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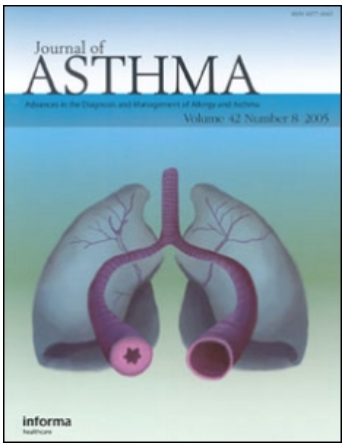

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Brooke Heidenfelder [a]; Markey Johnson [a]; Edward Hudgens [a]; Jefferson Inmon [a]; Robert G. Hamilton [b]; Lucas Neas [a]; Jane E. Gallagher [a]
[a] US Environmental Protection Agency (EPA), Human Studies Division, Research Triangle Park, North Carolina [b] Division of Allergy and Clinical Immunology, Departments of Medicine and Pathology Johns Hopkins University School of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

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ORIGINAL ARTICLE

Increased Plasma Reactive Oxidant Levels and Their Relationship to Blood Cells, Total IgE, and Allergen-specific IgE Levels in Asthmatic Children

Brooke Heidenfelder,[1] Markey Johnson,[1] Edward Hudgens,[1] Jefferson Inmon,[1] Robert G Hamilton,[2] Lucas Neas,[1] and Jane E. Gallagher, Ph.D.[1,*]

[1]*US Environmental Protection Agency (EPA), Human Studies Division, Research Triangle Park, North Carolina*
[2]*Division of Allergy and Clinical Immunology, Departments of Medicine and Pathology Johns Hopkins University School of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland*

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Asthma is a disorder characterized by inflammation of the airways. Oxidative stress may play a role in the pathophysiology of several diseases including asthma. Characterizing biomarkers of oxidative stress in the context of other systemic measures of immune function or inflammation could provide insight regarding underlying mechanisms inducing asthma. We evaluated whether oxidative stress in the form of plasma reactive oxidants differs between asthmatic and non-asthmatic children and elucidate relationships between plasma reactive oxidants and other asthma-related immunological markers. Plasma reactive oxidants, white blood cell counts, total serum immunoglobulin E (IgE), and a multi–allergen-specific IgE screen were measured in 74 asthmatic and 74 non-asthmatic children (9 to 13 years of age) from the Detroit, Michigan area. Plasma reactive oxidants were measured using a lucigenin-based chemiluminescence assay. Plasma reactive oxidants, eosinophils, and neutrophils (absolute counts and percent of total white blood cell counts), total IgE, and allergen-specific IgE levels were elevated in asthmatics after adjusting for age, gender, and ethnicity. IgE (total or allergen-specific), eosinophils and neutrophils were not significantly associated with plasma reactive oxidant levels. The association between plasma reactive oxidants and asthma status was similar when eosinophils, neutrophils, total IgE, or allergen-specific IgE were included as possible confounders in multivariate logistic regression models.

In conclusion, plasma reactive oxidants are elevated in asthmatics and appear to be an independent predictor of asthma status. Measurement of plasma reactive oxidants may be a useful adjunct diagnostic tool and potential mechanistic indicator relevant to the study of asthma and asthma exacerbation.

Keywords asthma, reactive oxidants, oxidative stress, immunoglobulin E, inflammation

Introduction

Asthma is a disease involving chronic airway inflammation and dysfunction.

