

Research Article



Clinicobiological Interrelations between Asthma Severity, Duration of Disease and Some Markers of Allergic Inflammation in Asthmatic Pediatric Population in Annaba (Algeria).

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ABSTRACT

The aim of this study was to study the relationships between the severity of asthma and associated allergic symptoms with the biological parameters of inflammation. 118 children were recruited: 68 asthmatics aged 2-15 years (sex ratio M/F: 1.72) and 50 healthy children aged 1-15 years (sex ratio M/F: 1.5). A standard questionnaire was completed by their GP. Total IgE levels, total protein, eosinophilia and blood levels of malondialdehyde (MDA) and glutathione peroxidase (GPx) were assayed. Atopic disease was present in 72% of children with asthma, in whose home environment high levels of allergens were found. All biological parameters measured were significantly higher in sick children (33% were in the third level GINA) than those of healthy children. Serum total IgE levels were very highly significantly correlated with asthma severity ($p < 0.001$) and MDA levels ($p < 0.001$) and highly significantly correlated with GPx levels ($p < 0.01$). The allergy score was correlated with eosinophilia only ($p < 0.05$). Atopic disease and an allergenic environment were strongly present in the paediatric population studied. The severity of the disease, proven clinically and through blood markers, is associated with many more allergic symptoms. Asthmatic disease, probably inadequately treated, has been present for a long time.

Keywords: Childhood asthma; severity; duration of disease; IgE.

INTRODUCTION

Allergic asthma is a public health problem. In the last few decades, the worldwide prevalence of this disease has doubled and it is estimated that 5 to 10 % of people suffer from this disease, in particular children¹. This is also the case in Algeria where 3.4 % of the population is asthmatic², a majority of whom are children. This marked increase in the incidence of asthma and of allergies cannot be explained by genetic factors alone and it is suggested that in addition to hereditary factors, environmental factors have a significant role in this pathology^{3,4}.

Although numerous studies, such as that conducted by Professor Isabelle Annesi Maesano's team⁵, have evoked phenotypical and environmental factors in severe asthma and allergy, few studies have precisely determined the interrelationships between asthma severity, the concomitant allergic manifestations and the biological markers of inflammation such as those of oxidative stress which characterise this disease.

This is the subject proposed in our study which analyses data from a clinical questionnaire including information about the indoor environment of a child population living in Annaba (Algeria).

We also performed blood assays of total IgE, total protein and eosinophilia and we assessed some oxidative stress parameters by blood assays of malondialdehyde (MDA) and glutathione peroxidase (GPx).

Lastly, statistical tests were used to examine the various relationships between the study parameters.

MATERIALS AND METHODS

Patients

This study involved 118 children: 68 asthmatics aged 2 to 15 years (sex ratio M/F: 1.72) and 50 healthy children aged 1 to 15 years (sex ratio M/F: 1.5). They were recruited between April 2011 and January 2012. All the children lived in the town of Annaba (far north-east Algeria) and all the parents of the children gave their informed consent. This study, complying with ethical requirements, was carried out in the context of a national research project PNR/MERSS.

Questionnaire

A standard questionnaire was completed by the patient's GP. It included clinical data and also information on the patient's home environment. These data were used to classify the asthmatic children into four levels of severity of the asthma according to the GINA scale. A second allergy score, also ranging from 1 to 4, and including the principal allergic manifestations of the disease, was established. Scores were considered positive from the 2nd level (GINA) and from the 3rd symptom for the allergy score.

Blood sampling

Blood was collected from fasting subjects by venous puncture into a heparinised tube for assay of total serum



IgE. Oxidative stress parameters were analysed in the globular sediment obtained after centrifugation of the same tube.

Determination of total IgE concentrations

Total IgE concentrations were assayed by ECLIA electrochemiluminescence immunoassay on Roche Elecsys analyser⁶. It is a two step sandwich method using two anti-IgE monoclonal antibodies, one specific for biotin and the other for ruthenium. After formation of the sandwich, the streptavidin-coated microparticles are held by a magnet. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplier. The results are read on a calibration curve. The recommended cut-off values are as follows: 60 IU/mL (from 1 to 5 years); 90 IU/mL (from 6 to 9 years); 200 IU/mL (from 10 to 15 years); 100 IU/mL (over 15 years).

