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Original Article

Serum Myeloperoxidase, Paraoxonase and Arylesterase Enzymes Levels in Children with Bronchiectasis

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Abstract ***Background:*** The aim of this study was to measure the serum levels of myeloperoxidase (MPO), paraoxonase (PON1) and arylesterase (ARE) in paediatric patients with non-cystic fibrosis bronchiectasis (non-CF BE), and to compare these results with healthy controls. ***Methods:*** A total of 101 paediatric patients with non-CF BE and 70 age- and sex-matched healthy controls were enrolled into the study between November 2010 and November 2015. The levels of MPO, PON1 and ARE were measured in all participants. ***Findings:*** Compared to controls, MPO levels were significantly lower (p=0.001), while PON1 and ARE levels were significantly higher in the patient group (p=0.001). ***Conclusion:*** Our study reports that the oxidant and anti-oxidant enzyme levels in non-CF BE differ from healthy controls; thus, suggesting a role in non-CF BE pathophysiology.

Key words *Arylesterase; Bronchiectasis; Myeloperoxidase; Oxidant; Paraoxonase*

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Introduction

Bronchiectasis (BE), is a disorder that causes dilatation in the bronchi and is characterised by abnormal intense sputum and coughing. Although BE is, for the most part, seen in patients with cystic fibrosis (CF), other underlying diseases, such as primary ciliary dyskinesia (PCD), recurrent and persistent lower respiratory tract infections etc. may cause chronic infection and inflammation and can result in BE (non-CF BE).[1]

The various enzymes produced by neutrophils and the secretion of cytokines are fundamental to the inflammatory process. Myeloperoxidase (MPO) is among the most important of neutrophilic enzymes, as it plays a crucial role in the 'oxidative burst' of neutrophils – the primary mechanism of neutrophil antibacterial defence. This mechanism results in a series of enzymatic reactions leading to the production of reactive oxygen species. These oxidant molecules are normally neutralised by the natural antioxidant defence system, enabling the levels of oxidants and antioxidants to be kept in a tight balance which protects tissues from exposure to excessive oxidant activity – while also enabling inflammation to take its physiological course of action. However, chronic exposure

to oxidative insult may cause significant metabolic, structural and functional damage in the interstitium and lung tissues, such as that observed in BE. Therefore, the levels of the oxidants and the antioxidants would be expected to have a role in the development and progression of BE.[2-4]

Paraoxonase 1 (PON1) is a multifunctional enzyme produced by the liver and is carried by the high density lipoprotein (HDL) particle in the circulation. In addition to its antioxidant and anti-inflammatory properties, the enzyme is known to have three primary functions: paraoxonase (PON), arylesterase (ARE) and lactonase. The antioxidant function of PON1 and its close association with the HDL particle makes it a crucial part of the defence against oxidative damage in macrophages and the low density lipoprotein and HDL particles. The level and activity of PON1 are known to respond to changes in the balance of oxidants and antioxidants in various pathologies involving inflammation.[5-8]

Enzymes such as MPO (as a measure of oxidant production), PON and ARE activities (as measures of antioxidant activity) have been shown to be altered in asthma, chronic obstructive pulmonary disease (COPD) and tuberculosis. The levels of these enzymes were studied in the serum in some studies, whereas they were studied in sputum and BAL in others. However, there are few studies which have evaluated the levels of these enzymes in patients with BE.

In our study, we aimed to determine MPO, PON and ARE serum levels in children with non-CF BE and to compare the results with healthy controls. Additionally, we aimed to determine factors which were associated with the levels of these enzymes (such as respiratory function test results, age, sex) in the patient group.