Inflammation in a majority of asthma cases is IgE antibody mediated and is thought to be perpetuated by the presence of elevated numbers of eosinophils (1), a hallmark of asthma status. Non-eosinophilic asthma has been proposed as a distinct asthma phenotype (2) and is often characterized by neutrophilic inflammation, which can act in cooperation with environmental factors and eosinophilic inflammation in severe asthmatics (2, 3). Activated inflammatory cells, including neutrophils and eosinophils, generate reactive oxidants (RO) that produce oxidative stress and can elicit a number of gene transcription and cell signaling changes (4). RO are thought to mediate inflammation through cell signaling in several disease processes throughout the body, including cancer (5). Granulocytic cells, and eosinophils in particular, spontaneously generate superoxide, a type of RO, at sites of antigen challenge, which contributes to inflammation-induced airway injury (6). Certain asthma medications have been shown to reduce superoxide production, which may contribute to their anti-inflammatory properties (7–9). Synthetic antioxidant mimetics that block RO-related damage are also being considered for asthma treatment (10). Asthmatics display increased generation of superoxide in inflammatory cells in both the lung and blood compartments (11–14). that correlates with measures of disease severity (15–17). Increased levels of RO from peripheral blood cells are present in both stable and exacerbated asthmatics relative to healthy control subjects (13–16). Recently Suzuki et al. (18) reported elevated levels of reactive oxygen metabolites in serum in subjects exhibiting acute exacerbation of asthma. These studies suggest that RO measurement may be used to distinguish asthma status.

Chemiluminescent measurement of RO is an effective and relatively simple procedure, Chemiluminescence (CL)-based RO measurements are generally evaluated in cell types relevant to the disease being studied, e.g., immune cells from patients with Crohn's disease (19), adult respiratory distress syndrome (19), or acute myeloid leukemia (20). Several

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*Corresponding author: Jane E. Gallagher, PhD, US EPA, Human Studies Division, MD 58 C, Research Triangle Park, 27711; E-mail: gallagher.jane@epa.gov

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studies have used lucigenin-based CL to detect superoxide production in peripheral blood cells collected from asthmatics (13, 14, 22) rather than using more invasive lung lavage techniques. Because diagnostic indicators such as cholesterol and triglycerides are measured in serum (23), it is of interest to ascertain whether RO is measurable in the same specimens. Plasma RO may reflect a more integrated measure of oxidative stress across multiple organ systems (24–26).

Eosinophils and neutrophils are associated with asthmatic inflammation. Higher blood eosinophil levels are associated with increased asthma severity (27), whereas non-eosinophilic asthmatics can exhibit elevated neutrophil levels (2). Allergic disease has been strongly associated with asthma. Allergen cross-linking of IgE antibody on effector cells induces allergic inflammation, one of the strongest susceptibility factors for the development of asthma (26). This study examines cell and serum measures of allergic inflammation in the context of plasma RO levels in asthmatic and non-asthmatic children. Plasma RO levels were evaluated in relation to other indicators of asthma symptomology (total serum IgE, a panel of allergen-specific serum IgEs, eosinophils, and neutrophils) to elucidate whether these markers are independent predictors of asthma.

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Methods

Study Population and Recruitment

The Environmental Protection Agency (EPA) conducted the Mechanistic Indicators of Childhood Asthma (MICA) in Detroit, Michigan: a subset of children who previously participated in the Detroit Children's Health Study (DCHS). For DCHS recruitment, questionnaires were mailed to members of the Henry Ford Health System (HFHS),Detroit, MI). MICA participants were recruited from households that returned DCHS questionnaires and had clinical visits between November 2006 and January 2007. MICA participants were prioritized to ensure equal participation of asthmatics and non-asthmatics.

Asthma status was based on both clinical records and a parental questionnaire. A child was considered asthmatic if the clinical record showed one or more asthma-related emergency department visits, two or more asthma-related outpatient visits, or two or more asthma-related medications. From the parental questionnaire, a child was considered asthmatic with a parental report of a physician's diagnosis of asthma. Children with conflicting or incomplete asthma status data were excluded from the analyses. The MICA study data include results from 148 asthmatic and non-asthmatic children 9 to 13 years of age residing in Detroit and Dearborn, Michigan. We classified asthmatic children who never used asthma medication (N = 6) or occasionally (N = 27) as mild asthmatics, while those who took one or more asthma medications on a daily basis (N = 41) were described as moderate to severe asthmatics. The study design and protocols were approved by the Institutional Review Boards at HFHS (Detroit, MI), Westat Inc. (Rockville, MD), and the University of North Carolina at Chapel Hill (Chapel Hill, NC–EPA's IRB of record). Written consent was obtained from guardians and child with an oral review of both consent and assent before drawing blood.

