

Altered oxidative state in schoolchildren with asthma and allergic rhinitis

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Oxidative stress may be defined as a disruption of the balance between the level of oxidants and reductants (antioxidants), and oxidative state in children may influence the risk of asthma and allergic disease. We investigated serum antioxidant levels: selenium, vitamin E, bilirubin, albumin, uric acid and transferrin as well as the oxidant ferritin and their association with asthma and allergic rhinitis. Children of 7–12 yr with asthma ($n = 50$) and no asthma (controls) ($n = 52$) underwent skin prick test, lung function, fractional exhaled nitric oxide (FeNO) measurements and blood sampling. Allergic rhinitis was found in 23 children, 19 with asthma and four controls. Healthy children were controls without rhinitis. Asthma was associated with reduced albumin (g/l), adjusted odds ratio (aOR) (95% CI) 0.81 (0.66, 0.99) ($p = 0.048$) compared with healthy children in a regression analysis adjusted for age and gender. Asthma with high FeNO ≥ 20 ppb was associated with reduced albumin, aOR 0.60 (0.40, 0.89) ($p = 0.012$) compared to controls with FeNO < 20 . Asthma with allergic rhinitis had reduced albumin, aOR = 0.70 (0.50, 0.99) ($p = 0.04$), and higher ferritin levels (mg/l) [aOR = 1.04 (1.00, 1.09) $p = 0.03$] compared to healthy children. Poorly controlled asthma was associated with lower vitamin E levels, aOR 0.79 (0.65, 0.95) ($p = 0.02$), lower transferrin levels, aOR 0.72 (0.57, 0.92) ($p < 0.01$), and higher albumin levels, aOR 1.53 (1.03, 2.28) ($p = 0.04$), compared to well controlled asthma. In conclusion, schoolchildren with asthma and rhinitis had reduced levels of the major serum antioxidant albumin, and poorly controlled asthma was associated with decreased vitamin E and transferrin levels. Reduced albumin was associated with increased FeNO, a marker of allergic inflammation in asthma, although the discriminatory value of this finding should be further assessed in population studies.

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Oxidative stress may be defined as a disruption of the balance between the level of oxidants and reductants (antioxidants) (1). Sites of inflammation are rich in reactive oxygen species and oxidant-producing enzymatic systems, which highlights the fact that oxidative stress exists

at nearly all sites undergoing inflammatory reactions (2).

The association between upper and lower allergic airways disease is increasingly focused upon (3), and both locations should ideally be assessed in studies of airway allergies. The respiratory tract has an antioxidant system protecting against exposure to noxious oxidants, and an imbalance between oxidants and antioxidants in favour of oxidants will lead to oxidative stress (4). Inflammatory disorders such as asthma and allergic rhinitis may be

Abbreviations: FeNO, Fractional exhaled nitric oxide; GA²LEN, Global Allergy and Asthma European Network; Hs-CRP, High-sensitivity CRP; ICS, Inhaled corticosteroids; MDI, Metered-dose inhaler; MEF₅₀, Mid-expiratory flow; ROS, reactive oxygen species; SPT, Skin prick test.

mediated by oxidative stress (5), depending partly on the antioxidant defences present within the respiratory tract lining fluids (6). The role of oxidative stress in allergic rhinitis is not well studied, but the pathogenesis may be similar to that of asthma (5). Existing epidemiological and immunological data propose that there are associations between antioxidants and allergic disease, and most observational studies report potentially beneficial associations between dietary antioxidants and allergic outcomes, but the exact nature of the relationships and the potential for therapeutic intervention remain unclear (7).

