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Original Article

Superoxide dismutase levels and peak expiratory flow in asthmatic children

Arie Kurniasih, Madarina Julia, Amalia Setyati

Abstract

Background Asthma is a chronic inflammatory process which involve variety of cells such as inflammatory mediators, reactive oxygen species (ROS), and cytokines. The inflammatory process would be exacerbated in the presence of oxidative stress. Superoxide dismutase (SOD) is the first important enzyme to protect the respiratory tract against oxidative stress. The decreased of SOD has a correlation with increased of airway obstruction and bronchospasm.

Objective To assess for a correlation between superoxide dismutase (SOD) levels and peak expiratory flow, as well as to determine the impact of SOD levels for predicting asthma attacks.

Methods We conducted a prospective cohort study at Dr. Sardjito Hospital, Yogyakarta, between February and April 2011 involving asthmatic children aged 5-18 years. Subjects' serum SOD levels and peak expiratory flow were measured at the same time point. We then performed a prospective study following up on the same subjects to find out if they had a recurrent asthma attack within one month of the tests. We also reassessed their peak expiratory flow one month after blood specimens were obtained.

Results Thirty-nine patients were enrolled in this study. There was no significant correlation between SOD level and peak expiratory flow [r=0.289; 95%CI -0.025 to 0.47; P=0.074]. However, older age was significantly associated with higher peak expiratory flow (β=0.5; 95%CI 3.10 to 11.57; P=0.01). Lower levels of SOD increased the risk of asthma attacks in a month following the initial measurements (RR=5.5; 95%CI 1.6 to 18.9; P=0.009).

Conclusion Superoxide dismutase (SOD) level is not significantly associated with peak expiratory flow. However, we find a relationship between older age and higher peak expiratory flow and a relationship between lower SOD levels and risk of asthma attacks within one month following the tests. **[Paediatr Indones. 2015;55:309-14.].**

Keywords: *asthma, superoxide dismutase, peak expiratory flow*

According to the *Global Initiative for Asthma* (GINA), asthma is defined as a chronic inflammatory airway disorder involving many cell types. In particular, mast cells, eosinophils, and T lymphocytes are associated with chronic inflammation that causes hyperresponsive episodes, recurrent wheezing, shortness of breath, chest tightness, and coughing, especially from night to early morning. The symptoms associated with airway constriction and lung obstruction are reversible.[1] The prevalence of asthma in children aged 0 to 17 years was 57 per 1,000 children.[2]

Chronic inflammation in asthma involves a complex interaction of cells and mediators, resulting in oxidative stress, such as increased levels of reactive oxygen species (ROS) and nitrogen species (NS).[3,4] The ROS causes the release of arachidonic acid which constricts airway smooth muscles, increases airway reactivity and gland secretion, as well as the synthesis and release of chemoattractant substances, tachykinins and neurokinins, lowers cholinesterase and production of endopeptide, as well as decreases

From the Department of Child Health, Gadjah Mada University Medical School/Dr. Sardjito Hospital, Yogyakarta.

Reprint requests to: Arie Kurniasih, MD, Department of Child Health, Gadjah Mada University Medical School/Dr. Sardjito Hospital, Jalan Kesehatan No.1, Yogyakarta 55284, Indonesia. Tel. +62-274-561616. Fax.: +62-274-583745. E-mail: ariekurniasih14@gmail.com.

the β-adrenergic receptor response. These pathological conditions cause bronchial hyperesponsiveness.[5,6] The SOD is the first-line enzyme to protect the respiratory tract against oxidative stress. It catalyzes the breakdown of free radicals into oxygen and hydrogen peroxide (H_2O_2). During asthma attacks, SOD activity decreases, along with an increase in the number of free radicals by the number of inflamed cells.[7] The decrease in SOD also illustrates the ongoing oxidative stress and causes a further increase of O_2 to form H_2O_2, or O_2 react with nitric oxide to form peroxynitrite and nitration of proteins, which can cause direct injury to the respiratory tract epithelium.[8,9]

Until recently, there has been a lack of knowledge regarding the relationship between SOD and peak expiratory flow in children with asthma. Most studies have been performed in adult populations.[5,10] Determining this relationship may help clinicians to prepare future interventions for asthmatic children. The objective of this study was to assess for a correlation between SOD levels and peak expiratory flow, and to determine the impact of SOD levels for predicting asthma attacks.