Immune Function Assessment

Whole blood (2 mL) was collected into EDTA-containing Vacutainer tubes (BD, Franklin Lakes, NJ) and a complete blood count with differentials was performed by the Henry Ford Health System clinical laboratory.

Serum specimens were prepared by collecting whole blood (8 mL) in a Vacutainer serum clot, red/gray tube (BD, Franklin Lakes, NJ) for each child. Serum was separated following centrifugation, and a 1.7 mL aliquot of serum was frozen at −80°C and sent to Johns Hopkins University School of Medicine Dermatology, Allergy and Clinical Immunology (DACI) Reference Laboratory. Total IgE and a multi-allergen screen for allergen-specific IgE antibody specific for a panel of aeroallergens were quantified in the ImmunoCAP 250 autoanalyzer (Phadia, Portage, Michigan). The multi-allergen screen detects the presence (positive/negative) and measures the relative level of IgE antibody specific for a panel of at least 15 common aeroallergens (Phadiatop). Each subject was tested with the same panel of aeroallergens. Phadiatop levels greater than 0.35 kIU/L indicate the presence of IgE antibody and provide a general assessment of the subject's atopic status. Phaditop levels exceeding 100 kIU/L were set to 100 kIU/L. Daily quality control was performed with Levey-Jennings plots for human sera that contained known amounts of IgE antibody.

Chemiluminescence Assay for Plasma Reactive Oxidants

Whole blood (2.5 mL) was collected in foil-wrapped Vacutainer tubes containing sodium citrate and was put on ice and plasma immediately separated from the whole blood. After centrifugation for 10 minutes at 2500 × g, a 250-μl aliquot of plasma was removed and protected from light exposure. The maximum time allowed between blood draw and the initiation of storage at −80°C was 60 minutes, but the time elapsed was 15 to 30 minutes for most samples. Plasma was stored for 72 to 79 days at −80°C before analysis.

Lucigenin reportedly reacts primarily with superoxide in human plasma (28, 29), however, the oxidant's status may have been altered as a result of freezing. Therefore, in this report, we refer to the substances reacting with lucigenin in this assay as "reactive oxidants" or RO. The chemiluminescence measurement of RO in plasma was adapted from Wu et al. (24). with minor modifications. Aliquots of 64 μl (in triplicate) of either plasma or 1× phosphate buffered saline (PBS) were added to a 96-well plate, mixed with 160-μl of freshly prepared lucigenin (2 mg/mL in 1× [PBS]; Sigma, St. Louis, MO). Chemiluminescence measurements were performed at 37°C using an LB96V MicroLumatPlus luminometer (Berthold Technologies). One minute after lucigenin addition, chemiluminescence was measured at 10-second intervals for a total of 10 minutes. Light emission units (relative luminescence units, RLU) were summed and averaged over the 10 minutes of measurement. Measurements from the PBS control subjects were subtracted from plasma RO measurements to adjust for background luminescence. Measurements less than background controls (N = 2) were set to equal the lowest detected value in the data set (0.6 RLU).

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Statistical Analyses and Variables

Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). Total serum IgE and allergen-specific IgE (Phadiatop) were modeled as their natural logarithms, whereas eosinophils and neutrophils variables were modeled as the percentage of absolute eosinophils or absolute neutrophils in relation to total white blood cell count. Differences in means were assessed using Student's t tests, applying the Satterthwaite method for groups with unequal variance and the pooled method for groups with equal or similar variance between the groups. Associations between biological indicators (RO, eosinophils, neutrophils, total serum IgE, and allergen-specific IgE) and asthma were examined using multivariate logistic regression analysis. We also examined whether the relationship between RO and asthma was independent of other clinical markers of asthma (eosinophils, neutrophils, total serum IgE, and allergen-specific IgE) using multivariate logistic regression models that included RO and other clinical markers as potential predictors of asthma status. All multivariate models included fixed covariate terms adjusting for age, sex, and ethnicity. Age was modeled as the natural logarithm of age in months. Odds ratios for all biological indicators were scaled to their respective interquartile ranges (IQR) to facilitate comparisons of the relationships between different biological indicators and asthma. All analyses were considered statistically significant at p value $\leq$ 0.05.