Epidemiological studies have shown that low intake of dietary antioxidants such as vitamin C, vitamin E and selenium is associated with respiratory symptoms and reduced lung function, but evidence determining the effects of diet on asthma is limited (8). Non-dietary hydrophilic endogenous serum antioxidants such as albumin and uric acid have a potential to protect tissues against oxidant injury (9), and albumin and uric acid are substantial contributors to the total serum antioxidant status with as much as 28% and 19.3%, respectively, in available assays (10). Bilirubin acts as an important co-antioxidant for vitamin E, thus inhibiting plasma and low-density lipoprotein lipid peroxidation (11). Free iron can act as an oxidant and catalyse the generation of highly reactive hydroxyl radicals with reports of positive association between asthma and iron levels (12). Transferrin may have antioxidant effects inhibiting iron-mediated oxidation through the capacity for binding free iron (13), whereas serum ferritin as a reliable biochemical marker of body iron stores is the preferred marker for iron-related oxidative stress (14).

The primary aim of this study was to assess whether asthma was associated with reduced levels of the dietary antioxidants vitamin E and selenium and the non-dietary antioxidants albumin, uric acid, bilirubin and transferrin or increased level of the oxidant ferritin. Secondary aims were to assess whether the level of fractional exhaled nitric oxide (FeNO), the presence of allergic rhinitis or different levels of asthma control were associated with reduced antioxidant levels or increased ferritin levels in children with asthma.

Methods

Study subjects

This study originates from the Norwegian study population from the GA²LEN (Global Allergy

and Asthma European Network) SARI multicentre case-control study across Europe to assess the relationship of plasma selenium with asthma (15). The present population was a case-control study of 102 schoolchildren with current asthma ($n = 50$) and non-asthmatic controls recruited from the outpatient clinic of our hospital or they were identified from a population-based postal questionnaire survey. The controls were identified through the questionnaire and were validated as having neither asthma nor wheeze by parental interview. Exclusion criteria were chronic inflammatory diseases other than asthma, allergic rhinitis and atopic dermatitis. Children with a history of asthma, but no current symptoms ($n = 8$), were excluded from this study. Mean age of the children was 9.7 (range 7–12), without significant age difference between the asthma and control children.

Study design

The subjects attended one clinical visit including a detailed parental questionnaire, height and weight measurements, lung function measurements by maximum forced expiratory flow-volume loops, collection of exhaled breath condensate (not further elaborated in this study), FeNO levels, salivary cortisol and skin prick tests to prevalent allergens. Serum was collected for storage analyses.

Ethics

Approval was obtained from the regional medical ethical committee as well as the Norwegian Data Inspectorate, and the study was registered in the Norwegian Bio Bank Registry. Each participant was provided with an information sheet explaining the study, and informed consent from the parents was given prior to study participation. The study was performed in accordance with the ethical standards of the Helsinki declaration.

Study methods

Information on treatment was obtained by structured parental interview. Regular use of inhaled corticosteroids (ICS) at the time of investigation was registered, and a low dose was defined as $<400 \mu\text{g}$ budesonide metered-dose inhaler (MDI) equivalents daily, whereas a medium dose was $400\text{--}800 \mu\text{g}$ and a high dose was $>800 \mu\text{g}$ budesonide MDI equivalents daily. No study subject used $>1000 \mu\text{g}$ budesonide per

day or had used oral corticosteroids within the last 12 months.

Spirometry was performed by the EasyOne spirometer according to the ERS/ATS guidelines (16) with the reference values of Zapletal (17). The study subjects withheld short-acting β -2-agonists and long-acting β -2-agonists for at least 6 and 12 h, respectively, prior to the investigation. Examinations were performed at least 1 wk after any respiratory infection. FeNO was measured by Ecomedis CLD 88, and the mean of the three best manoeuvres was assessed according to the ERS/ATS guidelines (18).

Skin prick tests (SPT) were undertaken for a common European panel (19) using ALK-Abello reagents against timothy pollen, cat dander, *Dermatophagoides farinae*, olive and birch pollen, parietaria, alternaria, histamine (10 mg/ml) and diluent controls. The widest diameter and its perpendicular diameter were measured, with the mean of these diameters registered. A positive SPT was defined as the wheal being equal to or more than 3 mm above the diluent control for any of the tested allergens. The regular intake of vitamin or mineral supplements was registered as yes or no following the question: 'Does your child take regular mineral or vitamin supplements?'