Methods

The study was conducted at the Children's Respirology Clinic, Dr. Sardjito General Hospital, Yogyakarta, from February until April 2011. Subjects were selected by consecutive random sampling. Inclusion criteria were patients with a diagnosis of asthma by clinical symptoms, aged 5 to 18 years, had complete medical records, and had parents who provided informed consent. We excluded patients who experienced an asthma attack at the time the measurements were taken, as characterized by the presence of wheezing and shortness of breath, as well as those who had other respiratory diseases such as tuberculosis or acute respiratory infection,[4] or who took antioxidant vitamins on a regular basis.[10]

Venous blood specimens were taken to measure SOD levels, at the same time point that peak expiratory flow was measured. Patients' data were recorded, including height, weight, and body mass index. Measurement of SOD level was performed with the *Cayman Chemical SOD kit*, an ELISA interpretation method, in the Biochemistry Laboratory, Gadjah Mada University Medical School, Yogyakarta. We defined SOD levels as low and high with cut off point 14,58 IU/mL.[13] The peak expiratory flow was measured by a Vitalograph electric peak flow meter. Pulmonary function testing with a peak flow meter was performed with the patient in a standing position, with lips tightly surrounding the mouthpiece. The patient took a deep breath, held it a few seconds, then exhaled firmly and quickly. The test was performed 3 times and the highest value was taken. We defined frequent episodic asthma when the asthma attack more than 1 time per month whereas rare episodic asthma if asthma attack less than 1 time per month. Steroid was defined as using inhalation steroid. Cigarette smoker was defined as smoker among in the family.

The relationship between SOD levels and peak expiration flow was analyzed by Pearson's correlation. Univariate and multivariate regression analyses were done to determine variables affecting peak expiratory flow. A prospective study was also performed, with children then were followed one month after the blood specimens were obtained, the occurrence of asthma attack was recorded. Independent T-test was used to evaluate for a relationship between SOD levels and the occurrence of asthma attack within the one month after SOD levels were measured. The protocol of the study was approved by the Research Ethics Committee of the Gadjah Mada University Medical School.

Results

The study design outline is described in **Figure 1**. **Table 1** shows the characteristics of study subjects. There was no correlation between levels of superoxide dismutase and peak expiratory flow as shown in **Figure 2** with r=0.289; 95%CI -0.025 to 0.47 (P=0.074). **Table 2** shows that older age was significantly associated with higher peak expiratory flow (β=0.5; 95%CI 3.10 to 11.57; P=0.01).

Children who experienced asthma attacks during the one month after the initial measurements had significantly lower SOD levels than children who did not experience an attack. Lower SOD level was a risk factor for asthma attack [RR=5.5; (95%CI 1.6 to 18.9); P = 0.009] (**Table 3**).