Results

A total of 148 children with concordant clinical and questionnaire reports of asthma status: 74 with a history of asthma and 74 without a history of asthma were used for this analysis. The cohort was predominantly African-American (88%) and had slightly more boys (59%) than girls.

Univariate distributions and t tests for the blood biological indicators by asthma status are shown in Table 1 . Mean levels of RO, eosinophils, total serum IgE, and allergen-specific IgE were significantly higher among asthmatics compared with non-asthmatics. There was no significant difference in neutrophil levels between asthmatics and non-asthmatics. Duration of blood plasma storage was not associated with plasma RO concentration (linear regression analysis coefficient: −0.0018, 95%CI: [−0.096, 0.092]). Using the frequency with which subjects took asthma medications as a surrogate measure of asthma severity, RO levels were 6.6 +/− 3.1 for mild asthmatics versus 8.8 +/− 4.5 for moderate to severe asthmatics ($p < 0.017$)

Associations between biological indicators and asthma are shown in Figure 1. RO was positively associated with asthma adjusting for age, sex, and ethnicity (odds ratio = 1.84; p = 0.0069). Eosinophils (odds ratio = 1.90; p = 0.0035), total serum IgE (odds ratio = 1.70; p = 0.016), and allergen-specific IgE (odds ratio = 2.65; p = 0.0076) were also positively associated with asthma in multivariate logistic regression models after adjusting for age, sex, and ethnicity. There was also a slight inverse association between neutrophils and asthma (odds ratio = 0.87); however, this association did not achieve statistical significance (p = 0.56).

The association between plasma reactive oxidants and asthma status did not vary when eosinophils, neutrophils, total IgE, or allergen-specific IgE were included as possible confounders in multivariate logistic regression models (Table 2).

Discussion

Asthma is a complex disease characterized by inflammation of the airways. Many cells and cellular constituents are thought to modulate the formation of RO, which in turn may mediate cellular signaling pathways related to immunity and inflammation (5). Characterizing new markers of inflammation in the context of well-established indicators of immune function may improve the diagnostic relevance and mechanistic significance of RO. Such studies may provide insight into the complex interactions between cells and immune and antioxidant factors transported in the plasma that can lead to increased levels of inflammation, a hallmark of many diseases including asthma.

RO levels are elevated in asthmatics and are believed to be involved in the pathogenesis of asthma. For example, resting blood cells from unstable asthmatics exhibit increased levels of oxidants compared with stable asthmatics, who in turn have higher oxidant levels than normal subjects (13). To our knowledge, the present study is the first to examine plasma levels of RO in asthmatic children, in relation to more established clinical indicators of immune function. While whole blood RO has been shown to be elevated in certain disease states (19, 30), including asthma (14), we hypothesized that plasma RO would be elevated in asthmatics owing to the chronic inflammation associated with this disease. A modified chemiluminescence assay (24) was used to measure plasma RO levels. The relationships between plasma RO, total and allergen specific IgE, and inflammatory cells (neutrophils and eosinophils) were evaluated in children 9 to 13 years of age. Elevated levels of plasma RO, total and allergen specific IgE, and eosinophils were observed in asthmatic versus non-asthmatic children and were statistically significant by t test and multivariate analysis models after adjusting for age, sex, and ethnicity. Total and allergen specific IgE levels, eosinophil and neutrophil levels were not significantly associated with plasma RO levels in this study population (data not shown). Relationships between RO and asthma were similar in statistical models adjusting for eosinophil, neutrophil, total IgE, or allergen-specific IgE levels. These results suggest that plasma RO predicts asthma independently from these serological markers. Our results are consistent with studies by Huang et al. (26) in which plasma RO was compared to two markers of inflammation, C-reactive protein and ferritin. Despite elevated levels of all three markers in febrile emergency room patients, no relationship between RO and these parameters was observed. The regulation of the immune system is complex, with molecular and cellular pathways impacted and modulated by cells and cellular constituents (31).