Vacutainer plastic blood collection tubes and 7-ml gel heparin tubes were used to collect plasma and serum, and the samples were, after storage in stable room temperature, centrifuged at 1100–1300 *g* for 10 min within 2 h of collection. The plasma for selenium analysis was subsequently stored at -20°C , and the other samples at -70°C . All samples were analysed in batches. Analyses of selenium were performed as described in the original GA²LEN study (15). Plasma vitamin E (α -tocopherol) was analysed at A/S Vitas, Oslo, by an HPLC system as described by Richenheimer et al. except that the fluorescence detector with excitation and emission wavelength was set to 295 and 330 nm, respectively (20). Serum concentrations of albumin, uric acid, transferrin and bilirubin were measured with the Cobas Integra 800 (Roche Diagnostics AS, Basel, Switzerland), ferritin was measured with Centaur (Bayer Diagnostica AS, Leverkusen, Germany), and high-sensitivity C reactive protein (Hs-CRP) was analysed by the immunoturbidimetric method Cobas Integra 800 (Roche Diagnostics AS) at Oslo University Hospital, Department of Clinical Chemistry.

The main explanatory variables were selenium, vitamin E, ferritin, transferrin, albumin, bilirubin and uric acid levels. Hs-CRP was analysed to

control for latent inflammatory disease or infections that potentially could bias the results.

Outcomes

The main outcome was *current asthma* (*asthma*) defined as having doctor's diagnosis of asthma and having had wheeze in the previous 12 months prior to study investigation.

Secondary outcomes were as follows:

- *Asthma with FeNO* ≥ 20 ppb, the cut-off value for FeNO corresponding to the upper reference limit for healthy children (21).
- *allergic rhinitis*: defined as doctor's diagnosis of allergic rhinitis and nasal allergic symptoms within the last 12 months prior to study investigation.
- *asthma control* (the last year): defined according to the GINA guidelines (22) as controlled vs. partly controlled or uncontrolled. Partly controlled or uncontrolled asthma was classified in children reporting any of the following: daytime symptoms more than twice a week, any limitations of activities, any nocturnal symptoms, need for reliever treatment more than twice a week, one or more asthma exacerbation per year or FEV₁ per cent predicted $< 80\%$. The other children with asthma were registered as having controlled asthma.

Controls were children without current asthma.

Healthy children were defined as having neither current asthma nor allergic rhinitis.

Statistical analyses

The normality of the distribution of data was assessed. Demographic results are given as mean, median values or per cent with standard deviation (SD), or 95% confidence intervals (95% CI), respectively, as stated. Relationships among the continuous variables were evaluated by Pearson's correlation analysis or by Spearman's correlation analysis when data were not normally distributed. Group comparisons employed Pearson's chi-square tests for categorical variables. Student's *t*-test, Mann-Whitney test (when data not normally distributed) and one-way Analysis of Variance (ANOVA) were used for continuous variables, with Tukey's and Dunnet's (when not normally distributed) *post hoc* tests for multiple comparisons. Stepwise backward logistic regression techniques were used to estimate associations between outcomes and the explanatory variables. The model assumptions were tested out using Hosmer's and Lemeshow's goodness of

fit test. Analyses were performed using Statistical Package for Social Sciences 15.0 (SPSS inc. Chicago, IL, USA). All tests were two-tailed, and p-values equal to or below 0.05 were considered significant.

Power calculation. Owing to a lack of relevant paediatric studies, the power calculation was based upon a previous study in Turkish adults (23) where the mean (SD) serum albumin level in patients with asthma and controls was 47.5 (3.0) g/l. Thus, 50 cases and 52 controls would give a minimum of 95% power to find a mean difference of 2.6 g/l between cases and controls with a significance level of 0.05. For regression analyses, a sample size of 102 would achieve 80% power to detect an R^2 of 0.15 attributed to 10 independent variables using an F -test with a significance level of 0.05.

