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# Relationship of Oxidant and Antioxidant Markers to Asthma Severity in Egyptian Asthmatic Children

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## Abstract

**BACKGROUND:** Asthma is a chronic airway disease which is characterized by oxidant antioxidant imbalance with the generation of oxidative stress related mediators.

**AIM:** The study aimed to evaluate the role of asymmetric dimethylarginine, and malondialdehyde as oxidant markers and serum paraoxonase activity as an antioxidant marker in asthma, and to determine their relationship to the asthma severity and lung function among asthmatic children in Egypt.

**PATIENTS AND METHODS:** This case control study was conducted on sixty patients with asthma compared with sixty apparently healthy children of matched age and sex.

**RESULTS:** Serum concentrations of oxidant markers as asymmetric dimethylarginine and malondialdehyde were significantly increased in asthmatic patients while anti-oxidant marker as paraoxonase activity was significantly decreased compared to healthy controls ($P < 0.05$). ANOVA test revealed highly significant elevation of the serum concentrations of oxidant markers while anti-oxidant marker was significantly decreased in severe asthmatic patients ($P < 0.001$) compared to the patients with moderate and mild asthma respectively. Serum malondialdehyde concentration was a strong predictor of asthma severity by multiple regression analysis ($P < 0.05$).

**CONCLUSION:** The study revealed an imbalance between oxidative and antioxidant defence systems in asthmatic children. Serum concentration of malondialdehyde was the most predictive biomarker having a significant association with asthma severity.

## Introduction

Asthma is the most frequent chronic inflammatory airway disease in children [1]. Its prevalence has increased during the last four decades, affecting more than one-third of children from the general population [2, 3]. Although the exact mechanism of the pathogenesis of asthma is unknown, asthma is characterized by oxidant-antioxidant defence systems imbalance with increased generation of oxidative stress related mediators in asthmatic patients [4] that cause an oxidative injury in asthma, and increased airway reactivity [5, 6].

Little is known about the role of asymmetric dimethylarginine (ADMA) in the pathogenesis of asthmatic airway inflammation [7]. The lung is a major source of ADMA [8] that can promote oxidative stress by a reduction in nitric oxide synthesis which would result in higher levels of peroxynitrite, that causes oxidative cell damage, and exacerbate airway inflammation [9]. ADMA can modify lung function, increase airway hyperreactivity even in non-inflamed airways, and promote lung collagen production and deposition [10]. Increased ADMA in serum has been found to be associated with the severity of symptoms of asthma in obese adults [11], and endothelial dysfunction [12-13].

Malondialdehyde (MDA) is an oxidant marker of pulmonary oxidative stress, and lipid peroxidation [14]. Paraoxonase, an antioxidant enzyme may play a protective role in asthma. It hydrolyzes lipid peroxides and prevents low-density lipoprotein (LDL) oxidation [15].

Therefore, the aim of our present study was to evaluate the role of ADMA, malondialdehyde, and paraoxonase activity in asthma among asthmatic children and to determine the relationship of these biomarkers to the underlying aetiology, the clinical severity and lung function among asthmatic children in Egypt.

## Subjects and Methods

### *Subjects*

This descriptive cross sectional case control study was carried out on a total of sixty asthmatic children aged between 6 to 12 years (mean 8.35± 3.53 years) were studied. They were classified according to the severity of asthma that was determined using pulmonary function tests into three subgroups as follows; twenty patients had mild persistent asthma in group I, twenty patients had moderate persistent asthma in group II, and twenty patients had severe persistent asthma in group III. The inclusion criteria for selection included all known asthmatic patients who had been regularly attending the Pediatric Allergy and Immunology clinic, Abo El-Rish Children's Hospital, Cairo University, Egypt. Exclusion criteria included obese children, the presence of chronic heart, liver and kidney diseases, concomitant inflammatory disease such as infections and autoimmune disorders, and Diabetes mellitus. Patients taking antioxidant drugs, vitamins, diuretics, hormone replacement therapy were also excluded. All the children involved in the study did not have any symptoms of lower or upper respiratory tract infection or asthma exacerbation within the previous four weeks.

Sixty apparently healthy non-asthmatic children from public schools of matched age and sex were recruited as a control group with no history of atopic dermatitis, or allergic rhinitis/ conjunctivitis. They were selected from the outpatient's clinic at the National Research Center. They were attending the clinic for follow-up. The study was approved by the Research Ethics Committee of the National Research Center with ethical approval number 17022. Written informed consent was obtained from the parents of the participating patients and controls.

