

Blood Plasminogen Activator Inhibitor-1 in Children with Persistent Asthma

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Abstract

Objectives: Plasminogen activator inhibitor (PAI)- 1 is the main inhibitor of the fibrinolytic system and is thought to play an essential role in tissue remodeling and fibrosis. We sought to investigate the expression of PAI- 1 in blood of a sample of atopic children with persistent asthma in relation to other clinical and laboratory parameters.

Methods: We enrolled 45 atopic children (6- 12 years old) with physician- diagnosed persistent bronchial asthma from the Pediatric Allergy and Immunology Unit of Ain Shams University Children's Hospital as well as 45 age and sex- matched healthy children. They were subjected to clinical evaluation, skin prick testing (SPT) with five common environmental allergens, complete blood counting, serum total IgE assay, and measurement of PAI- 1 by enzymatic immune assays.

Results: The blood PAI- 1 levels during acute exacerbation of bronchial asthma were significantly higher than the corresponding values after quiescence of symptoms and signs. The healthy controls showed a significantly lower PAI- 1 expression as compared to the patients' data whether during asthma exacerbation or remission. Blood PAI- 1 expression did not vary with asthma severity. However, being enrolled consecutively, the sample was not evenly distributed on various grades of severity. Blood PAI- 1 expression did not bear any significant relation to the serum total IgE, absolute eosinophil count, or the use of inhaled corticosteroid therapy in our series. The findings are indeed limited by the sample size.

Conclusion: We report the over- expression of PAI- 1 in the blood of a group of atopic school- aged children with persistent asthma that was still elevated after resolution of exacerbation. Wider scale studies are needed to verify the current conclusions. Study of PAI- 1 expression in other obstructive and/or restrictive pulmonary diseases and in early life wheeze could be worthwhile.

مُثَبِّطُ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ فِي دَمِ الْأَطْفَالِ الْمَصَابِينِ بِالرَّبْوِ الشَّعْبِيِّ الْمُسْتَمِرِّ

المقدمة والهدف: مُثَبِّطُ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ هُوَ الْمَانِعُ الرَّئِيسِيُّ لِنِظَامِ تَحْلَالِ الْفَيْبرِينِ وَهُوَ يَلْعَبُ دَوْرًا أَسَاسِيًّا فِي إِعَادَةِ تَشْكِيلِ الْأَسْجَةِ وَتَطَوُّرِ التَّخْلِيفِ الرَّئْوِيِّ. سَعِينَا لِلتَّحْقِيقِ فِي التَّعْبِيرِ عَنِ مَنَشَطِ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ فِي الدَّمِ لِعَيِّنَةٍ مِنَ الْأَطْفَالِ الْمَصْرِيِّينَ الْمَصَابِينِ بِالْحَسَّاسِيَّةِ وَيَعَانُونَ مِنْ أَزْمَاتِ الرَّبْوِ الشَّعْبِيِّ الْمُسْتَمِرِّ وَعِلَاقَتِهِ بِالْعَلَامَاتِ السَّرِيرِيَّةِ وَالْمَخْبِرِيَّةِ الْأُخْرَى.