Results

Demographic and clinical characteristics of the study children are shown in Table 1. Allergic rhinitis was found in 19 (38%) children with current asthma and in four control children (8%). Among children with both asthma and allergic rhinitis, seven children were sensitized to seasonal allergens, five to perennial allergens, five to both perennial and seasonal allergens, and two had no sensitization.

Asthma was well controlled in 26 subjects (six with low-dose ICS and six with moderate- or high-dose ICS) and partly controlled or uncontrolled in 24 (eight with low-dose ICS and six with moderate- or high-dose ICS). FeNO was significantly higher in children with asthma compared with controls, as well as in children

with compared to without allergic rhinitis [mean 18.1 ppb (95% CI 10.7, 25.5) vs. 9.6 (7.3, 11.2) ($p = 0.005$), respectively].

Antioxidants and ferritin

The mean or median values (95% CI) of selenium, vitamin E, ferritin, transferrin, albumin, bilirubin, uric acid and high-sensitivity CRP (Hs-CRP) are listed for current asthma and controls in Table 2. Albumin was lower in current asthma (mean difference) (-0.8 g/l); ($p = 0.05$), but without significant differences regarding the other explanatory variables.

The children with asthma were separated into two groups by FeNO levels with a cut-off level of ≥ 20 ppb and compared with controls. As only one control had a FeNO level ≥ 20 ppb, the remaining 51 controls with a FeNO < 20 ppb were employed for the analyses. The levels of antioxidants, ferritin and Hs-CRP for the three groups are shown in Table 3. Albumin was significantly reduced (mean difference -1.6 g/l); ($p = 0.02$) in asthmatic children with FeNO ≥ 20.0 , whereas Hs-CRP was increased (median difference 0.5 mg/l); ($p = 0.02$) compared to controls with FeNO < 20 (Tukey's or Dunnett's *post hoc* test). The distribution of albumin levels in relation to FeNO groups is demonstrated in Fig. 1.

As only four control children had allergic rhinitis, we compared asthmatic children with healthy children (controls without allergic rhinitis), and separate analyses (Tukey's or Dunnett's *post hoc* tests) of asthma with allergic rhinitis ($n = 19$) demonstrated merely increased uric acid levels in contrast to asthma only [asthma without allergic rhinitis ($n = 31$)] (median

Table 1. Demographic and clinical characteristics of the study children

n	Current asthma 50	Controls 52	p-values
Boys, n (%)	28 (56)	26 (50)	0.54
Age, yr mean (SD)	9.6 (1.5)	9.7 (1.2)	0.74*
Vitamin or mineral supplements, n (%)	28 (56)	27 (52)	0.68
Parental smoking, n (%)	15 (30)	8 (15)	0.08
Parental asthma, n (%)	13 (26)	10 (19)	0.71
Parental allergy, n (%)	33 (66)	32 (62)	0.89
SPT any positive, n (%)	24 (48)	7 (13)	<0.001
Allergic rhinitis, n (%)	19 (38)	4 (8)	<0.001
FEV ₁ % predicted Mean (SD)	90.3 (13.4)	97.9 (12.2)	0.02*
FEV ₁ /FVC% predicted Mean (SD)	96.0 (11.7)	100.5 (6.8)	0.02*
MEF ₅₀ % predicted Mean (SD)	78.4 (21.9)	91.0 (23.4)	0.03*
Fractional exhaled nitric oxide ppb Mean (95% CI)	20.0 (12.1, 21.3)	6.6 (5.5, 7.7)	<0.001*

Significant p-values in bold.

MEF₅₀, Mid-expiratory flow; SPT, Skin prick test.

p-values given by Pearson's chi-square-test or by *Student's t -test.