Determination of eosinophilia

A blood smear was prepared by placing a drop of blood on a microscope slide. This smear was then fixed with methanol for 2 to 3 minutes and then stained with May-Grünwald-Giemsa.

A count is then performed under the microscope, of 200 blood cells, all types. The generally accepted cut-off value for children is 500 cells/ μl ⁷.

Total protein assay

Protein concentration was determined using the BRADFORD method which employs Coomassie blue G250 as reagent. This reagent reacts with the amine groups of protein forming a blue coloured complex.

Optical density (OD) at 595 nm is then read with a spectrophotometer. The protein concentration is determined by comparison with a standard range of bovine serum albumin (BSA) (1mg/ml) previously measured under the same conditions.

Assay of oxidative stress parameters

The globular sediment obtained after removal of the serum is milled, washed in two aliquots of an isotonic solution, and cold centrifuged.

The supernatant or the lysate of erythrocytes obtained is collected for the assay of oxidative stress parameters.

The marker of lipid peroxidation, or malondialdehyde (MDA), is evaluated by measuring substances reacting with thiobarbituric acid (TBA) according to the method of Esterbauer.⁸

MDA concentration is expressed in nmol/mg of protein. Glutathione peroxidase (GPx) activity was determined by the method of Flohe & Gunzler (1984)⁹, based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH).

The concentration of GPx is expressed in μmol GSH/mg of protein.

Statistical Analysis

Table 1: Descriptive statistics

	Healthy population	Asthmatic population
Number of subjects	50	68
Mean age (years)	7.09	8.57
Sex ratio (M/F)	30/20	43/25
Familial atopy (%)	14	72
Duration of the disease (mean)	-	4.53
GINA score (%)		
Intermittent asthma	-	13
Mild persistent asthma	-	29
Moderate persistent asthma	-	33
Severe persistent asthma	-	23
Location of the homes (urban area/ rural area)	30/20	45/23
Presence of smokers (%)	10	26
Presence of pets (%)	2	5
Presence of moulds (%)	10	89

Student's *t* test was used to compare the means of the two groups (asthmatic children and healthy children). The impact of allergic asthma on oxidative stress was obtained by the Chi2 test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Description of the population

The descriptive statistics of the population, compiled from the questionnaires are given in table 1. We observed that familial atopy was present in 72 % of asthmatic children and in 14 % of healthy children. Over 73 % of asthmatic children had a history of at least one personal atopic manifestation other than allergic asthma, the most significant of which was allergic rhinitis which was found in 73.52 % of them (figure 1).

According to the GINA classification, we found that the majority, amounting to 33 % of asthmatic children, had moderate persistent asthma.

The indoor environment of asthmatic children was rich in aeroallergens, as shown by the percentages displayed in table 1 which gives the figures for animal hair, moulds and passive smoking. Sixty six percent of asthmatic children lived in the urban zone of the town of Annaba.

Clinical scores and biological parameters

Mean values for the biological parameters for the two populations are shown in table 2. Our results show that total IgE levels in asthmatic children are high compared with those in healthy children. This difference is highly significant ($p < 0.01$). Eosinophilia is also significantly higher in sick children compared with healthy children ($p < 0.05$).



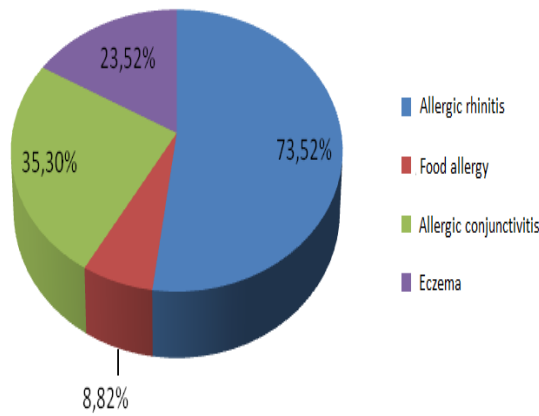


Figure 1: Distribution of personal allergic manifestations in the asthmatic population.

