514,47)	
Ig E	Negative	394,81±107,55	426,58 (239,79 - 486,31)	0,670	188,12±100,84	186,51 (76,3 - 303,16)	0,733
	Positive	461,04±133,01	429,61 (227,88 - 775,43)		220,4±125,11	192,76 (62,32 - 494,69)	

a-b: There is no difference between values with the same letter.

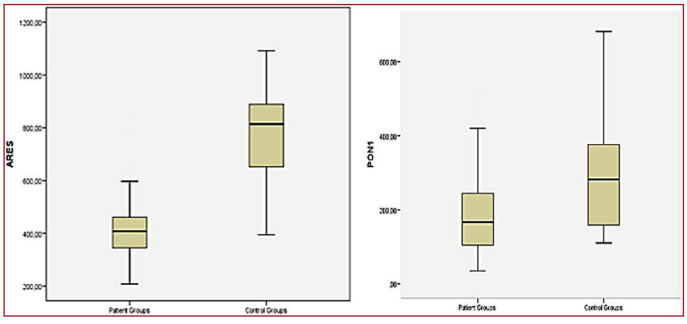

Figure 1. Comparison of serum PON-1 and ARES Activity Levels of Patients and the Control Group

Although there was a negative correlation between age and serum ARES activity levels in correlation analysis, no statistical significance was detected (r: -0.096, p> 0.05). A statistically significantly positive correlation was found between serum PON1 activity levels and age (r: 0,208, p: 0.048). The number of bronchiolitis attacks showed positive correlation with serum PON1 activity levels, but this was not statistically significant (r=0.111, p> 0.05). Despite, there was a negative correlation between the number of bronchiolitis attacks and serum ARES activity levels, no statistically significance (r: -0.157, p> 0.05) was observed.A negative correlation was found between the scores of the patients Wang score and PON1 activity levels, however, this correlation was not statistically significant (r: -0.105, p> 0.05). Similarly, there was a negative correlation between patients' Wang score and serum ARES activity levels, which was also not statistically significant (r:-0.11, p> 0.05). A moderate positive correlation was found between the activity levels of PON1 and ARES, with statistically significance (r:0.297, p<0.001) (**Table 4**).

Table 4. The correlation Analysis of PON1 and ARES Activity Levels

	ARES		PON1	
	p	r	p	r
Age	0,365	-0,096	0,211	0,132
Duration of Hospitalization	0,731	-0,054	0,845	-0,031
Number of Bronchiolitis Attacks	0,138	-0,157	0,293	0,111
Corticosteroid Need	0,408	0,117	0,957	0,008
Attack Score	0,377	0,094	0,322	-0,105
Exclusive Breastfeeding in the first 6 month of life	0,322	-0,106	0,188	0,14
White Blood Cell Count	0,163	-0,147	0,702	0,041
C-Reactive Protein Level	0,509	-0,071	0,891	0,015
Erythrocyte Sedimentation Rate	0,137	-0,178	0,444	0,092
Immunglobuline E Level	0,838	-0,044	0,948	0,014
Total Eosinophil Count	0,632	0,055	0,444	-0,089
ARES			<0,001	0,297
PON1	<0,001	0,297		

DISCUSSION

Oxidative stress contributes to the pathogenesis of various respiratory diseases such as asthma, cystic fibrosis, pulmonary tuberculosis, allergic rhinitis and chronic neonatal lung disease in children.[6-10,13-16] There is a lot of evidence that oxidative stress formation is linked to the pathogenesis of various acute and chronic inflammatory lung diseases. However, little is known about the role of oxidative stress in lung diseases caused by viruses.[17] In this study, PON1 and ARES activities, which are indicators of oxidative status, were investigated in children with acute bronchiolitis. This study showed that PON1 and ARES activities were statistically

significantly lower in patients with acute bronchiolitis than in the control group.

Free oxygen radicals usually play an important role in cellular signaling, regulation of cytokines, growth factors, transcription, immunomodulation and apoptosis. However, when there is an excessive production of free oxygen radicals, damage of DNA, lipids, and proteins can occur. This causes a loss of cellular integrity and functionality.[18] Oxidative stress is referred as an imbalance in the production of free oxygen radicals and insufficient function of detoxfying these radicals. Cells have antioxidant defense systems that they use to protect them from free oxygen radicals.[19] Antioxidants are protective against free oxygen radicals, but sometimes this is not completely sufficient. Reactive oxygen radicals (RS) often form after viral infections. Free oxygen radicals may also have the potential to facilitate viral replication in virus infections. The effect of free oxygen radicals on cellular functions depends on the amount of radicals and how long the cell is exposed to these molecules.[18,20]