Table 2. Levels of antioxidants, ferritin and high-sensitivity C reactive protein (Hs-CRP) in current asthma and controls

	Current asthma (n = 50)	Controls (n = 52)	p-values
Selenium $\mu\text{mol/l}$ mean (95% CI)	0.70 (0.67, 0.74)	0.71 (0.68, 0.74)	0.72
Vitamin E $\mu\text{mol/l}$ mean (95% CI)	23 (22, 25)	23 (22, 24)	0.63
Ferritin $\mu\text{g/l}$ (95% CI) mean (95% CI)	32.5 (26.9, 38.2)	29.7 (26.6, 32.8)	0.38
Transferrin g/l mean (95% CI)	3.00 (2.91, 3.10)	2.94 (2.86, 3.02)	0.26
Albumin g/l mean (95% CI)	46.6 (46.0, 47.2)	47.4 (46.8, 47.9)	0.05
Bilirubin $\mu\text{mol/l}$ median (95% CI)	7.0 (5.7, 7.4)	6.8 (6.9, 7.9)	0.63*
Uric acid $\mu\text{mol/l}$ median (95% CI)	224 (205, 245)	216 (196, 238)	0.30*
Hs-CRP mg/l median (95% CI)	3.6 (3.4, 3.9)	3.4 (3.1, 3.6)	0.10*

Significant p-values in bold.

p-values given by Student's *t*-test or by *Mann-Whitney test.

Table 3. Levels of antioxidants, ferritin and high-sensitivity C reactive protein (Hs-CRP) in asthma with fractional exhaled nitric oxide (FeNO) ≥ 20.0 (ppb), asthma with FeNO <20.0 and controls with FeNO <20.0 , respectively

	Asthma FeNO ≥ 20.0 (n = 16)	Asthma FeNO <20.0 (n = 34)	Controls FeNO <20.0 (n = 51)	p-values for comparison of three groups (ANOVA test)
Selenium $\mu\text{mol/l}$ mean (95% CI)	0.74 (0.68, 0.80)	0.68 (0.64, 0.72)	0.70 (0.67, 0.73)	0.30
Vitamin E $\mu\text{mol/l}$ mean (95% CI)	22 (19, 25)	24 (22, 26)	23 (22, 24)	0.29
Ferritin $\mu\text{g/l}$ mean (95% CI)	28.0 (18.1, 37.9)	34.7 (27.5, 41.8)	29.9 (26.6, 33.1)	0.29
Transferrin g/l mean (95% CI)	3.11 (2.91, 3.30)	2.96 (2.85, 3.07)	2.94 (2.86, 3.02)	0.18
Albumin g/l mean (95% CI)	45.8 (44.8, 46.7)	47.0 (46.1, 47.8)	47.4 (46.9, 47.9)	0.03
Bilirubin $\mu\text{mol/l}$ median (95% CI)	6.7 (5.2, 8.6)	7.0 (5.2, 7.4)	6.8 (6.0, 7.9)	0.82
Uric acid $\mu\text{mol/l}$ median (95% CI)	244 (228, 278)	207 (191, 224)	217 (205, 238)	0.07
Hs-CRP mg/l median (95% CI)	4.0 (3.3, 4.3)	3.4 (3.3, 3.8)	3.5 (3.2, 3.6)	0.03

Significant p-values in bold.

difference +40 $\mu\text{mol/l}$); ($p = 0.01$), but not compared to healthy children ($n = 48$); ($p = 0.08$). No other significant differences were found for the other antioxidants, ferritin or Hs-CRP (Table 4).

Children with partly controlled or uncontrolled asthma had significantly lower levels of vitamin E (mean difference $-3 \mu\text{mol/l}$); ($p = 0.03$) and transferrin (mean difference -0.21 g/l); ($p = 0.02$) than children with controlled asthma (Table 5), but as shown in Table 2, these differences were not present between children with asthma and controls.

Ferritin and transferrin correlated inversely (Pearson $r = -0.28$; $p = 0.004$), whereas transferrin and Hs-CRP were positively correlated (Spearman's $\rho = 0.22$; $p = 0.03$). There were no significant correlations between the other explanatory variables.