طرق البحث: تَمَّ إِجْرَاءُ الدَّرَاسَةِ عَلَى ٤٥ طِفْلٍ مَصَابٍ بِالرَّبْوِ الشَّعْبِيِّ الْمُسْتَمِرِّ يَنْتَمُونَ إِلَى الْفَنَةِ الْعَمْرِيَّةِ (٦-١٢) سَنَةً مِمَّنْ يَتَابَعُونَ بِوَحْدَةٍ حَسَّاسِيَّةٍ وَمَنَاعَةِ الْإِطْفَالِ بِمَسْتَشْفَى الْأَطْفَالِ جَامِعَةِ عَيْنِ شَمْسٍ وَكَذَلِكَ ٤٥ مِنَ الْأَطْفَالِ الْأَصْحَاءِ مُمْتَاثِلِينَ فِي السِّنِّ وَالْجِنْسِ لِلْمَجْمُوعَةِ الْأُولَى كَمَجْمُوعَةٍ ضَابِطَةٍ. تَمَّ عَمَلُ تَقْيِيمِ إِكْلِينِيكِيِّ وَاخْتِبَارَاتِ الْحَسَّاسِيَّةِ بِالْوَخْزِ الْجُلْدِيِّ (SPT) لِمَسَبِّبَاتِ الْحَسَّاسِيَّةِ الْبِنِيَّةِ وَتَحَالِيلِ طَبِيبَةٍ تَشْمَلُ عَدَدَ دَمٍ كَلِّيٍّ وَقِيَاسَ تَرْكِيْزِ الْأَجْسَامِ الْمَنَاعِيَّةِ هـ وَمَثَبِّطِ الْمَحْفَظِ لِلْبِلَازْمِينُوجِينِ-١ فِي الدَّمِ.

النتائج: أَوْضَحَتِ النَّتَائِجُ أَنَّ مَسْتَوِيَّاتِ مَثَبِّطِ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ فِي دَمِ الْأَطْفَالِ الْمَصَابِينِ بِالرَّبْوِ خِلَالَ نَوْبَةِ الرَّبْوِ الْحَادَةِ أَعْلَى بِكَثِيرٍ مِنَ الْقِيَمِ الْمُنَاطَرَةِ لِنَفْسِ الْمَرْضَى بَعْدَ سَكُونِ الْأَزْمَةِ الرَّبْوِيَّةِ. وَكَانَ مَثَبِّطُ مَحْفَظِ الْبِلَازْمِينُوجِينِ-١ أَقَلَّ بِكَثِيرٍ فِي الْأَصْحَاءِ بِالْمَقَارَنَةِ بِالْمَرْضَى سِوَاهُ أَثْنَاءِ نَوْبَاتِ الرَّبْوِ أَوْ السَّكُونِ. لَمْ تَوْجَدْ عِلَاقَةً ذَاتَ دَلَالَةٍ إِحْصَائِيَّةٍ بَيْنَ مَسْتَوَى الْبِلَازْمِينُوجِينِ-١ فِي الدَّمِ وَبَيْنَ تَرْكِيْزِ الْأَجْسَامِ الْمَنَاعِيَّةِ هـ أَوْ عَدَدِ كُرَاتِ الدَّمِ الْبَيْضَاءِ مِنْ نَوْعِ الْإِيْزِينُوفِيلِ كَمَا لَمْ يَوْجَدْ إِرتِبَاطٌ بَيْنَ الْعِلَاجِ الْمُنْتَظَمِ بِمَرْكِبَاتِ الْكُورْتِيْزُونِ الْمَسْتَشَقِّ وَبَيْنَ مَسْتَوَى هَذَا الْمَعَامَلِ فِي الْمَرْضَى الْمُدْرَجِينَ بِالْبَحْثِ.