Table 2: Mean values of the biological parameters in the two populations

Parameters	Healthy children	Asthmatic children
Total IgE (UI/ml)	122.48	1857.66**
Eosinophilia (cells/ μ l)	450	520*
MDA (nmol/mg of protein)	0.49	0.86*
GPx (μ mol GSH/mg of protein)	0.20	0.09*
Total protein (mg/ml)	7.26	10.67

*: $p < 0.05$ significant difference

** : $p < 0.01$ highly significant difference

Our results show that the concentration of MDA is higher in asthmatic children compared with that in healthy children ($p < 0.05$). Furthermore, glutathione peroxidase (GPx) enzyme activity is significantly decreased in the asthmatic population ($p < 0.05$).

The percentage of asthmatic children with total IgE concentrations and eosinophilia above normal values is high for the two scores, exceeding 50 % (see figure 2).

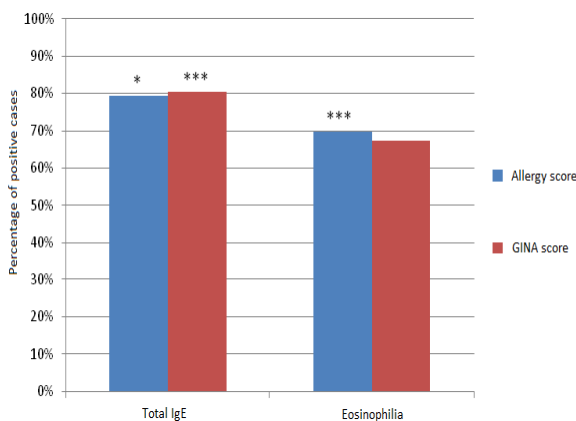


Figure 2: Percentage of cases positive for total IgE and eosinophilia in children with positive clinical scores.

The increase in the percentages of total IgE is highly significant ($p < 0.05$) and that of the eosinophilia is very highly significant ($p < 0.001$) for the allergy score.

We investigated the relationship between the GINA score and the percentage of positive cases for total IgE and eosinophilia. The results obtained show that the severity of the asthma is strongly related to the percentage of total IgE ($p < 0.001$). In contrast, there is no relationship with the percentage of positive cases for eosinophilia ($p > 0.05$).

Biological parameters and oxidative stress

The statistical analysis shows an association between total IgE levels and MDA concentration. This correlation is very highly significant ($\chi^2, p = 0.001$). Total IgE levels are also associated with GPx concentration in a highly significant ($\chi^2, p < 0.01$) manner. However, we did not find a correlation between eosinophilia and these two parameters (MDA and GPx).

Oxidative stress and clinical scores

We also investigated the relationship between the oxidative stress parameters and clinical scores (score GINA and allergy score). Figure 3, displaying the changes in the MDA and GPx levels according to severity of the asthma, shows that MDA concentration increases with the severity of the asthma and reaches its maximum levels in severe persistent asthma. In contrast, we note that GPx concentration decreases with asthma severity. The statistical analysis shows that MDA concentration is significantly related to asthma severity ($\chi^2, p = 0.05$). On the other hand, the decrease in GPx concentration is not related to asthma severity ($\chi^2, p > 0.05$). We also found that the allergy score is not statistically related to MDA concentration, nor to that of GPx ($\chi^2, p > 0.05$).