Regression analyses

Current asthma was inversely associated with albumin (g/l), adjusted odds ratio (aOR) [0.81 (0.66, 0.99) $p = 0.048$] compared to healthy children in a backward stepwise logistic regression model adjusting for age and gender. Current

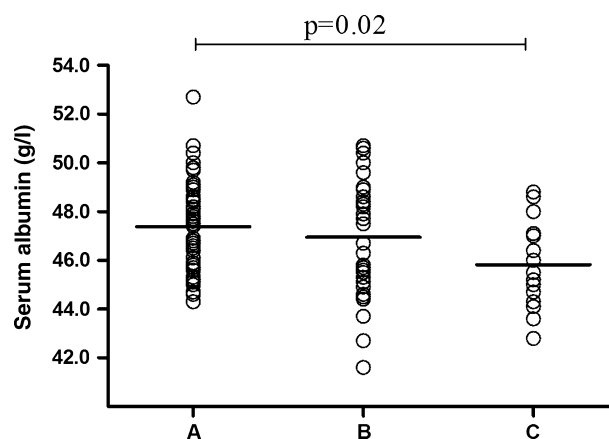


Fig. 1. Levels of serum albumin in controls with fractional exhaled nitric oxide (FeNO) <20.0 (ppb) (a), asthma with FeNO <20.0 (b) and asthma with FeNO ≥ 20.0 (c). Bold horizontal lines indicate mean values. Only significant p-values are shown (Tukey's *post hoc* test).

asthma was, in this model, not associated with selenium, vitamin E, bilirubin, uric acid, transferrin, ferritin or Hs-CRP compared to healthy children.

Likewise asthma with a FeNO ≥ 20 was associated with reduced albumin only [aOR = 0.60

Table 4. Levels of antioxidants, ferritin and high-sensitivity C reactive protein (Hs-CRP) in asthma with rhinitis, asthma only and healthy children

	Asthma with rhinitis (n = 19)	Asthma only (n = 31)	Healthy (n = 48)	p-values for comparison of three groups (ANOVA test)
Selenium $\mu\text{mol/l}$ mean (95% CI)	0.69 (0.64, 0.75)	0.71 (0.66, 1.29)	0.70 (0.67, 0.72)	0.90
Vitamin E $\mu\text{mol/l}$ mean (95% CI)	23 (20, 25)	24 (22, 26)	23 (22, 24)	0.55
Ferritin $\mu\text{g/l}$ mean (95% CI)	37.3 (25.1, 49.6)	29.6 (24.0, 35.2)	29.1 (25.9, 32.2)	0.15
Transferrin g/l mean (95% CI)	3.01 (2.86, 3.16)	3.01 (2.88, 3.14)	2.96 (2.87, 3.04)	0.70
Albumin g/l mean (95% CI)	46.2 (45.1, 47.8)	46.8 (46.0, 47.6)	47.4 (46.9, 47.9)	0.08
Bilirubin $\mu\text{mol/l}$ median (95% CI)	7.0 (5.2, 8.6)	7.0 (5.2, 8.2)	6.8 (6.0, 8.2)	0.74
Uric acid $\mu\text{mol/l}$ median (95% CI)	254 (216, 286)	214 (192, 224)	218 (205, 238)	0.006
Hs-CRP mg/l median (95% CI)	3.8 (3.1, 4.1)	3.4 (3.4, 3.9)	3.4 (3.2, 3.6)	0.18

Significant p-values in bold.