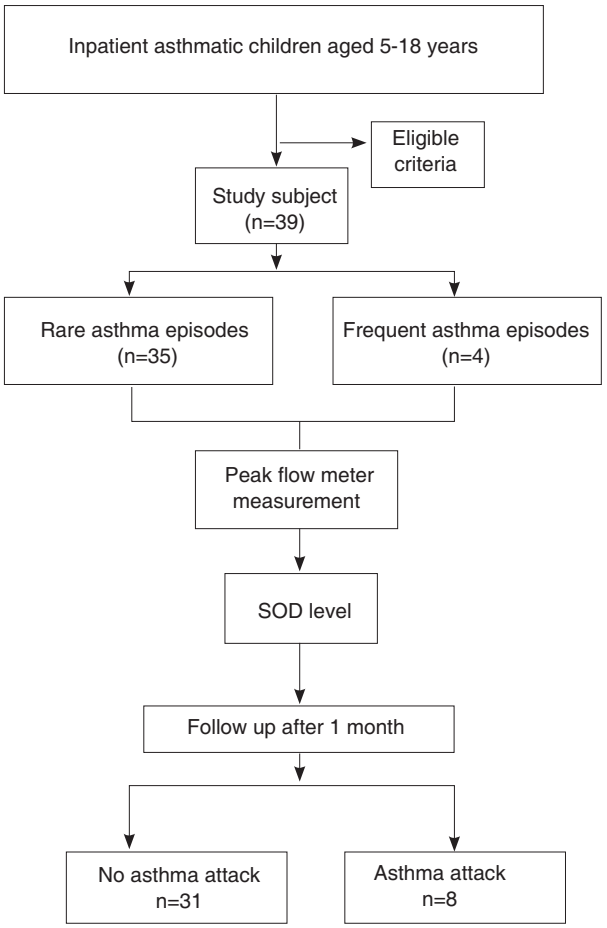

Figure 1. Flow chart of study participants

Table 1. Characteristics of subjects

Characteristics	N=39
Mean age (SD), years	9 (2.63)
Gender, n	
Male	26
Female	13
Frequency of asthma attacks, n	
Rare	34
Frequent	5
Severity of asthma attack, n	
Mild	34
Moderate	5
Mean body mass index/age (SD), Z-score	0.54 (1.59)
Mean height/age (SD), Z-score	-0.35 (1.66)
Mean SOD level (SD), U/mL	16.39 (3.14)
Mean peak expiratory flow (SD), L/min	166 (38.9)
Steroid therapy, n	
No	35
Yes	4
Cigarette smoke exposure, n	
No	22
Yes	17

Discussion

We found no significant relationship between SOD level and peak expiratory flow in inpatient asthmatic children. This result was consistent with that of a previous study which indicated no association

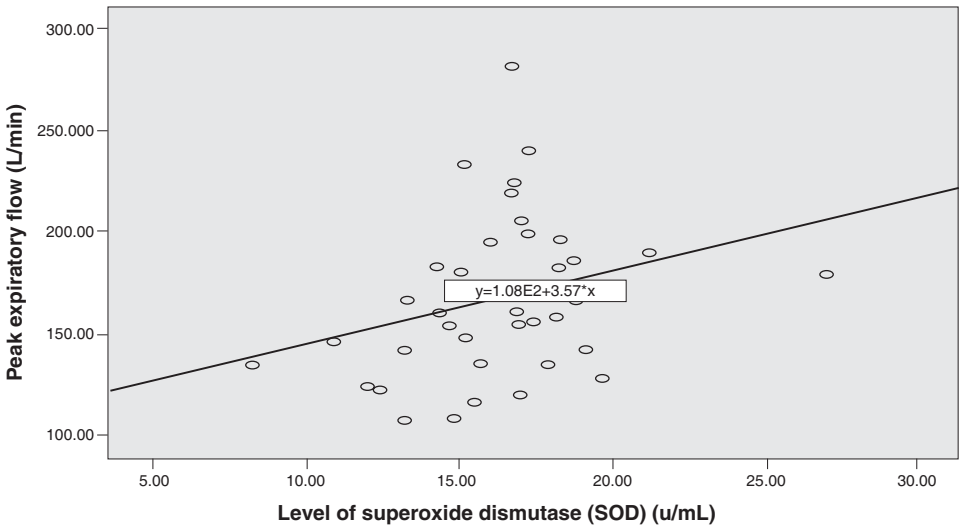

Figure 2. Correlation between SOD levels with peak expiratory flow

Table 2. Linear regression analysis of peak expiratory flow as the dependent variable

Variables	Univariate linear regression ß (95%CI)	P value	Multivariate linear regression β (95%CI)	P value
SOD level	0.29 (-0.37 to7.5)	0.74		
Gender	0.47 (-36.1 to 17.7)	0.47		
Age, years	0.51 (3.24 to 11.71)	0.001	0.5 (3.10 to 11.57)	0.01
Height for age, Z-score	-1.49 (-11.17 to 4.2)	0.36		
BMI for age, Z-score	0.28 (-7.46 to 8.88)	0.87		
Frequency of asthma attacks (1= rare, 2 = frequent)	3.4 (-2.5 to 58.04)	0.07	-0.11(-46.47 to 21.63)	0.46
Steroid therapy (1=no, 2=yes)	0.19 (-34.47 to 22.21)	0.66		
Cigarette smoke (1= no, 2 = yes)	2.5 (-44.74 to 5.09)	0.12	-0.25(-42.28 to 2.74)	0.08

Note: variables with P value <0.25 in univariate analysis were further tested in multivariate linear regression.