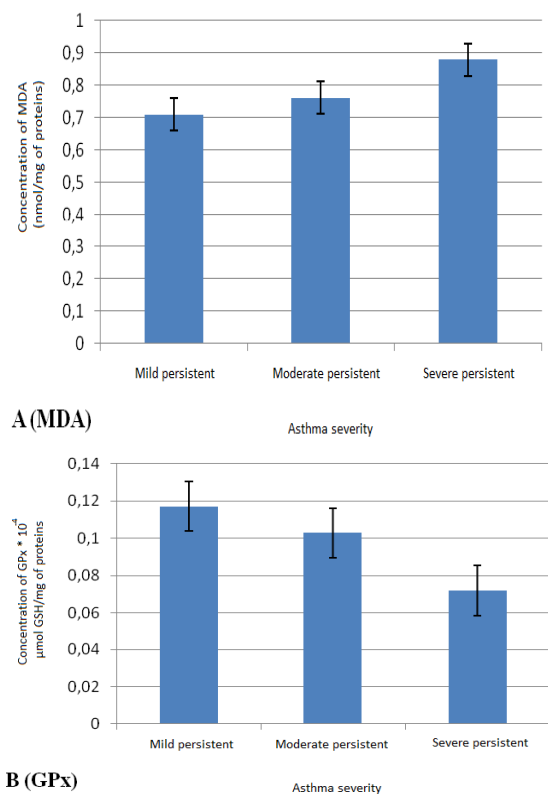


Figure 3: Variation in MDA (A) and GPx (B) concentrations in relation to asthma severity.

Clinical score and duration of the disease

Figure 4 shows the duration of the disease in relation to the clinical scores. We note that children with a longer duration of the disease (5.19 years) also have a higher number of allergic manifestations (score 4). Similarly, children who have been sick for 4.53 years and 4.7 years, have more severe asthma levels (GINA scores of 4 and 3, respectively).

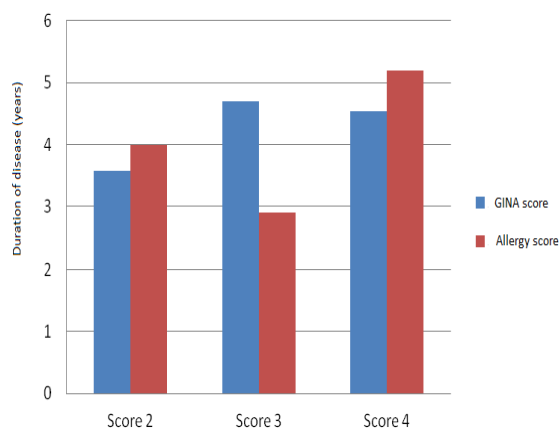


Figure 4: Variation in the duration of the disease in relation to clinical score in the asthmatic population.

DISCUSSION

The general description of the population reveals the existence of familial atopy in asthmatic children and this at a frequency which is very high (72 %), compared to that found in the control population (14%). This genetically determined tendency to produce IgE-type antibodies is already considered as a risk factor for the development of an allergic disease. However it is true that it is above all the interaction genes/environment which will determine the development of chronic inflammatory diseases, their onset, their progression and the associated remodelling, due to the effect of several aggressive factors [10].

In this context we confirmed that the home environment of asthmatic children contained several sources of aeroallergens likely to exacerbate the pathology by prolonging it. We found these to be moulds which are present in high quantities compared with the homes of healthy children (89% vs 10%), animal hair (pets present in 5% vs 2% of houses of healthy children) and passive smoking (26% vs 10%). Taken together these factors are thus considered to contribute not only to the development of the asthmatic disease but also to the onset of other associated allergic manifestations. Indeed, 73 % of them had a history of at least one personal atopic manifestation. In most cases this was allergic rhinitis.

In our study, after scoring the sick children according to the severity of their asthma (GINA score), we note that the majority fraction (33%) is in the 3rd GINA level, i.e. children with moderate persistent asthma. Boumendjel¹¹ also found that most asthmatic children in the Annaba region (study carried out in 2005) had moderate

persistent asthma. We regret to observe that the situation in Annaba has not really changed over a period of about ten years.

As we assayed serum total IgE levels, eosinophilia and total protein in all the children recruited, it was tempting to study various associations between these biological parameters and certain other markers of inflammation. Firstly, we remarked that sick children had markedly higher concentrations than healthy children for all the above mentioned biological parameters. In fact this finding is widely described in the literature because their increase is thought to reflect an increase in proinflammatory mediators in pathological conditions^{12,13}. Moreover, our results again confirm that serum total IgE level is closely associated with asthma severity as evaluated by the GINA score ($p < 0.001$) and although we found no correlation between asthma severity and eosinophilia, this level and that of total IgE are nevertheless both related to the allergy score ($p < 0.05$).