### *Methods*

All patients and controls were subjected to a complete physical examination and anthropometric measures. All measurements were made according to techniques described in the Anthropometric Standardization Reference Manual [16]. Weight (in kg) was taken using a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height (in cm) was measured using a Seca 225 stadiometer to the nearest 0.1 cm with the children dressed in minimal clothes and without shoes. Each measurement was taken as the mean of three consecutive readings following the recommendations of the International Biological program [17]. BMI and growth percentiles for all children were calculated based on the WHO growth standards with the help of Anthro-Program [18].

Information on age, sex, parental consanguinity, family history of bronchial asthma, duration of illness and treatment modalities were collected via a questionnaire from parents. Patients were diagnosed according to the clinical manifestations of asthma (a cough, wheezing, shortness of breath, and exercise intolerance) according to GINA [19], and the clinical severity of the asthma was determined by dynamic spirometry.

Dynamic spirometry (Master screen Pneumo, Erich Jaeger GmbH, Germany) was performed in all the patients and the healthy controls. The following data were obtained; forced vital capacity FVC (litre), forced expiratory volume in the first, second FEV1 (litre), FEV1/FVC ratio or FEV1%. For every parameter obtained, actual and predicted values for age, sex, height, weight and percentage (%) of the predicted were calculated. The highest values of three forced expiratory manoeuvres were used. The ratio FEV1/FVC is a measure of airflow obstruction. These measurements were performed according to the standards of the European Respiratory Society and the American Thoracic Society [20].

Skin prick testing for aeroallergen sensitivity was done using lancets (Staller point, Paris, France) providing a standard puncture of 1 mm. Commercial allergen solutions manufactured by Allergopharma (Joachim Ganzer KG, Reinbeck, Germany) were used for the skin test. Forty-four different allergens consisting of house dust mite, grass, wild grass, tree pollens, fungi, animal dander, and insects were tested. Test sites were evaluated 20 min after allergen application using European Academy of Allergy and Clinical Immunology criteria [21].

Overnight fasting venous blood samples were collected from all patients and controls into empty tubes, and the separated serum was stored at -80 ºC until they were used. Serum ADMA concentrations were determined by Sandwich- enzyme linked immunosorbent assay (ELISA) according to the method described by Schulze et al., [22]. In addition, malondialdehyde measurement was assayed according to the method described by Hunter et al., [23], and results are expressed as nmol/ml. Paraoxonase activities were measured according to Gan et al., [24]. Serum paraoxonase activity was expressed as U/L. The cutoff value was calculated from healthy control samples. Quantitative determination of serum total immunoglobulin E (IgE) was performed using ELISA according to the manufacturer's guidelines [25]. Also, C-reactive

protein and erythrocyte sedimentation rate were also assayed for all children.

### *Statistical Analysis*

Statistical analyses were performed using the SPSS statistical package software for Windows version 21 (SPSS Inc., Chicago, Illinois, USA). Parametric variables are expressed as the mean ± SD. Differences between parametric variables among the controls and the studied patient's groups were analysed using two tailed unpaired t-tests. Pearson's correlation coefficients were used to evaluate correlations between the data exhibiting parametric distribution. The comparison between groups was performed with one-way analysis of variance (ANOVA). Multiple regression analysis was performed to examine the relationship between serum levels of asymmetric dimethylarginine, malondialdehyde and paraoxonase activity with asthma severity. A P value < 0.05 was considered significant and p < 0.005 was considered highly significant.

## Results

A total of sixty asthmatic children aged between 6 to 12 years (mean 8.35± 3.53 years) were studied. Positive family history of asthma was present in 15 patients (25%), and positive history of recurrent hospitalization was present in 18 patients (30%). According to the parents' history, none of them was exposed to secondhand smoke.

**Table 1: Comparison of anthropometric measures, pulmonary function parameters, and laboratory findings of the studied patients and the control groups**

