

Original article

Plasma concentration of thymus and activation-regulated chemokine in childhood asthma

Background: Thymus and activation-regulated chemokine (TARC) is responsible for trafficking of T helper 2 lymphocytes into sites of allergic inflammation. However, its role in assessing the severity of acute asthma in children is still unclear.

Objective: We sought to evaluate plasma TARC as a marker for monitoring asthma exacerbation in terms of asthma attack severity.

Methods: Plasma TARC concentration was estimated in 24 asthmatic children aged between 2 and 17 years attending the Pediatric Allergy and Immunology Unit of Children's Hospital, Ain Shams University, and 23 age and sex-matched healthy children using a sandwich enzyme immunoassay technique. For asthmatic patients, the measurement was performed during and after the resolution of acute asthma attack. In addition, complete hemogram and plasma total IgE were evaluated and peak expiratory flow rate was assessed in asthmatic patients during and after acute asthma exacerbation.

Results: Plasma TARC mean concentration was significantly higher during acute asthma (839.2 ± 453.6 pg/ml) than after resolution of symptoms (416.5 ± 324.8 pg/ml) and both were statistically higher than the control value (165.7 ± 135.2 pg/ml). During acute attacks of asthma, plasma TARC level was significantly elevated among patients with severe attacks of wheezing (1336.3 ± 431.2 pg/ml) than in those with moderate (743.8 ± 91.8 pg/ml) and mild (437.5 ± 66.1 pg/ml) attacks and inversely related to PEFr measurements during attacks ($r = -0.98$, $P < 0.001$). Meanwhile, no significant relationship was found between plasma TARC levels and either plasma total IgE levels or the absolute eosinophil count. Neither the history of other atopic symptoms nor family history of atopy influenced plasma TARC levels. A significant reduction in plasma TARC level was observed after treatment with inhaled β_2 agonist drugs either alone or in conjunction with inhaled glucocorticoids.

Conclusion: Our findings support the concept that TARC may be implicated in the pathogenesis of asthma. Plasma TARC is a useful marker in monitoring the severity of asthma exacerbation and in assessing the degree of allergic inflammation in the asthmatic airway. This would help physicians to design appropriate therapy in terms of dose and duration of treatment especially among children with quiescent asthma. Future studies should focus on using TARC antagonists as a new approach to asthma immunotherapy.

Key words: bronchial asthma, acute attacks, remission, TARC, atopy, inhaled glucocorticoids, β_2 agonists.

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INTRODUCTION

T helper 2 (Th2) lymphocytes play a crucial role in the pathogenesis of bronchial asthma¹. This subset of cells has been functionally identified by the production of a profile of cytokines namely IL-4, IL-5, IL-9 and IL-13² and by the preferential expression of CC chemokine receptor 3 (CCR3), CCR4 and CCR8³.

Thymus and activation-regulated chemokine (TARC; CCL 17) is the ligand for CCR4 receptor;

Imai and associates⁴, in 1996, first described the isolation and molecular characterization of TARC by cloning the D3A gene from peripheral blood mononuclear cells (PBMCs) after stimulation with phytohemagglutinin (PHA). This chemokine is constitutively expressed in the thymus and is produced by monocyte-derived dendritic cells (DCs) and endothelial cells. The gene encoding TARC is located on chromosome region 16q13⁵. The gene product is a basic protein with 71 amino

acids and a predicted mass of 8 kd. It acts on the chemokine receptor CCR4, which is expressed on PBMCs and human T lymphocytes⁶.

In vitro studies showed that TARC could induce selective migration of lymphocytes, especially with the phenotype of Th2 cells⁷. The expression of CCR4 on Th2 lymphocytes and CCR4-specific ligand TARC on airway epithelial cells was strongly upregulated in endobronchial biopsy specimens from asthmatic patients after allergen challenge^{8,9}. In addition, tracking of effector Th cells that were transplanted into naïve mice revealed that these cells predominantly used the CCR4-related pathway to recruit Th2 cells to the lung after repeated antigen stimulation¹⁰. CCR4/TARC interaction is thus important in regulating the trafficking of Th2 lymphocytes into sites of allergic inflammation. TARC has also been implicated in the pathogenesis of hypereosinophilic syndrome^{11,12}, allergic rhinitis¹³ and atopic dermatitis¹⁴. Elevated levels were found in the serum of patients with atopic dermatitis as compared with control subjects and patients with psoriasis. Furthermore, the serum level of TARC has been found to correlate with the severity of atopic dermatitis¹⁵.

The aim of this study was to investigate plasma TARC concentrations in children with asthma in relation to the severity of acute asthma attack.