الخلاصة: مِنْ هَذِهِ الدَّرَاسَةِ التَّجْرِيْبِيَّةِ، يُمْكِنُ إِسْتِنْتِاجُ أَنَّ مَثَبِّطُ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ كَانَ أَعْلَى بِكَثِيرٍ فِي مَجْمُوعَةٍ مِنَ الْأَطْفَالِ الْمَصَابِينِ بِالرَّبْوِ الشَّعْبِيِّ الْمَزْمَنِ بِالْمَقَارَنَةِ مَعَ مَجْمُوعَةٍ مُطَابِقَةٍ مِنَ الْأَصْحَاءِ. وَكَانَتْ مَسْتَوِيَّاتِ الْأَرْتِفَاعِ أَعْلَى بِوَضُوحٍ خِلَالَ نَوْبَاتِ الرَّبْوِ الْحَادَةِ عِنْدَ سَكُونِ الْأَعْرَاضِ. نَوَصِي بِإِجْرَاءِ دَرَسَاتٍ عَلَى نِطَاقٍ أَوْسَعٍ لِلتَّحْقِيقِ مِنَ الْإِسْتِنْتِاجَاتِ الْحَالِيَّةِ. كَمَا نَوَصِي بِدَرَسَةِ مَثَبِّطِ مَنَشَطِ الْبِلَازْمِينُوجِينِ-١ فِي الْأَمْرَاضِ الرَّئْوِيَّةِ الْإِنْسَادِيَّةِ الْأُخْرَى وَفِي الرُّضْعِ الَّتِي يَعْانُونَ مِنْ الْإِيْزِينُوفِيلِ الرَّئْوِيِّ مِمَّا قَدْ يَوْضِحُ الْقِيَمَةَ الْمُحْتَمَلَةَ لِهَذِهِ الدَّلَالَاتِ فِي التَّتَبُّؤِ بِدَرَجَةِ تَطَوُّرِ الْحَسَّاسِيَّةِ الرَّبْوِيَّةِ النَّاشِئَةِ.

Introduction:

Asthma is associated with structural changes to airways such as extracellular matrix deposition and epithelial damage. Evidence suggests that asthmatic airway epithelial repair is abnormal and that elevated plasminogen activator inhibitor- 1 (PAI- 1) levels observed in asthma may be involved in the epithelial repair process and in excessive matrix accumulation (Stevens et al., 2008). While many consider the inflammatory process in asthma to derive from products of inflammatory cells, such as mast cells, eosinophils, and lymphocytes, it has been shown that mechanical stimulation of the airway epithelium, as would occur during bronchoconstriction, is itself a potent stimulus and can activate profibrotic pathways (Wiggs et al., 1997; Tschumperlin et al., 2003).

PAI- 1, a 45- kDa serine proteinase inhibitor, is the main physiological plasminogen activator inhibitor. It occurs in human plasma at an antigen concentration of about 20 ng/ml (Collen, 1999). Allergen- induced upregulation of PAI- 1 synthesis may also participate in the development of bronchial hyperreactivity (Kowal et al., 2007). As a matter of fact, the asthmatic airway epithelial cells are inherently dysfunctional in their ability to repair wounds; plasminogen activator inhibitor- 1 mRNA and protein activity are constitutively up- regulated in asthmatic epithelium and play functional roles in both proliferation and repair of healthy cells. In asthmatic cells, elevated plasminogen activator inhibitors- 1 levels fail to stimulate epithelial repair (Stevens et al., 2008).

With this as a background, we sought to investigate the expression of PAI- 1 in the blood and of a sample of Egyptian atopic children with persistent asthma in relation to other clinical and laboratory parameters. The ultimate objective was to open a new path towards understanding the pathogenic mechanisms of asthma and paving the way for adjuvant non- conventional lines of therapy.

Subjects And Methods:

This follow up study comprised 45 asthmatic and 45 clinically healthy children enrolled consecutively from the Pediatric Allergy and Immunology Unit and General Outpatient Clinic of the Children’s Hospital, Ain Shams University, Cairo. They were enrolled after obtaining an informed consent from their parents or care- givers.

The 45 asthmatic children comprised 22 males and 23 females. Their ages ranged from 6 to 12 years with a mean (SD) of 8.53 (1.2) years. The diagnosis of bronchial asthma was established according to the criteria of the American Thoracic Society (ATS, 1993) and the severity judged by the GINA guidelines of the American National Heart Lung and Blood Institutes (NHLBI) of the National Institutes for Health (GINA, 2012). The control group comprised 45 clinically healthy children. They comprised 24 males and 21 females. They presented in companionship of sibs with minor acute illness. Their ages ranged from 6 to 12 years with the mean (SD) value of 8.53 (1.2) years. They underwent the same study measurements as the patients at enrollment.