Free oxygen radicals and lipid peroxidation products can affect viral replication by modulating the activation state of cells, regulating host inflammatory and immune responses, causing oxidative damage in host tissues and virus components.[21,22] PON1 and ARES have the ability to hydrolyze organophosphate compounds and aromatic carboxylic esters. In addition, it is thought to have an antioxidant effect as it protects high density lipoprotein (HDL) and low density lipoprotein (LDL) from lipid peroxidation.[4]

Studies show that oxidative stress supports lung damage and inflammation after Influenza A infection.[23] It has been shown that damage to the lung tissue is a consequence of the virus-induced cytopathic effect and also due to the cytotoxic effects of excessive inflammation.[24] In our study, it was found that both PON1 and ARES activity levels were statistically significantly lower in patients with bronchiolitis group compared to the control group. Although the results were lowest in patients with severe bronchiolitis, no stattistically significant difference was found among the severity of bronchiolitis. The lower levels of the enzyme activities in patients with acute bronchiolitis supports the view that the damage to lung tissue is caused by the cytotoxic effects of excessive inflammation.

In the study of Hennet et al.[25] endogenous concentrations of antioxidants such as glutathione, vitamin C and E in the lungs, liver and blood plasma of mice infected with Influenza A / PR8 / 34 virus were found to be lower than the control group. Lin et al.[26] reported that the antioxidant properties of superoxide dismutase 1 (SOD1) may decrease apoptosis, pro-inflammatory response and mitochondrial dysfunction, and this may play a positive role in controlling H5N1 infection. As mentioned in these aforementioned studies, infections due to Influenza virus can induce oxidative stress and contribute to viral pathogenesis. It is known that use of antioxidants can have a protective effect against viral infections. In the literature, there are no studies investigating PON1 and ARES activity levels in patients with acute bronchiolitis. Therefore, we think that our study will contribute to the literature.

Oxidative stress also plays an important role in the pathogenesis of pulmonary inflammation caused by RSV. Mochizuki et al.[27] evaluated changes in intracellular glutathione reduction status in healthy airway epithelial cells and RSV-infected bronchial epithelial cells, and showed that RSV-induced oxidative stress may cause airway inflammation. In their study, Moreno-Solis et al.[28] reported that oxidized glutathione levels showed disease severity in patients with RSV-induced bronchiolitis, and this infection increased oxidative stress. Hosakote et al.[17] reported that RSV infection causes down-regulation in the airway antioxidant system and this causes in vivo oxidative damage, in children with bronchiolitis. According to the results of our study, PON1 activity levels were found to be lower than the control group and this has been shown to contribute to oxidative stress. In our study, in the nasal swab samples, 8 (47.1%) of 17 patients with RSV type A + RSV type B were found to be moderate bronchiolitis and 9 (52.9%) were statistically significant. Although the PON1 and ARES activity levels of these patients were lower than the controls, they were not statistically significant. The reason for this may be due to the fact that none of these patients had severe bronchiolitis.

In the study of Ozkaya et al.[6] PON1 activity levels were significantly lower in patients with allergic rhinitis compared to the control group, and it was reported that it may be a predictor in showing the severity of the disease. Emin et al.[7] found that PON1 levels were lower than the control group in their study in children with asthma and reported that PON1 measurement could be used as a systemic marker in uncontrolled asthma in children. Rumora et al.[10] showed that PON1 and ARES activity levels in patients with chronic obstructive pulmonary disease had decreased independently from HDL concentrations compared to the control group. For this reason, it has been reported that PON1 and ARES activity levels can be used in the diagnosis of disease. According to the results of our study, as the severity of bronchiolitis worsened, PON1 and ARES activity levels decreased. Although larger studies are needed to for an exact suggestion, our results may suggest that PON1 and ARES activity levels may be used as predictors in determining the severity of the disease in patients with acute bronchiolitis.

Our study had some limitations. The number of patients and controls is rather small which affects the power of the study. While PON1 enzyme functions as an antioxidant by inhibiting the oxidation of LDL, absence of serum lipid levels makes another limitation of the study.

CONCLUSION

We determined that PON1 and ARES activity levels were lower in patients with acute bronchiolitis. The results of our study show that PON 1 and ARES activities may play a role

in pathogenesis of acute bronchiolitis which indicates the association of increased oxidative stress and development of bronchiolitis, in pediatric patients. However, prospective studies with larger case series are needed to better understand how the host response to viral infection occurs and the effects of oxidative stress on the cell.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was conducted with the approval of Necmettin Erbakan University Clinical Research Ethics Committee (Date: 24.02.2017, Number: 2017-825).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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