Table 3. Relationship of SOD level and asthma attacks in the month after the initial measurements

SOD levels \ Asthma attack	Yes	No	Total	RR (95%CI)	P value
Low SOD level, n	5	4	9	5.5 (1.6 to 18.9)	0.009
High SOD level, n	3	27	30		

between an increase in SOD levels and an increase in forced expiratory volume 1 (FEV_1), although that study showed higher SOD levels in asthmatic patients compared to normal patients.[11] Increased SOD levels, however, may have a negative relationship with the degree of airway obstruction, where higher levels of SOD can be found in asthma patients with more severe degrees of obstruction.[12] Comhair et al. reported a consistent relationship between greater oxidative stress and inactivation of SOD, which aggravates inflammation, progressing to airway obstruction.[13] Lack of airway obstruction, as measured by peak expiratory flow, positively correlated to SOD level, with higher SOD level resulting in greater peak expiratory flow. Katsoulis et al. concluded that lower serum antioxidant level in asthmatic patients had a positive correlation to decreased FEV_1, measured at the days of admission and discharge.[14] Ochs-Balcolm et al. who studied asthmatic and COPD patients, reported that imbalanced antioxidant and oxidant states lead to chronic airway obstruction, and may be affected by lifestyle and diet.[15] In 2001, Uzuner et al. studied 40 asthmatic children in Turkey, and found that increased oxidative stress and decreased antioxidants lead to inflammation and hyperreactivity of airways.[16]

We found that age was associated with peak expiratory flow. This finding is consistent with a study by Hellberg in 2008 which showed that peak expiratory flow is influenced by age of onset of asthma and asthma duration.[17] The results of other similar studies concluded that peak expiratory flow was influenced by age and weight in girls and by height in boys.[18,19] In 2006, Mohammadzadeh et al. examined 1,056 children in Iran and documented that peak expiratory flow had a strong correlation with height, compared to the weight and age.[20] In addition, Manjunath et al. reported that peak expiratory flow was influenced by the height of boys and girls.[21]

In this study, we also conducted a prospective observation one month after the SOD examination. Lower levels of SOD increased the risk of asthma attack more often than did higher levels of SOD. Therefore, high levels of SOD may contribute to a protective effect from asthma attacks. Consistent with this finding, research by Psarras et al. mentioned that the increase in oxidative stress in asthma indicates the role of an oxidant and antioxidant imbalance that could aggravate asthma disease pathogenesis and increase the frequency of attacks.[22] Furthermore, Sackesen et al. also stated that asthmatic children

tended to have decreased total body antioxidants, both enzymatic and non-enzymatic, including superoxide dismutase and glutathione peroxidase.[23] Fenech et al. reported that in moderate and severe asthma attacks, patients' plasma SOD levels are lower than in normal people, and routine use of steroids in patients with asthma affects SOD levels.[24] Fitzpatrick et al. observed that children who had severe asthma attacks tended to have high levels of oxidative stress in the epithelial lining fluid (ELF). In turn, this would increase the conversion of glutathione (GSH) and glutathione disulfide (GSSG) to glutathione redox. Hence, the ELF and GSH levels were lower in children with asthma. Glutathione is the body's first line of enzymatic defense against inflammatory processes in the ELF. The decrease in GSH may increase the risk for an asthma attack.[25]

In conclusion, there is no significant relationship between SOD levels and peak expiratory flow. However, there are significant correlations between older age and higher peak expiratory flow, and between lower SOD level and the risk of asthma attacks in the one month following testing.

Conflict of interest

None declared.