In addition, it is well known that allergic asthma is characterised by chronic inflammation of the airways¹⁴. During this inflammation, cells recruited in the bronchial mucosa such as the eosinophils secrete chemical mediators, essentially reactive oxygen species (ROS)¹⁵. This increase in the production of ROS provokes an imbalance in the oxidant/antioxidant balance. The result is oxidative stress¹⁶. Reactive oxygen species can modify various biological molecules including the polyunsaturated fatty acids of the cell membranes principally, resulting in a product of lipid peroxidation, malondialdehyde (MDA)¹⁷.

In our study we evaluated this oxidative stress by assay of MDA and GPx. The results obtained show that asthmatic children have higher concentrations of MDA than those of healthy children ($p < 0.05$). This raised MDA level may be explained, among other things, by the increase in the number of inflammatory cells activated in the pulmonary alveoli which release large quantities of superoxide anions and hydrogen peroxide by various mechanisms. The superoxide anion can interact with NO• generating highly toxic peroxynitrites (ONOOH)¹⁸. Moreover, several studies have shown high concentrations of oxidative stress parameters in asthmatics^{19,20}. As it is well known that GPx is a key antioxidant enzyme which regulates ROS levels, it is therefore suggested that it would protect cells from damage caused by aeroallergens²¹. In fact we showed that GPx activity was significantly decreased in asthmatic children compared with healthy children ($p < 0.05$). Similarly, Sackesen reported a fall in GPx concentration in the serum and in the erythrocytes of asthmatic children¹⁹. It is thought that loss of GPx activity could amplify the exacerbation of inflammation in asthmatic patients because glutathione peroxidase prevents prostaglandin synthesis.

In doing this it reduces the expression of proinflammatory mediators known to play a major role in the pathogenicity of allergic asthma²².

Lastly, we found total IgE levels to be very highly significantly correlated to MDA concentration ($p < 0.001$) and highly significantly correlated to GPx ($p < 0.01$), whereas eosinophilia was not correlated with these two parameters. We also investigated the clinical-biological link with oxidative stress parameters and clinical scores. The results obtained show that MDA and GPx are related to the severity of the asthma and are not related to the allergy score. A recent study²³ showed that the product of lipid peroxidation (MDA) is related to asthma severity and the highest concentrations of it are observed in children with severe asthma. Ercan also showed that the increase in MDA concentrations and the decrease in GPx concentrations are linked to the severity of the asthma²⁴.

In addition, we show that our various calculated scores varied with the duration of the disease. That is, the longer the duration of the disease, the greater the severity and the more numerous are the allergic manifestations. These results suggest that these children with severe asthma and/or a large number of allergic manifestations have suffered from asthma for a long time. This may be evidence of poor treatment compliance or due to denial of the disease by young people living mainly, in this case, in urban areas or in the town.

CONCLUSION

We first remarked the existence of familial atopy in asthmatic children and this at a frequency which was very high (72 %), compared to that found in the control population (14%). We also remarked that the home environment of asthmatic children contained several sources of aeroallergens likely to exacerbate the pathology by prolonging it and contributing to the onset of other associated allergic manifestations, including allergic rhinitis (observed in 73 % of children). These findings meant that the majority of sick children (33%) were scored as having severe asthma, in the 3rd GINA level.

This clinical approach was strengthened by the result of the biological parameters tested. We found the sick children to have markedly higher levels than healthy children for all the biological parameters assayed (total IgE, eosinophilia and total protein). This inflammatory condition is manifested by excess oxidative stress, which is characterised by changed in certain specific markers such as MDA the concentration of which was significantly higher in sick children, contrary to GPx activity.

Several clinical-biological relationships were revealed. In particular, the fact that serum total IgE is closely related to asthma severity, MDA and GPx. The latter two markers are also related to asthma severity and not related to allergy score. In a similar way this latter score is related to total IgE and also to eosinophilia. Lastly, we showed that the two scores vary in relation to duration of the disease.

These results suggest that children with severe asthma and/or numerous allergic manifestations have been suffering from poorly controlled asthma for a long time.

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