METHODS

Studied sample

This case-control study comprised 24 asthmatic children (17 boys and 7 girls) aged between 2 and 17 years (mean \pm SD: 7.71 \pm 4.69 years). These patients were enrolled from the Pediatric Allergy and Immunology Unit of Ain Shams University, Children's Hospital. Of these patients, 12 had mild persistent asthma and 12 were suffering from moderate persistent asthma according to the global initiative for asthma guidelines¹⁶. The patients were enrolled and evaluated during acute asthmatic attacks and followed up until the attack subsided clinically and then reevaluated. During asthma exacerbation, the severity of the acute attack was determined clinically and by measuring the peak expiratory flow rate (PEFR)¹⁷ where 6 patients had mild attack, 10 had moderate attack and 8 had severe attack. The asthma exacerbation was triggered by exposure to allergens (food or animal allergens) in 15 children, exercise induced in 8 children and upper respiratory tract infection in one child. Among these patients, 12 had history of other atopic manifestations such as nasal or skin allergy and 18 patients gave history of allergic

diseases within the family members. At the time of the study, none of patients had acute lower respiratory tract infection and none were receiving oral glucocorticoids. After obtaining the first blood sample, all patients started regular inhalation of β 2 agonist drugs (salbutamole inhaler; dose: 400-800 μ g/day) and inhaled GCCs (beclomethasone dipropionate; dose: 200-400 μ g/day) were additionally administered to 10 patients (8 patients with severe and 2 patients with moderate attacks).

In addition, 23 age and sex-matched healthy children were enrolled in the study to serve as a control group. They were recruited from the Out-patient Clinic of the Children's Hospital at Ain Shams University while presenting with minor complaints. None of these children had a personal or family history of allergic diseases.

Study measurements

Detailed history was taken for the duration and severity of symptoms, drug therapy, and family history of atopy. Children with data suggestive of any concomitant disease were excluded from the study. Patients were subjected to a general clinical examination, including weight and height measurements. Chest examination for the assessment of the severity of wheezing and chest tightness was performed on the day of sampling. Patients under study underwent a complete hemogram, including total and differential leukocytic counts (Coulter counter T660, Coultronics, France).

Plasma total IgE concentration

EDTA-anticoagulated peripheral venous blood was collected from patients and control subjects and centrifuged within 30 minutes of collection at 3000 rpm for 10 minutes. Plasma concentration of total immunoglobulin (Ig) E was measured by enzyme linked immunosorbent assay (Pathozyne IgE, Omega Diagnostics Limited, UK) and the value of IgE used for data analysis was the percentage from the highest normal for age¹⁷.

Plasma TARC concentration

EDTA plasma samples from the studied subjects were stored at -20°C until analysis for TARC concentration in batch. We used a 96-well polystyrene microplate coated with a murine monoclonal antibody (mAb) against human TARC. The levels of TARC in plasma were measured in duplicate by a sandwich enzyme immunoassay technique¹⁸ using a commercially available kit (Quantikin, R&D System, Minneapolis, USA). The sensitivity of the kit in detecting TARC was 7 pg/ml. The intra-assay and inter-assay coefficients of variance for measuring plasma TARC concentration were 4.0% and 8.3%, respectively.

Plasma TARC was measured twice for each asthmatic child (during and after resolution of acute asthma attack), and the intervals between the first and second samples in all patients ranged from 7 to 10 days. In the healthy control children, plasma TARC was measured only once at enrollment.

Statistical analysis

Statistica for Windows version 5 (Stat soft, Tulsa, OK, USA) was used for all statistical analyses. All numeric data were expressed as mean \pm standard deviation and median values. Non-parametric data were analyzed using the Mann-Whitney U-test or Wilcoxon rank test for paired data. Correlation coefficients and their statistical significance were determined with Spearman's rank correlation test. Probability values of less than 0.05 were regarded as statistically significant.

RESULTS

Plasma TARC concentrations were significantly higher in asthmatic children than healthy controls. The mean concentration during acute asthma (839.2 ± 453.6 pg/ml) and that of the same children after resolution of the attack (416.5 ± 324.8 pg/ml) were statistically higher than the control value (165.7 ± 135.2 pg/ml). Also, the difference between plasma TARC during and after quiescence of asthma attack was statistically significant (table 1).

Plasma TARC concentration in relation to the severity of acute asthma attacks

During acute attacks of asthma, the mean plasma TARC level was significantly elevated among patients with severe attacks of wheezing (1336.3 ± 431.2 pg/ml) than those with moderate attacks (743.8 ± 91.8 pg/ml) and patients with mild attacks (437.5 ± 66.1 pg/ml). Further, the difference between plasma TARC levels in patients with moderate attacks and those with mild attacks of wheezing was significant (Figure 1). Also, an inverse relationship between plasma TARC concentrations and PEFr was observed among asthmatic children during acute attacks of asthma ($r = -0.98$, $p < 0.001$).

Plasma TARC levels in relation to asthma severity grade in remission

After the resolution of acute asthma symptoms, plasma TARC level among patients classified as

having mild persistent asthma (378.3 ± 387.4 pg/ml) was comparable with those of moderate persistent asthma (454.6 ± 277.3 pg/ml) and both were significantly higher than the control values (165.6 ± 135.2 pg/ml). Also, plasma TARC levels did not significantly correlate with the PEFr measurements during the quiescence of asthma ($r = 0.45$, $p > 0.05$).