Table 5. Levels of antioxidants, ferritin and high-sensitivity C reactive protein (Hs-CRP) in controlled and partly controlled or uncontrolled asthma

	Controlled asthma (n = 26)	Partly controlled or uncontrolled asthma (n = 24)	p-values
Selenium $\mu\text{mol/l}$ mean (95% CI)	0.67 (0.62, 0.72)	0.73 (0.68, 0.78)	0.13
Vitamin E $\mu\text{mol/l}$ mean (95% CI)	25 (23, 27)	22 (20, 24)	0.03
Ferritin $\mu\text{g/l}$ mean (95% CI)	33.8 (24.5, 43.1)	31.2 (24.3, 38.0)	0.65
Transferrin g/l mean (95% CI)	3.11 (3.00, 3.21)	2.90 (2.74, 3.06)	0.02
Albumin g/l mean (95% CI)	46.4 (45.5, 47.2)	46.8 (45.9, 47.8)	0.44
Bilirubin $\mu\text{mol/l}$ median (95% CI)	7.3 (5.2, 10.0)	6.1 (5.0, 7.2)	0.12*
Uric acid $\mu\text{mol/l}$ median (95% CI)	220 (192, 254)	224 (193, 242)	0.51*
Hs-CRP mg/l median (95% CI)	3.8 (3.2, 3.9)	3.6 (3.3, 3.9)	0.62*

Significant p-values in bold.

p-values given by Students *t*-test or by *Mann–Whitney test.

(0.40, 0.89) $p = 0.012$] compared to controls with $\text{FeNO} < 20$.

Asthma with allergic rhinitis ($n = 19$) was associated with reduced albumin [$a\text{OR} = 0.70$ (0.50, 0.99) $p = 0.04$] and higher ferritin levels (mg/l) [$a\text{OR} = 1.04$ (1.00, 1.09) $p = 0.03$] compared to healthy children.

Partly controlled or uncontrolled asthma was associated with lower vitamin E levels ($\mu\text{mol/l}$) [$a\text{OR} = 0.79$ (0.65, 0.95) $p = 0.02$], lower transferrin levels (per 0.1 g/l) [$a\text{OR} = 0.72$ (0.57, 0.92) $p < 0.01$] and higher albumin levels [$a\text{OR} = 1.53$ (1.03, 2.28) $p = 0.04$] compared to controlled asthma.

The use of low, moderate or high doses of ICS or regular intake of vitamins and minerals showed no associations with the levels of selenium, vitamin E, albumin, bilirubin, uric acid, ferritin, transferrin or Hs-CRP after adjusting for age and gender.

Discussion

The major serum antioxidant albumin was significantly reduced in schoolchildren with current asthma, and having asthma with FeNO levels ≥ 20 (ppb) comprised the most compelling reduction in albumin levels. Poorly controlled

asthma was associated with reduced levels of the antioxidants vitamin E and transferrin. The children with asthma and allergic rhinitis had higher levels of the oxidant ferritin compared to healthy children when adjusting for albumin, age and gender.

The reduced albumin levels in the present study of children with asthma are in line with results from Japanese schoolchildren and Turkish adults with asthma (24, 25). In Australian adults, albumin was lower in more severe asthma, and albumin correlated positively with lung function (9). When assessing total antioxidant status, commonly albumin contributes up to 28% (10), and in US children, plasma reactive oxidants were elevated in asthmatics and appeared to be an independent predictor of asthma status (26). Albumin has antioxidant properties protecting against lipid peroxidation and thiol-dependent oxidation, as well as anti-inflammatory functions in controlling inflammatory gene transcription (27). As albumin levels in the present study were lowest in children with increased levels of FeNO , this implicates an inverse association between albumin levels and inflammatory activity. FeNO , as an objective measure of inflammation in asthma, was more strongly associated with

reduced albumin than the presence of allergic rhinitis.

Hs-CRP is proposed a surrogate marker of airway inflammation in asthma (28), and the slightly increased levels of Hs-CRP (within the normal range of CRP) found in asthmatic children with FeNO ≥ 20 ppb emphasize an increased intensity of allergic inflammation in these subjects.

The increased uric acid levels found in asthma with allergic rhinitis in the present study may reflect an up-regulation to compensate for lower levels of other antioxidants or increased levels of oxidants (10).