Subjects and Methods

Paediatric patients who were diagnosed with non-CF BE by HRCT imaging were included in the study. Patients with acute exacerbations during the last month, patients with malnutrition and history of using immunosuppressive medications were excluded from the study. A total of 101 patients with non-CF BE were enrolled as the patient group. Ninety-three percent (n=94) of the patients had cough, 92.1% (n=93) had increased sputum, 33.7 (n=34) had clubbing and 5.9% (n=6) had haemoptysis. The final diagnoses were PCD in 19.8% (n=20), idiopathic in 48.5% (n=49) and 'other diagnoses' (post-infectious BE, bronchiolitis obliterans, immune deficiency, etc.) in 31.7% (n=32) (Table 1).

In patients who were older than five years of age (n=131), forced vital capacity (FVC), forced expiration volume in the first second (FEV1), peak expiratory flow rate (PEF) and medium expiratory flow rate (MEF25-75) parameters were measured using a spirometer (Spirolab III, Medical International Research, Italy). The values were recorded as the percentage of expected values with regard to age and height. FEV1 values higher than 80% were considered to show mild disease, 60% to 80% were identified as moderate, while values lower than 60% were accepted to show severe disease.[9]

Control Group

The control group was selected from children who applied to the Child Health Monitoring Outpatient Clinic for routine paediatric check-up. A total of 70 age- and sex-matched paediatric patients without malnutrition, chronic/systemic diseases, anaemia and history of immunosuppressive drug use were enrolled into the study as the control group.

Enzyme Measurements

Venous blood samples were obtained from the patient and the control groups after 12 hours of fasting. The blood was centrifuged at 1000 g for 10 minutes to obtain serum.

Table 1 Distribution of the findings and BE final diagnosis of the patient group

	n	%
Cough	94	93.1
Sputum	93	92.1
Haemoptysis	6	5.9
Clubbing	34	33.7
Final diagnosis		
PCD *	20	19.8
Idiopathic	49	48.5
Other	32	31.7

*All patients had situs inversus totalis. PICADAR score was over 10.

These samples were frozen and stored at -80^{0}C until measurements were performed. MPO, PON, and ARE levels were determined by the photometric method via an Abbott Architect C 16000 (Japan) device. In measuring PON activity in serum, paraoxon was used as the substrate. The p-nitrophenol resulting from the reaction gives absorbance at 405 nm in the visible region. Enzyme activity was identified by measuring the absorption of this product.

Phenyl acetate was used as a substrate to measure the ARE activity of the PON1 enzyme. The principle of the experiment is based on measuring the absorbance of the phenol produced by disintegration with the PON1 enzyme to phenol and acetic acid, at 545 nm.[10]

MPO activity was quantified by the modified *o*-dianisidine method.[11] The mixture for measurement was composed of 0.1 M phosphate buffer (pH 6.0), 0.01 M hydrogen peroxide and 0.02 M *o*-dianisidine. Sample serum was added and the absorbance change was measured at 460 nm.[12]

Statistical Analysis

The NCSS 2007 software (NCSS Statistical Software, Kaysville, UT, USA) was used for all statistical analyses. Data obtained by descriptive statistical methods (given as: mean, standard deviation, median, frequency, ratio, minimum, maximum) and showed normal Gaussian distribution were evaluated using the Student's t test in two-group comparisons. Data with a non-normal distribution were evaluated using the Mann Whitney U test for two-group comparisons. In >2 group comparisons, the One Way ANOVA and the Kruskal Wallis tests were used for comparison, with regard to normality of distribution. The Pearson's Chi-square test and Yates' continuity correction tests (Yates corrected chi-square) were used to compare qualitative data between groups. The correlations between parameters were evaluated using the Spearman's or Pearson's correlation analyses according to normality of distribution. P values <0.05 were considered to indicate statistical significance.

Results

Our study was conducted with a total of 171 subjects (101 in the patient group and 70 in the control group) aged between 1 and 16 years. Demographic characteristics of the groups are given in Table 2. Groups were similar in regard to age and sex (p>0.05 for both). MPO levels of the patient group were found to be significantly lower than controls (p=0.001). The serum levels of PON1 and ARE were found to be significantly higher in the patient group compared to the control group (p=0.001).

