

Decreased glutathione and low catalase activity contribute to oxidative stress in children with α -1 antitrypsin deficiency

ABSTRACT

Background Recent investigations in animal models have revealed oxidative stress and oxidative damage in the pathogenesis of alpha-1 antitrypsin deficiency (AATD). However, no data are available on the oxidative stress status and antioxidant enzyme activity in these patients. This study was aimed to analyse the oxidative stress profile and enzymatic antioxidant defence mechanisms in children with AATD.

Methods Oxidative stress parameters and the activity of the main antioxidant enzymes were prospectively measured in serum of fifty-one children diagnosed with AATD and thirty-eight control individuals.

Results Oxidative stress was increased in the serum of children with intermediate- (MZ; SZ) and high-risk (ZZ) phenotypes for developing AATD-related emphysema and/or liver disease. When compared with the control group, intermediate- and high-risk groups showed significantly lower total glutathione and reduced glutathione levels, decreased catalase activity and increased glutathione peroxidase activity leading to an accumulation of hydrogen peroxide that would explain the significantly increased levels of oxidative stress biomarkers observed in these patients. No differences were observed between the control (MM) and the low-risk (MS; SS) groups. A gradation in oxidative stress parameters was observed when patients were compared among themselves, in that the expression of the Z allele produces a higher

oxidative stress status in homozygous (ZZ) than in heterozygous (MZ; SZ) patients.

Conclusions Increased oxidative stress, together with reduced antioxidant defence are involved in the pathophysiology of AATD at early stages, opening up a new rationale for the use of antioxidant therapies in the treatment of the disease.

α -1 Antitrypsin deficiency (AATD) is a rare genetic and hereditary condition characterised by low circulating levels of the α -1 antitrypsin (AAT) protein, a serine protease inhibitor that protects lung tissues from damage caused by proteolytic enzymes, such as neutrophil elastase and proteinase-3. Deficiency of circulating AAT is associated with an increased risk of developing emphysema, hepatic cirrhosis, panniculitis, bronchiectasis and vasculitis.¹

Clinical data indicate that there is a great variability in the severity of the symptoms found in patients with AATD, and neither plasma AAT levels nor phenotype are sufficient to identify those patients who will develop severe lung or liver disease, indicating that there may be other mechanisms, in addition to the protease-antiprotease imbalance, that contribute to the development of emphysema or liver disease.² Recent studies in animal models have shown an association between oxidative stress (OS) and the pathophysiology of AATD.^{3,4}

We hypothesised that OS is increased in patients with AATD at early stages before the development of severe clinical manifestations. Thus, the present study was undertaken to investigate the role of OS in children with AATD. The OS status was evaluated in plasma by monitoring the total glutathione, the oxidised versus reduced glutathione ratio (GSSG/GSH), the oxidation products 8-hydroxydeoxyguanosine

(8-OHdG), malonyldialdehyde (MDA) and protein carbonyl (PC), and the activity of the main antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRd).

Fifty-one children diagnosed with AATD, and 38 control individuals, were prospectively included in the study. Demographic and clinical characteristics of patients included in the study are presented in online supplementary table S1. There were no differences in age, sex, Body Mass Index and lung or liver damage markers, and all the children were asymptomatic according to their physical status and the pulmonary and liver tests. Intermediate- (MZ; SZ) and high-risk (ZZ) patients showed significantly higher GSSG/GSH ratio ($p=0.001$; $p<0.001$, respectively), MDA ($p=0.004$; $p<0.001$), 8-OHdG ($p<0.001$; $p<0.001$) and PC ($p=0.010$; $p=0.002$) than the control group (MM). When compared with the control group, intermediate and high-risk patients showed significantly lower total glutathione ($p<0.001$; $p<0.001$), GSH levels ($p<0.001$; $p<0.001$), decreased CAT activity ($p=0.003$; $p<0.001$) and increased GPx activity ($p=0.040$; $p<0.001$) leading to an accumulation of hydrogen peroxide ($p=0.040$; $p=0.001$) (table 1 and see online supplementary figures E1–E4) that would explain the significantly increased levels of OS biomarkers observed in these patients (see online supplementary figure E5). No differences were observed between the control and the low-risk (MS; SS) groups. A gradation in OS parameters was observed when patients were compared among themselves, in that the expression of the Z allele produces a higher OS status in homozygous (ZZ) than in heterozygous (MZ;SZ) patients (see online supplement

Table 1 Biomarkers of oxidative stress in AATD and control children

Oxidative stress biomarkers	Control (MM)	Low risk (MS; SS)	Intermediate risk (MZ; SZ)	High risk (ZZ)	p Value
GSSG/GSH (%)	2.42 (1.97–3.57)	2.62 (1.07–3.77)	4.14 (2.60–6.32)	6.47 (4.56–9.50)	<0.001
TG (μ M)	2930 (2592–3687)	2684 (2261–2831)	1818 (1474–2535)	1630 (1292–1800)	<0.001
GSH (μ M)	2864 (2532–3620)	2183 (1947–2622)	1741 (1464–2326)	1713 (1376–1937)	<0.001
GSSG (μ M)	72.98 (63.96–91.81)	85.86 (75.32–108.5)	64.0 (32.11–102.2)	75.60 (68.05–101.6)	0.254
MDA (pmol/mg)	0.85 (0.75–1.07)	0.72 (0.52–1.85)	1.15 (0.85–1.68)	1.94 (1.44–3.76)	<0.001
PC (nmol/mg)	0.15 (0.12–0.20)	0.16 (0.13–0.23)	0.17 (0.16–0.18)	0.25 (0.19–0.26)	0.010
8-OHdG (ng/mL)	0.61 (0.54–0.71)	0.68 (0.60–0.82)	0.97 (0.68–1.11)	2.00 (1.16–2.54)	<0.001
SOD (U/mL)	5.38 (4.56–5.61)	5.90 (4.38–6.30)	6.16 (5.41–7.53)	5.76 (3.78–6.17)	0.080
CAT (nmol/min/mL)	37.66 (32.05–40.57)	36.95 (31.39–43.01)	26.92 (16.47–38.89)	17.67 (14.05–23.26)	<0.001
GPx (nmol/min/mL)	21.52 (18.32–23.18)	22.16 (20.79–24.48)	24.52 (19.37–26.79)	32.35 (29.16–37.05)	<0.001
GRd (nmol/min/mL)	3.69 (3.41–4.38)	3.04 (2.79–4.97)	4.46 (2.67–5.22)	4.20 (2.86–4.87)	0.610
H ₂ O ₂ (fluorescence units)	198.50 (138.3–333–3)	251.0 (212.0–335.0)	323.0 (258.5–350.0)	584.0 (369.0–601.0)	0.001

All values are expressed as median (IQR). Statistic significance after application of Kruskal-Wallis test. p Values lower than 0.05 were statistically significant (labelled in bold).

AATD, α -1 antitrypsin deficiency; 8-OHdG, 8-hydroxydeoxyguanosine; CAT, catalase; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, reduced glutathione; GSSG, oxidised glutathione; MDA, malonyldialdehyde; PC, protein carbonyls; SOD, superoxide dismutase; TG, total glutathione.

for results and discussion on multiple hypothesis testing).

In conclusion, our study supports the hypothesis that OS is a feature of AATD at early stages, and is associated with the presence of AAT Z protein. An increased chronic oxidative status could contribute to the higher risk of lung and liver damage observed in these patients, suggesting a rationale for the use of antioxidant therapies in the treatment of the disease.⁵

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