| Variables | | Total patients group N = 60 Mean ± SD | Control Group N = 60 Mean ± SD | Total Patients vs controls P-value |
|---|---|---|---|---|
| | BMI | 19.27 ± 3.05 | 17.35 ± 1.98 | 0.90 |
| Anthropometric measures | Height/age percentile | 13.98 ± 15.72 | 39.05 ± 5.18 | 0.000** |
| | Weight/age percentile | 40.06 ± 24.24 | 42.15 ± 14.73 | 0.95 |
| Pulmonary function parameters | FVC (% of predicted) | 71.9 ± 1.31 | 95.28 ± 2.65 | 0.000** |
| | FEV1 (% of predicted) | 72.6 ± 1.44 | 95.4 ± 2.47 | 0.000** |
| | FEV1/FVC ratio | 72.6 ± 1.44 | 94.96 ± 2.73 | 0.000** |
| | Serum paraoxonase (U/ml) | 143.23 ± 18.67 | 212.14 ± 19.15 | 0.000** |
| Laboratory findings | Serum MDA (nmol/ml) | 2.48 ± 0.59 | 1.23 ± 0.19 | 0.000** |
| | Serum ADMA (mg/dl) | 2.96 ± 0.83 | 1.17 ± 0.45 | 0.001** |
| | Serum IgE (U/ml) | 51.7 ± 16.56 | 8.45 ± 5.46 | 0.000** |
| | ESR (mm/hr) | 18 ± 3.41 | 4.37 ± 0.86 | 0.000** |
| | CRP(mg/ml) | 51.7 ± 16.56 | 4.5 ± 0.81 | 0.000** |

*Significant difference at p < 0.05, **highly significant difference at p < 0.005.

The mean BMI and weight for age percentile of the studied patients were within normal range. The mean height for age percentile of the studied patients' group was highly significant lower compared to the control group (13.98 ± 15.72 versus 39.05 ± 5.18 respectively, P < 0.001). The mean forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and FVC/FEV1 ratio of the total patient's group was found to be highly significantly lower compared to the control group (P < 0.05). Serum concentrations of ADMA, MDA, IgE, ESR, and CRP were highly significantly increased among asthmatic patients compared to healthy controls (P < 0.001). Serum paraoxonase activity was significantly decreased in asthmatic patients compared to healthy controls (P < 0.05) as shown in Table 1.

The studied patients were divided according to the degree of severity (mild, moderate, and severe). Significant differences in height for age percentile between the three groups with the highest value were present in severe asthmatic patients (F = 3.35, P < 0.05). No significant differences were present between patients' subgroups as regards the mean BMI, and weight for age percentiles (P > 0.05). ANOVA test revealed highly significant elevation of the serum concentrations of ADMA, MDA, and CRP in severe asthmatic patients compared to patients with moderate and mild asthma respectively (P < 0.001). The mean serum paraoxonase activity was found to be highly significantly lower in the patients with severe asthma compared to those with moderate and mild asthma (P < 0.05), as shown in Table 2. Figure 1 showed the comparison of laboratory findings of the studied patients' subgroups.

**Table 2: Comparison of the anthropometric measures, and laboratory findings of the studied patients' subgroups (ANOVA)**

| Variables | | Mild- (group I) N = 20 Mean±SD | moderate (group II) N = 20 Mean±SD | Severe (group III) N = 20 Mean±SD | F | P-value |
|---|---|---|---|---|---|---|
| Anthropo-metric measures | BMI | 19.48±2.72 | 19.26±3.78 | 19.14±2.79 | 0.036 | 0.965 |
| | Height / age percentile | 21.05±18.83 | 13.91±9 | 10.64 ±8.75 | 3.35 | 0.048** |
| | Weight / age percentile | 22.36±16.82 | 29.44±22.52 | 36.29± 29.08 | 1.22 | 0.306 |
| Laboratory findings | Serum paraoxonase (U/ml) | 149.79±19.96 | 142.52±13.85 | 135.49±18.21 | 2.31 | 0.049* |
| | Serum MDA (nmol/ml) | 2.07±0.15 | 2.44±0.46 | 3.07±0.7 | 12.59 | 0.000** |
| | Serum ADMA (mg/dl) | 1.37±2.5 | 2.36± 1.43 | 3.28±1.28 | 6.96 | 0.003** |
| | IgE (U/ml) | 46.6± 15.73 | 37.84±7.93 | 65.29±10.67 | 22.69 | 0.000** |
| | CRP (mg/dl) | 37.84±7.93 | 55.3±10.67 | 66.6±15.73 | 22.69 | 0.000** |

*Significant difference at p < 0.05; **highly significant difference at p < 0.005.

Serum concentrations of ADMA and MDA were significantly increased with increasing severity of asthmatic attack (P < 0.001), while serum paraoxonase activity was significantly decreased with increasing severity of asthmatic attack (P < 0.05). Serum ADMA concentrations were significant negative correlated with the percent predicted forced expiratory volume in one second (FEV1). Serum MDA concentration was significant negative correlated with the percent predicted forced vital capacity, and FEV1/FVC ratio, while serum paraoxonase activity was found to be significant positive correlated with the percent predicted forced vital capacity (FVC), and the FEV1/FVC ratio (P < 0.05), as shown in Table 3.