We also evaluated the results of the patient group with regard to age, sex, respiratory function test results, final

Table 2 Characteristics of the groups and the evaluation of the variables

	Group		
	Patient (n=101)	**Control (n=70)**	**p**
Sex			
Girl, n (%)	56 (55.4)	35 (50.0)	0.483[a]
Boy, n (%)	45 (44.6)	35 (50.0)	
Age group (months)			
0-24, n (%)	5 (5.0)	0 (0.0)	
25-72, n (%)	8 (7.9)	27 (38.6)	
73 and over, n (%)	88 (87.1)	43 (61.4)	
Age (months) Mean ± SD (median)	119.41±45.32 (120)	107.11±43.35 (108)	0.078[c]
MPO Mean ± SD (median)	104.26±148.97 (58.74)	317.19±485.30 (155)	0.001[b]
ARES Mean ± SD	534.25±73.41	25.19±11.48	0.001[c]
PON1 Mean ± SD (median)	113.86±77.41 (112.11)	56.89±36.77 (48)	0.001[b]

[a]Pearson Chi-Square Test; [b]Mann-Whitney Test; [c]Student-t Test; p<0.05

diagnosis and smoking exposure. While there was a statistically significant difference between MPO levels (p=0.009) in terms of age, PON and ARE levels did not show such differences. The MPO values in children aged between 0-24 months were found to be significantly higher compared to other age groups (Table 3). Regarding exposure to smoking, the serum levels of MPO, PON and ARE did not show a statistically significant difference (Table 4). The MPO and PON levels of subgroups according to patients' final diagnoses were similar (p>0.05); however, ARE levels showed significant difference according to the patients' final diagnoses (p=0.047). Analysis with the post-hoc Bonferroni correction revealed that the ARE levels of subjects diagnosed with PCD were significantly lower compared to those diagnosed with idiopathic or 'other' diseases (p=0.014 and p=0.038, respectively). When results were evaluated according to respiratory function tests, the levels of MPO, PON and ARE were found not to be associated with any functional parameters.

Discussion

In our study, MPO levels in the patient group were found to be significantly lower compared to control group, while PON and ARE levels were found to be significantly higher in the patient group.

To our knowledge, this is the first study to evaluate the

Table 3 Evaluation of the Variables among the Ages of the Patient Group

	Age Groups (months)			
Patient (n=101)	**0-24**	**25-72**	**73 and over**	**p**
Sex				
Girl, n (%)	1 (1.8)	6 (10.7)	49 (87.5)	0.207[h]
Boy, n (%)	4 (8.9)	2 (4.4)	39 (86.7)	
MPO				
Mean ± SD (median)	354.77±382.88 (163.00)	125.21±118.78 (77.52)	88.12±117.14 (55.49)	0.009[f]**
ARES				
Mean ± SD (median)	515.21±60.08 (544.06)	539.72±61.33 (553.89)	534.83±75.51 (543.01)	0.777[f]
PON				
Mean ± SD (median)	100.92±75.01 (55.41)	86.84±47.75 (81.36)	117.06±79.74 (120.23)	0.689[f]

[h]Fisher Freeman Halton Test (Monte Carlo)

[f]Kruskal Wallis Test; **p<0.01

Table 4 Evaluations according to the smoking status in the patient group

	ARES		**MPO**		**PON**	
	Mean ± SD		**Mean ± SD (Median)**		**Mean ± SD (Median)**	
Smoking by the patient						
No (n=74)	529.30±76.59		92.02±112.02 (61.88)		115.86±78.98 (116.14)	
Yes (n=26)	546.69±64.21		136.95±224.60 (53.08)		110.50±74.75 (113.0)	
p	0.303[c]		0.844[b]		0.869[b]	
	r	**p**	**r**	**p**	**r**	**p**
Monthly income	-0.017	**0.871**	0.028	**0.786**	-0.206	**0.041***