The inverse relationship between albumin and ferritin found in our study may express an oxidative imbalance or even oxidative stress in children with both asthma and allergic disease, with the increased ferritin levels possibly involved in the allergic inflammation. The literature support for our finding is limited. Only few studies describe the relationship between asthma and ferritin, with conflicting results (12, 25). However, ferritin has been found to correlate negatively with plasma vitamin antioxidants in South-Korean adults (14). In iron overload, albumin with the supportive effect of bilirubin has the potential of offering antioxidant protection (27).

The reduced levels of vitamin E in partly controlled or uncontrolled asthma compared to controlled asthma may correspond to lower antioxidative ability in less controlled asthma, in line with the reports of increased oxidative stress in acute exacerbations of asthma (29). A plausible biological support for the observation is that vitamin E is a fat-soluble antioxidant with a defence role against oxidant-induced membrane injury (30). In Australian adults, vitamin E was reduced in severe asthma, as well as in mild to moderate asthma compared to healthy subjects (9). A recent systematic review and meta-analysis of the association between antioxidant vitamins and asthma outcome measures found that vitamin E intake was associated with the severity of asthma, but not asthma as outcome (31).

The observed association in the present study between reduced transferrin and partly controlled or uncontrolled asthma compared to controlled asthma lacks support in the literature, with few reports of the association between transferrin and allergic disease (23). The negative correlation between transferrin and ferritin, however, was expected owing to their inversely related effects in regulating body iron stores (32).

Selenium, which is necessary for adequate glutathione peroxidase protection of cells from oxidative damage, was, in line with other studies (33), not associated with any of the outcomes. However, it is noticeable that the Norwegian children in the present study were found to have the lowest levels of plasma selenium throughout Europe in the original SARI study (15).

Bilirubin was not significantly related to the outcomes reported in our study, and there was no confirmation of associations between bilirubin with vitamin E or albumin as co-antioxidants (11, 27). Transient relief of severe asthma symptoms during hyperbilirubinemia has been reported (34), but we found no relationship between bilirubin levels and asthma control.

Strengths and limitations of this study

The present case-control study was sufficiently powered to detect significant differences in albumin levels; thus, the lack of associations with other analysed antioxidants is unlikely to be explained by type II errors alone. Owing to the cross-sectional design, we were unable to detect possible causative associations but could observe temporal associations between clinically expressed disease and markers of inflammation and markers of oxidant and antioxidant activity. We chose to perform separate analyses of antioxidants and ferritin rather than in a compiled kit of plasma reactive oxidants, as it strengthens the understanding of the analyses and biological associations. The present study was not designed to observe possible discriminatory capacity of the inflammatory markers for asthma and allergic rhinitis in a general population, and the potential clinical value of the observed associations must be assessed in further studies.

FeNO is a marker of both airway inflammation and oxidative stress (35); thus, we decided priori to investigate whether a high FeNO, as an inflammatory marker, was associated with oxidative state. The cut-off level for FeNO of 20 (ppb) was obtained based upon a previous study from Israel (21) and appeared to be an appropriate marker of inflammation in asthma, as only one control subject (with allergic rhinitis) had levels above this cut-off. This conservative cut-off estimate is supported by a recently published reference limit for healthy 10-yr-old children in Oslo assessed at 16.7 (ppb) (36). Lung function is found to be of limited discriminatory value for asthma in school-aged children (37) and was thus not chosen as one of the outcomes.

As only 4 of the controls had allergic rhinitis, this group was too small for relevant statistical analyses of associations between control children with allergic rhinitis and levels of antioxidants, ferritin and Hs-CRP.

Conclusion

Children with asthma had reduced levels of the major serum antioxidant albumin, and reduced albumin was associated with increased FeNO, a marker of allergic inflammation in asthma. Poorly controlled asthma was associated with further imbalance in oxidative state. Complex associations between oxidative state and inflammation in asthma and allergic disease need further elaboration.

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