Serum paraoxonase activity was found to be significant negative correlated with serum concentrations of ADMA, malondialdehyde, IgE, CRP

and, ESR. Serum concentrations of ADMA and malondialdehyde were significantly positive correlated with IgE, CRP and, ESR, as shown in Table 4.

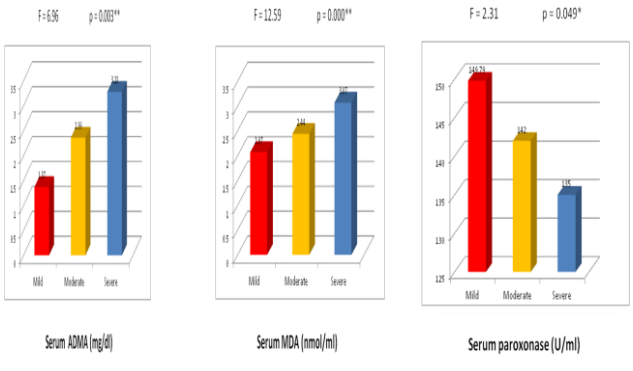


*Figure 1: Comparison of laboratory findings of the studied patients' subgroups*

To confirm the previous correlations, multiple regression analysis was done showing the association between the different biomarkers studied and disease severity in Table 5. Serum concentration of malondialdehyde was the only predictive biomarker having a significant association with disease severity (OR = 11.278, 95% CI = 1.145-111.082 and P = 0.038).

**Table 3: Correlations between parameters of the pulmonary function and serum biomarkers of the oxidative status in the asthmatic patients**

| Variables | FEV1 | FVC | FEV1/ FVC ratio |
|---|---|---|---|
| Serum paraoxonase (U/ml) | 0.142 | 0.38* | 0.35* |
| Serum MDA (nmol/ml) | 0.188 | -0.32* | -0.33* |
| Serum ADMA (mg/dl) | -0.491** | -0.007 | 0.28 |
| Serum IgE (U/ml) | -0.859** | -0. 87** | -0. 88** |
| ESR (mm/h) | 0.433** | -0.394* | -0.0005 |
| CRP (mg/dl) | -0.89 | -0.90** | -0.92** |

*Significant difference at p<0.05, **highly significant difference at p<0.005.

## Discussion

Asthma is a chronic airway inflammation which characterized by recurrent episodes of wheezing and coughing which related to increased oxidative stress [2]. To the best of our knowledge, there is no available information in the literature discussing the role of malondialdehyde, asymmetric dimethylarginine, and paraoxonase activity in airway inflammatory diseases among asthmatic children in Egypt. Therefore, this current study is considered to be the first clinical study that was carried out to determine the role of these biomarkers in asthma pathogenesis and to determine their relationship to the underlying aetiology, the clinical severity and lung function among asthmatic children in Egypt.

In our present study, shortness of breath, chronic recurrent episodes of wheezing and chest tightness, coughing, chest discomfort, and loss of appetite were the main symptoms among children with asthma. The symptoms score were significantly higher among the patients with severe asthma compared to those with mild and moderate asthma. This is in agreement with Koterba and Saltoun [26].

**Table 4: Correlations between the laboratory findings of the studied patients**

| Variables | Serum MDA | Serum ADMA | Serum IgE | ESR | CRP |
|---|---|---|---|---|---|
| Serum paraoxonase (U/ml) | -0.7** | -0.4** | -0.737** | -0.783** | -0.761** |
| Serum MDA (nmol/ml) | 1 | 0.138 | 0.688** | 0.805** | 0.712** |
| Serum ADMA (mg/dl) | 0.138 | 1 | 0.201* | 0.289** | 0.218* |

*Significant difference at p < 0.05; **highly significant difference at p < 0.005.

Regarding anthropometric measures, in our present study, the mean height for age percentile of the studied patients' group was highly significantly lower compared to the control group (13.98 ± 15.72 versus 39.05 ± 5.18 respectively, P < 0.001), and were highly significantly lower in the patients with severe asthma compared to patients with moderate and mild asthma respectively by ANOVA test. This is in agreement with Elnady et al., [27], who reported that the mean height for age percentile of the asthmatic group was highly significantly lower compared to the control group. Our explanation is that stunting in asthmatic patients could be due to a chronic disease condition, and the effect of long-term corticosteroid therapy on bone.