References

1. Global Initiative for Asthma (GINA). 2011. Global strategy for asthma management and prevention, National Institutes of Health. [cited October 2012]. Available from: www.ginaasthma.com/download.asp?=214.
2. Kartasamita CB. Asma. In: Rahajoe N, Supriyatno B, Setyanto DB, editors. Buku ajar respirologi anak. 2nd ed. Jakarta: Ikatan Dokter Anak Indonesia; 2008. p. 162-213.
3. UKK Pulmonologi PP IDAI. Pedoman nasional asma anak. Proceeding of the Indonesian Pediatric Respiratory Meeting I: focus on asthma; 2003 Aug 8-10; Jakarta, Indonesia.
4. Nadeem, A, Raj HG, Chhabra SK. Increased oxidative stress in acute exacerbations of asthma. J Asthma. 2005;42:45-50.
5. Dweik RA, Comhair SA, Gaston B, Thunnissen FB, Farver C, Thomassen MJ, *et al.* NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. Proc Natl Acad Sci. 2001;98:2622-7.
6. Rahman I, MacNee W. Reactive oxygen species from asthma and COPD: basic mechanism and clinical management. London : Elsevier Academic Press; 2002. p. 243-54.
7. Kinnula VL, Crapo JD. Superoxide dismutase in the lung and human lung diseases. Am J Respir Crit Care Med. 2003;167:1600-19.
8. Granot E, Kohen R. Oxidative stress in childhood--in health and disease states. Clin Nutr. 2004;23:3-11.
9. Christofidou-Solomidou M, Muzykantov VR. Antioxidant strategies in respiratory medicine. Treat Respir Med. 2006;5:47-78.
10. Misso NL, Brooks-Wildhaber J, Ray S, Vally H, Thompson PJ. Plasma concentrations of dietary and nondietary antioxidants are low in severe asthma. Eur Respir J. 2005;26:257-64.
11. Hanta I, Kuleci S, Canacankatan N, Kocabas A. The oxidant-antioxidant balance in mild asthmatic patients. Lung. 2003;181:347-52.
12. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. Eur Respir J. 2003;21:177–86.
13. Comhair SA, Ricci KS, Arroliga M, Lara AR, Dweik RA, Song W, *et al.* Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. Am J Respir Crit Care Med. 2005;172:306-13.
14. Katsoulis K, Kontakiotis T, Leonardopoulous I, Kotsovili A, Legakis N, Patakas D. Serum total antioxidant status in severe exacerbation of asthma: correlation with the severity of the disease. J Asthma. 2003;40:847–54.
15. Ochs-Balcom HM, Grant BJ, Muti P, Sempos CT, Freudenheim JL, Browne RW, *et al.* Antioxidants, oxidative stress, and pulmonary function in individuals diagnosed with asthma or COPD. Eur J Clin Nutr. 2006;60:991-9.
16. Uzuner N, Karaman Z, Coker C, Turgut S, Uzuner H, Onvural B. Serum trace element levels in bronchial asthma. Turkish Resp J. 2001;2:10-5.
17. Hellberg J. Factors associated with lung function impairment in children and adults with obstructive lung disease. [Thesis]. [Stockholm, Sweden]: Karolinska Institutet; 2008.
18. Vijayan VK, Reetha AM, Kuppurao KV, Venkatesan P, Thilakavathy S. Pulmonary function in normal south Indian children aged 7 to 19 years. Indian J Chest Dis Allied Sci. 2000;42:147-56.
19. Zhang QL, Zheng JP, Yuan BT, He H, Wang J, An JY, *et al.* Feasibility and predicted equations of spirometry in Shenzhen preschool children. Zhonghua Er Ke Za Zhi. 2005;43:843-8.
20. Mohammadzadeh I, Gharagozlou M, Fatemil SA. Normal

values of peak expiratory flow rate in children from the town of Babol, Iran. Iran J Allergy Asthma Immunol. 2006;5:195-8.
21. Manjunath CB, Kotinatot SC, Babu M. Peak expiratory flow rate in healthy rural school going children (5-16 years) of Bellur region for construction of nomogram. J Clin Diagn Res. 2013;7:2844-6.
22. Psarras S, Caramori G, Contoli M, Papadopoulos N, Papi A. Oxidants in asthma and in chronic obstructive pulmonary disease (COPD). Curr Pharm Des. 2005;11:2053-62.
23. Sackesen C, Ercan H, Dizdar E, Soyer O, Gumus P, Tosun BN, et al. A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. J Allergy Clin Immunol. 2008;122:78-85.
24. Fenech AG, Ellul-Micallef R. Selenium, glutathione peroxidase and superoxide dismutase in maltese asthmatic patients: effect of glucocorticoid administration. Pulm Pharmacol Ther. 1998;11:301-8.
25. Fitzpatrick AM, Teague WG, Holguin F, Yeh M, Brown LA. Airway glutathione homeostasis is altered in children with severe asthma: evidence for oxidant stress. J Allergy Clin Immunol. 2009;123:146-52.