Consistent with the published literature (1) eosinophil levels were elevated in the asthmatic group. Eosinophils are involved in the inflammatory process and can produce free radicals when activated (32), to a much higher degree than neutrophils (33). This study could not evaluate the extent to which neutrophils and eosinophils act in concert to induce the formation/release of antioxidants or RO.

Total and allergen-specific IgE levels were elevated in asthmatics. Allergen-sensitized individuals are thought to be

more susceptible to RO-induced inflammation. This susceptibility appears to be linked to polymorphisms in glutathione-S-transferase that reduce the protein's ability to protect against RO (34). No significant relationship between plasma RO levels and total or allergen-specific IgE levels was observed in the present study. Given that over 95% of the asthmatic subjects in the clinical study were stable, the present study could not evaluate asthma severity or symptoms in terms of their levels of RO or immune function indicators.

Neutrophils are elevated in severe asthmatics, during severe exacerbations, and in asthmatic smokers (27). Asthma characterized by high levels of neutrophils typically manifests among middle-age, non-atopic, female adults (2). Taken together, most children in this study would not have a neutrophilic form of asthma. In the present study, no association was observed between neutrophil and plasma RO levels, consistent with a study by Huang et al. (26) of febrile patients but inconsistent with the findings of Mattheyse et al. (35), where RO released from neutrophils diminished during episodes of asthmatic airway obstruction. The authors suggested that the activated cells may preferentially sequester in the lung.

The current study has several limitations. Given the single time point blood measurement, intra-individual variability, an important criterion when validating a new biologic or clinical indicator of exposure or a disease state, could not be assessed. Plasma RO levels were not evaluated in relation to other potential indicators of inflammation, e.g., C-reactive protein, ferritin, or cytokines, nor were RO levels studied in relation to lung function. We are intrigued by the detection of RO in frozen plasma, suggesting that the specific RO measured in this assay are stable and therefore not like the commonly repudiated superoxide anion. The detection of RO in frozen plasma by lucigenin- induced chemiluminescence analysis has been reported by Park et al. (25). There is debate as to whether the lucigenin probe overestimates the superoxide levels due to possible redox cycling of lucigenin itself. Ex vivo generation of superoxide can be potentiated via interactions with various cofactors and or substrates in the plasma (28, 29). Thus, the possibility exists that we are measuring plasma RO and or RO generation by plasma constituents (cofactors and or substrates) that predispose to RO formation in the presence of our chemiluminescence agent. The convenience of storing plasma before RO analysis extends the application towards larger clinical or epidemiological studies. Using the frequency with which subjects took asthma medication as a surrogate indicator of asthma severity, RO measurements were significantly higher in moderate to severe asthmatics compared to mild asthmatics. However, the classification of asthma severity based on asthma medication usage alone is limited because we did not control for variation in asthma medication dose, type, and route of administration.

In conclusion, few clinical and environmentally relevant indicators of systemic inflammation have been developed despite the existence of chemicals known to induce reactive oxidants (36) and the association of inflammation with many disease states. Our data indicate that a simple measure of systemic reactive oxidant burden may be clinically useful in assessing asthmatic children. Plasma RO levels were independent of eosinophil and neutrophil concentrations, total IgE, or the presence of allergen-specific IgE antibody; and the association between RO and asthma appeared to be independent of these clinical indicators of asthma. Measuring RO in plasma obviates the need for ex vivo challenge of isolated blood cells with environmental or pro-inflammatory stimuli and may provide a useful adjunct to other serum markers of vascular inflammation. For example, eosinophil cationic protein, a potential biomarker of airway inflammation, is elevated in the serum, plasma, induced sputum, saliva, and bronchoalveolar lavage fluid of stable asthmatics and used to assess asthma severity and management (37). Whether measurement of RO will be useful from a diagnostic or asthma management perspective will require further evaluation. We are presently evaluating RO in other biological fluids to evaluate whether plasma RO is a sensitive marker of exposure to environmentally relevant contaminants in water and air.