**Table 5: Multiple logistic regression between disease severity and serum biomarkers**

| Variables | B coefficient | OR | 95% CI | P |
|---|---|---|---|---|
| Serum paraoxonase (U/ml) | -0.036 | 0.964 | 0.916-1.015 | 0.167 |
| Serum MDA (nmol/ml) | 2.423 | 11.278 | 1.145-111.082 | 0.038* |
| Serum ADMA (mg/dl) | 0.372 | 1.451 | 0.033-644.261 | 0.905 |

Dependent variable: disease severity. *Significant difference at p<0.05.

Our present study revealed a highly significantly elevation of the mean serum level ADMA among all asthmatic children studied compared to controls (P < 0.005). This is in agreement with the study conducted by Lau, et al., [28], who reported that the concentrations of asymmetric dimethylarginine (ADMA) had been increased in serum of adults and sputum samples, as well as exhaled breath condensates of pediatric patients with asthma, compared to controls.

In our present study, serum ADMA concentration was found to be highly significantly increased in the patients with severe asthma (P < 0.001) compared to those with moderate and mild asthma, respectively. This is in agreement with Scott et al., [29], who mentioned that increased serum ADMA had been discovered to be related to the severity of asthma.

In this current study, serum ADMA

concentration was found to be significant negative correlated with the percent predicted forced expiratory volume in one second (FEV1). This argument is consistent with the findings of Lara, et al., [30], and Holguin et al., [11], who pronounced that ADMA concentration in peripheral plasma samples from patients with severe asthma correlated with worsening of the symptom scores, predicted FEV1%, and FVC%. Also, Carraro, et al., [31] observed the changes in pulmonary function testing causing a decline in FEV1% predicted in asthmatic patients, together with a significant increase in sputum ADMA ($P < 0.05$), especially after allergen challenge in asthmatic patients with mild allergic asthma, and concomitant with the onset of airways obstruction.

Serum malondialdehyde concentration is one of the most frequently used indicators of lipid peroxidation of the membranes that results from oxidative damage. This implies that children during an acute asthmatic attack are exhibit enhanced lipid peroxidation [32]. Given the present data, the serum concentration of MDA was highly significantly increased in the studied patients compared to controls ($P < 0.001$), and in severe asthma compared to the studied patients with moderate and mild asthma ($P < 0.001$). Serum MDA concentration showed significant negative correlation with the percent predicted FVC and FEV1/FVC ratio. Serum MDA level was the most predictive biomarker having a significant association with asthma severity ($P < 0.05$).

In our present study, the mean paraoxonase activity of the studied patients was found to be highly significantly lower compared to the healthy control group, ($P < 0.001$). This is in agreement with the study conducted by Cakmak, et al., [33], who determined that the enzyme paraoxonase activity had an antioxidant function and was low in asthmatic children. Also, our current study is in agreement with the study conducted by Can et al., [34] who observed that asthmatic patients had reduced paraoxonase activity. They explain the evidence of elevated paraoxonase activity in asthmatic patients after treatment, may indicate that paraoxonase could play a role in asthma.

Our present study revealed a highly significantly lower serum paraoxonase activity in the patients with severe asthma compared to those with moderate and mild asthma respectively ($P < 0.05$). Its serum activity was positively correlated with the percent predicted FVC and FEV1/FVC ratio. It became notably decreased with increasing severity of asthmatic attack ($P < 0.001$). Our study is in agreement with the study conducted by Górnicka et al., [35], who suggested that serum paraoxonase activity was significantly decreased with increasing severity of the asthmatic attack.

In our asthmatic children, serum paraoxonase activity showed significant negative correlation with serum concentrations of ADMA and MDA. It means that increased serum oxidative stress mediators as ADMA and malondialdehyde concentrations were associated with reduced paraoxonase activity in asthmatic children supports their role for these mediators in asthma pathogenesis (in all, $P < 0.05$). This is in agreement with Ekmekci et al., [36], and Kabaroglu et al., [37], who mentioned that an inverse relationship between reduced serum paraoxonase activity and increased oxidative stress biomarkers in asthmatic patients. An increase in oxidative stress and a lower in paraoxonase activity may be important contributors to the acceleration of the progression of attack in asthmatic patients [38].

In conclusion, our results revealed that an imbalance between oxidative (ADMA, and MDA) and antioxidant biomarkers (paraoxonase activity) in asthmatic children. Serum malondialdehyde concentration was the most predictive biomarker having a significant association with asthma severity. Early identification of these biomarkers may help in identifying those at risk of severe asthma.

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