[b]Mann Whitney U Test; [c]Student-t Test

r=Spearman Correlation Factor; *p<0.05

levels of MPO, PON and ARE in the serum of paediatric patients with non-CF BE. However, there are a few studies which have evaluated these enzymes in other lung diseases. In the literature, studies focusing on the association between PON1 levels and respiratory diseases in the paediatric population are rather scarce. In a study performed with a group of paediatric patients, the serum PON1 levels of patients with pulmonary tuberculosis were found to be lower compared to healthy controls. The authors concluded that, in this patient group, low PON1 level might emerge as a result of the down-regulation of oxidative stress.[13] In another study performed in a paediatric population diagnosed with asthma, serum PON1 levels were found to be decreased.[14] In adults with COPD, Rumora et al reported significantly lower PON1 and ARE levels compared to controls. Rumora et al also explained this decrease with the down-regulation of oxidative stress.[15] Another study, carried out by Acay and colleagues, found that PON1 and ARE levels were notably low compared to controls in adults with COPD and asthma.[16]

In the present study, PON1 and ARE levels were found to be significantly higher in paediatric patients with non-CF BE. Normally, this enzyme is produced by the liver, released into the circulation, and functions in the defence against free radicals by acting at the tissue level or systemically in chronic inflammation. As BE is pathophysiologically different from COPD, asthma and tuberculosis infection in terms of mucus production and inflammatory activity, the increase in the blood levels of these enzymes among patients diagnosed with BE may be explained by the increase in the production of these enzymes secondary to the continuous inflammation observed in BE. Furthermore, despite the fact that these enzymes are produced at a high rate with an aim to counteract oxidative stress, it is possible to speculate that there could be other impairments of the antioxidant mechanism in patients with BE. Since our study is the first of its nature in patients with non-CF BE, further studies are required to elucidate the mechanism of changes in these enzymes. Future studies would benefit from measuring the levels of these enzymes in bronchoalveolar lavage samples or lung tissues in order to obtain a more specific understanding of the source of change.

Many studies investigating the association between smoking and PON1 demonstrated that smoking reduced both the level and the activity of PON1 in the serum.[17-19] It was reported that PON1 activity was significantly lower in smokers compared to non-smokers and showed a negative correlation with the number of cigarettes smoked per day.[18] In our study, no association was found between exposure to cigarette smoking and PON1 and ARE enzyme levels in the group of patients diagnosed with non-CF BE.

As mentioned before, MPO plays a critical role in the defence against microorganisms, exemplified by the development of recurrent infections MPO-deficient inidivduals. It is thought that prolonged airway inflammation is due to the deficient MPO hydrogen peroxide-CI system activity in these patients. Reportedly, patients who were detected to have total and subtotal MPO enzyme deficiency suffered from increased frequency of cardiovascular damage and serious infections.[20] It was demonstrated in an animal study that the risk of pneumonia and death increased in animals with genetically reduced MPO enzyme activity, following intra-tracheal *Candida albicans* infection.[21] In our study, MPO levels were found to be lower in those with non-CF BE compared to the control group. With this data, it may be speculated that reduced MPO activity may be associated with reduced neutrophil activity, leading to continuation of chronic infection and ultimately resulting in BE development.

In our study, MPO enzyme levels were found to be significantly lower in the non-CF BE group; therefore, suggesting a link between decreased bactericidal activity and BE. However, further studies are necessary to draw clear conclusions, as there are no other studies that have assessed MPO levels in paediatric patients with non-CF BE.

Conclusion

In conclusion, although there is a lack of sufficient research on the levels of MPO, PON and ARE in paediatric patients with non-CF BE, our study shows that the activities of these enzymes are altered in BE. Further studies, preferably evaluating tissue and bronchoalveolar lavage samples, are required to increase our understanding of the roles of these enzymes in the pathophysiology of BE.

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Disclosure Statement

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