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References

1. Trivedi SG, Lloyd CM. Eosinophils in the pathogenesis of allergic airways disease. Cell Mol Life Sci 2007; 64:1269–1289.
2. Haldar P, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. J Allergy Clin Immunol 2007; 119:1043–1052; quiz 53–4.
3. Barnes PJ. New molecular targets for the treatment of neutrophilic diseases. J Allergy Clin Immunol 2007; 119:1055–62; quiz 63–4.
4. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. J Biochem Mol Biol 2003; 36:95–109.
5. Bubici C, Papa S, Dean K, Franzoso G. Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance. Oncogene 2006; 25:6731–6748.
6. Sanders SP, Zweier JL, Harrison SJ, Trush MA, Rembish SJ, Liu MC. Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects. Am J Respir Crit Care Med 1995; 151:1725–1733.
7. Ezeamuzie CI, Al-Hage M. Effects of some anti-asthma drugs on human eosinophil superoxide anions release and degranulation. Int Arch Allergy Immunol 1998; 115:162–168.
8. Sadeghi-Hashjin G, Nijkamp FP, Henricks PA, Folkerts G. Sodium cromoglycate and doxantrazole are oxygen radical scavengers. Eur Respir J 2002; 20:867–872.
9. Persdotter S, Lindahl M, Malm-Erjefalt M, von Wachenfeldt K, Korn SH, Stevens T, Miller-Larsson A. Cooperative inhibitory effects of budesonide and formoterol on eosinophil superoxide production stimulated by bronchial epithelial cell conditioned medium. Int Arch Allergy Immunol 2007; 143:201–210.
10. Kinnula VL. Production and degradation of oxygen metabolites during inflammatory states in the human lung. Curr Drug Targets Inflamm Allergy 2005; 4:465–470.
11. Rahman I, Biswas SK, Kode A. Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol 2006; 533:222–239.