Assessment of atopy was performed by skin prick testing (SPT) using five common environmental allergens (house dust, mite, cockroach, Aspergillus fumigatus, eggs, and cat dander), as well as positive histamine control, and negative saline control. A positive response was defined as a wheal size equal to or greater than 3 mm above the negative control.

Laboratory Investigations:

1. Complete blood counting especially for the absolute eosinophil count

using coulter counter (Coulter Microdiff 18, fullerton, CA, USA).

2. Serum total IgE by the ELISA technique (Medix Biotech, Inc., Agenzyme Company, Industrial Road, San Carlos, CA, USA).
3. Measurement of the PAI- 1 was done by enzymatic immune assays (Zymutest PAI- 1 Antigen, #RK012A, Hyphen BioMed, France). This was performed to all subjects at enrollment and after quiescence of exacerbation in the patients’ group.

Results:

Plasma PAI- 1 levels of the asthmatic children during acute asthma exacerbation ranged from 34 to 80 pg/ ml [mean (SD)=5 4 (11.84) pg/ml]. These values were significantly higher than the corresponding values of the same patients when studied after subsidence of symptoms and signs which ranged from 20 to 213 pg/ml [mean (SD)= 46.9 (27.4) pg/ml]. The healthy controls had significantly lower plasma PAI- 1 levels [range= 3- 28 pg/ml; mean (SD)= 17.7 (6.3) pg/ml] as compared to patients’ data whether during asthma exacerbation or after quiescence Figure (1).

Plasma PAI- 1 levels did not vary according to asthma severity grades during stability. In patients with mild persistent asthma, the mean (SD) value was 46.8 (10.42) pg/ml. The corresponding values in patients with moderate persistent and severe persistent asthma were 40.55 (10.9) pg/ml and 61.5 (57.45) pg/ml, respectively ($p > 0.05$).

Plasma PAI- 1 did not show significant variation with the use of inhaled corticosteroid (ICS) therapy in our series. Its mean (SD) level in children receiving ICS was 54.07 (11.77) pg/ml in comparison to 55 (50) pg/ml in the rest of the studied sample Figure (2).

Plasma PAI- 1 during asthma exacerbation had a significant positive correlation with its level after remission. Otherwise, we could not detect any significant linear relations between plasma PAI- 1 and other numerical variables studied including the age, absolute eosinophil count, and s. total IgE.

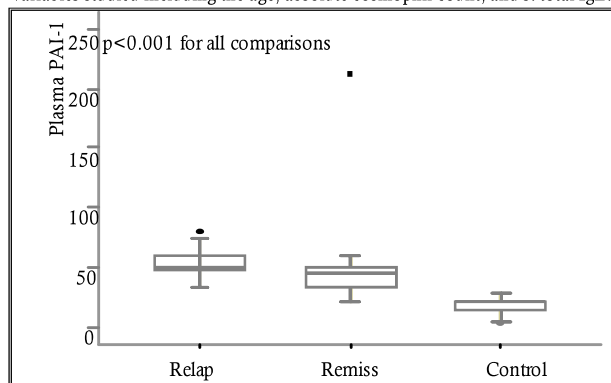


Fig. (1) Plasma PAI- 1 results in the studied sample

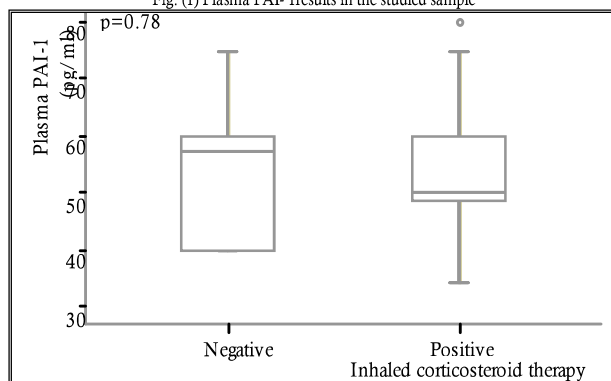


Fig. (2) Variation of plasma PAI- 1 levels with inhaled corticosteroid therapy

Discussion:

The plasma PAI- 1 levels of the asthmatic children during acute exacerbation of bronchial asthma were significantly higher than the corresponding values of the same patients when studied after quiescence. The healthy controls had a significantly lower PAI- 1 expression as compared to the patients' data whether during asthma exacerbation or quiescence. The upregulation of PAI- 1 after subsidence of asthma exacerbation points to the continuing inflammatory process in this disease and the risk of remodeling even during quiescence of symptoms.

Increased expression of plasma PAI- 1 in asthma was reported in other studies; mostly in adults. Kowal and colleagues (2007) sought to evaluate the effect of allergen challenge on plasma PAI- 1 concentration in 54 atopic asthmatic adults and 54 healthy non- atopic controls. Plasma samples were collected before, as well as 30 min, 6h and 24 h after HDM allergen challenge. The mean baseline plasma PAI- 1 concentration was greater in patients than controls. A year later, Kowal and Coworkers (2008) reported that concentrations of PAI- 1 were elevated in sputum supernatants in adult asthmatics as compared to controls.

PAI- 1 expression did not vary in our series with the grade of asthma severity during stability. However, the severe persistent asthma category was limited to nine children in our sample being enrolled consecutively. Gender did not influence the expression of PAI- 1 in the blood and there are no published data that support any impact of gender on fibrinolysis inhibitors.

We recruited only children with atopy as evidenced by positive SPT results to common environmental allergens denoting IgE sensitization. SPT reactivity was an exclusion criterion in the control group to avoid enrollment of atopic subjects. The number of positive SPTs could not be correlated to PAI- 1 expression in our series. Also, the SPT wheal diameter showed no linear relationship to the levels of PAI- 1 in the blood. This comes in concordance with the findings of Ozbek et al. (2009) in their study on Turkish children with asthma and allergic rhinitis where the results of skin prick testing did not correlate with PAI- 1 gene polymorphisms. Another study from Turkey as well did not find any relationship between PAI- 1 status and the degree of SPT reactivity (Bora et al., 2012). It seems that the PAI- 1 upregulation is related to the mere presence of atopy without respect to the extent of sensitization. PAI- 1 expression needs to be explored in non- atopic allergic diseases such as intrinsic asthma to know whether it is related to remodeling per se regardless of the atopic status.

The plasma PAI- 1 levels were comparable between asthmatics with normal total serum IgE and those with elevated levels during asthma exacerbation. Similarly, the presence of elevated levels of plasma PAI- 1 failed to have a significant relation to the absolute eosinophil counts during exacerbation. PAI- 1 expression did not bore any significant variation with the use of inhaled corticosteroid therapy. The latter finding was in agreement with Cho et al. (2011) who found no significant association between plasma PAI- 1 and inhaled steroids. It seems that control of PAI- 1 expression, and hence its effect on remodeling and pulmonary fibrosis, needs new non- conventional lines of therapy.

From this pilot study, we report the over- expression of PAI- 1 in a group of atopic school- aged children with persistent asthma as compared to a matched group of healthy controls. The levels were significantly higher during asthma exacerbations than after quiescence of symptoms and signs. The latter,

however, did not decline to the control levels pointing to the continuing asthmatic inflammatory process between exacerbations.

We recommend wider scale studies to verify the current conclusions. Study of PAI- 1 expression in other obstructive and/or restrictive pulmonary diseases will outline its exact relationship to asthma and whether linked to the atopic predisposition or the fibrosis that ensues. Evaluation of PAI- 1 and other markers of fibrinolysis in a cohort of early life wheezers with follow up through childhood might illuminate their potential prognostic value for developing asthma.

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