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12. Nadeem A, Chhabra SK, Masood A, Raj HG. Increased oxidative stress and altered levels of antioxidants in asthma. J Allergy Clin Immunol 2003; 111:72–78.
13. Vachier I, Chanez P, Le Doucen C, Damon M, Descomps B, Godard P. Enhancement of reactive oxygen species formation in stable and unstable asthmatic patients. Eur Respir J 1994; 7:1585–1592.
14. Teramoto S, Shu CY, Ouchi Y, Fukuchi Y. Increased spontaneous production and generation of superoxide anion by blood neutrophils in patients with asthma. J Asthma 1996; 33:149–155.
15. Kanazawa H, Kurihara N, Hirata K, Takeda T. The role of free radicals in airway obstruction in asthmatic patients. Chest 1991; 100:1319–1322.
16. Shanmugasundaram KR, Kumar SS, Rajajee S. Excessive free radical generation in the blood of children suffering from asthma. Clin Chim Acta 2001; 305:107–114.
17. Neijens HJ, Raatgeep RE, Degenhart HJ, Duiverman EJ, Kerrebijn KF. Altered leukocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. Am Rev Respir Dis 1984; 130:744–747.
18. Suzuki S, Matsukura S, Takeuchi H, Kawaguchi M, Ieki K, Odaka M, Watanabe S, Homma T, Dohi K, Aruga T, Sato M, Kurokawa M, Kokubu F, Adachi M. Increase in reactive oxygen metabolite level in acute exacerbations of asthma. Int Arch Allergy Immunol 2008;146 (suppl 1):67–72. Epub 2008 May 27.
19. Kitahora T, Suzuki K, Asakura H, Yoshida T, Suematsu M, Watanabe M, Aiso S, Tsuchiya M. Active oxygen species generated by monocytes and polymorphonuclear cells in Crohn's disease. Dig Dis Sci 1988; 33:951–955.
20. Tagan MC, Markert M, Schaller MD, Feihl F, Chiolero R, Perret CH. Oxidative metabolism of circulating granulocytes in adult respiratory distress syndrome. Am J Med 1991; 91:72S–8S.
21. Er TK, Tsai SM, Wu SH, Chiang W, Lin HC, Lin SF, Wu SH, Tsai LY, Liu TZ. Antioxidant status and superoxide anion radical generation in acute myeloid leukemia. Clin Biochem 2007; 40:1015–1019.
22. Woschnagg C, Garcia R, Rak S, Venge P. IL-5 priming of the PMA-induced oxidative metabolism of human eosinophils from allergic and normal subjects during a pollen season. Clin Exp Allergy 2001; 31:555–564.
23. Benlian P, Cansier C, Hennache G, Khallouf O, Bayer P, Duron F, Carrat F, Couderc R, Chazouillères O, Bardet J, Bouchard P, Poupon R, Masliah J, Béréziat G. Comparison of a new method for the direct and simultaneous assessment of LDL- and HDL-cholesterol with ultracentrifugation and established methods. Clin Chem 2000 Apr; 46(4):493–505.
24. Wu MM, Chiou HY, Wang TW, Hsueh YM, Wang IH, Chen CJ, Lee TC. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. Environ Health Perspect 2001; 109:1011–1017.
25. Park YK, Park E, Kim JS, Kang MH. Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. Mutat Res 2003; 529:77–86.
26. Huang HH, Yan HC, Han CL, Yu FC, Kao WY, Chen WT. Association of in vitro oxidative stress, serum ferritin concentration and C-reactive protein in febrile emergency room patients. Clin Invest Med 2005; 28:48–54.
27. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol 2007; 120:S94–138.
28. Chung KY, Lee SJ, Chung SM, Lee MY, Bae ON, Chung JH. Generation of free radical by interaction of iron with thiols in human plasma and its possible significance. Thromb Res 2005; 116:157–164.
29. Chen WT, Lin YF, Yu FC, Kao WY, Huang WH, Yan HC. Effect of ascorbic acid administration in hemodialysis patients on in vitro oxidative stress parameters: influence of serum ferritin levels. Am J Kidney Dis 2003; 42:158–166.
30. Oldenburg B, van Kats-Renaud H, Koningsberger JC, van Berge Henegouwen GP, van Asbeck BS. Chemiluminescence in inflammatory bowel disease patients: a parameter of inflammatory activity. Clin Chim Acta 2001; 310:151–156.
31. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2008; 8:183–192.
32. Nagata M. Inflammatory cells and oxygen radicals. Curr Drug Targets Inflamm Allergy 2005; 4:503–504.
33. Lacy P, Abdel-Latif D, Steward M, Musat-Marcu S, Man SF, Moqbel R. Divergence of mechanisms regulating respiratory burst in blood and sputum eosinophils and neutrophils from atopic subjects. J Immunol 2003; 170:2670–2679.
34. Spiteri MA, Bianco A, Strange RC, Fryer AA. Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation. Allergy 2000; 55 (suppl 61):15–20.
35. Mattheyse FJ, van Heerden K, Mattheyse M, Williams Z, Bouic P, van Schalkwyk EM, Bardin PG. Reduced activation of peripheral blood neutrophils after late-phase asthmatic responses but not in mild stable asthma. Respiration 2001; 68:471–479.
36. Becker S, Soukup JM, Gallagher JE. Differential particulate air pollution induced oxidant stress in human granulocytes, monocytes and alveolar macrophages. Toxicol In Vitro 2002; 16:209–218.
37. Koh GCH, Shek LPC, Goh DYT, Van Bever H, Koh DSQ. Eosinophil cationic protein: Is it useful in asthma? A systematic review. Respir Med 2007; 101:696–705.

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