Plasma TARC concentration in relation to history of other atopic diseases

Twelve asthmatic patients enrolled in the study had personal history of other atopic disorders (3 patients with nasal allergy and 9 patients with skin allergy). Although the mean plasma TARC level of these patients (970 ± 584.6 pg/ml) tended to be higher than that of patients with no history of other atopic diseases (708.3 ± 225.8 pg/ml) yet, no statistical significant difference was observed between both groups. Also, no significant difference in plasma TARC concentrations was noted between asthmatic children with family history of allergic diseases (414.8 ± 344.1 pg/ml) and those with no family history of allergic diseases (425 ± 242.4 pg/ml).

Moreover, no significant correlation between plasma concentrations of TARC and total IgE was detected either during acute asthma exacerbation or after remission of the attack ($r = 0.08$, $p > 0.05$ and $r = 0.22$, $p > 0.05$ respectively). Peripheral blood eosinophil count did not correlate with plasma TARC concentration either during acute asthma exacerbation or after the resolution of asthma attack ($r = 0.03$, $p > 0.05$ and $r = 0.33$, $p > 0.05$ respectively).

Plasma TARC concentration in relation to drug therapy

During acute asthma attacks, ten patients were treated with β_2 agonists and inhaled glucocorticoids (GCCs). Among these patients, the mean plasma TARC concentration significantly decreased after treatment (352.5 ± 223.5 pg/ml) as compared to the corresponding value observed before treatment (711 ± 259.3 pg/ml). A similar observation was noticed among patients who only received inhaled β_2 agonist drugs. These patients showed a significantly ($p < 0.05$) lower plasma TARC values after treatment (462.1 ± 383 pg/ml) than before treatment (930.7 ± 543.8 pg/ml) (Figure 2).

Table 1: Plasma TARC in the studied sample.

Studied children	Plasma TARC (pg/ml)					Z value	p
	N	Range	Median	Mean	±SD		
Asthmatic children							
During acute attack	24	350- 2000	725	839.2	453.6	5.71*	<0.0001
In remission	24	30- 1500	350	416.5	324.8	3.35*	<0.001
Control group	23	24-450	130	165.7	135.2	4.29†	<0.0001

* Versus the control group.

† Versus asthmatic children during acute attack.

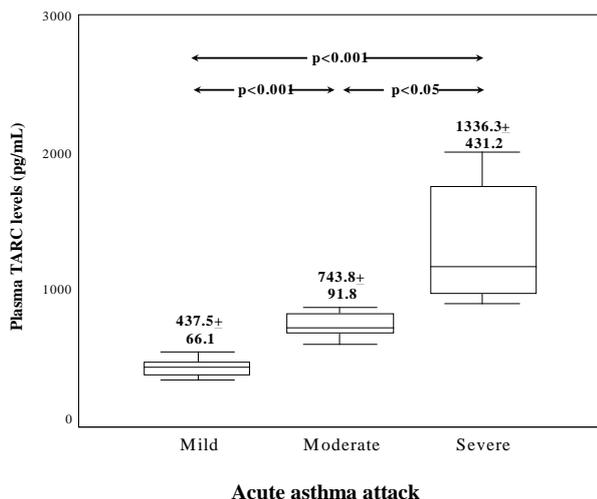


Figure 1: Plasma TARC levels in relation to severity of acute asthma attacks. Written values represent the mean ± standard deviation; horizontal lines indicate the median values and boxes enclose the interquartiles. The ranges are marked as maximum and minimum. Mann-Whitney test was employed for comparison between groups.

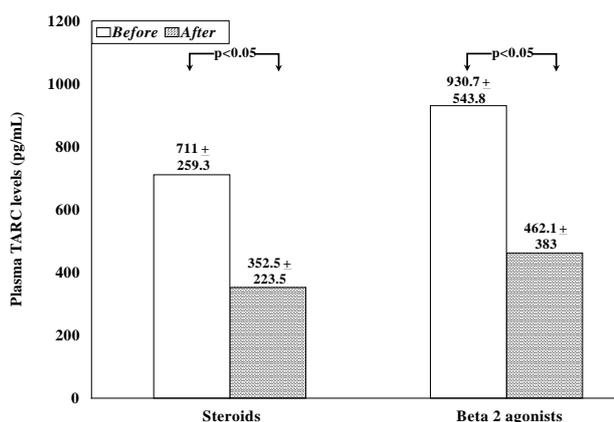


Figure 2: Mean plasma TARC concentration in asthmatic children before and after treatment with inhaled steroids and beta 2 agonists.

DISCUSSION

The evidence accumulated to date strongly support that TARC plays a dominant role in Th2-type

disease conditions by recruiting Th2 cells into inflammatory sites¹⁹. In fact, the role of TARC was evident in our results since plasma TARC concentrations were significantly increased in asthmatic children as compared with the control group. This finding is in agreement with that of Sekiya and co-workers²⁰ who reported increased TARC concentration in both sputum and serum samples of asthmatic patients as compared to healthy subjects indicating that the elevated levels of circulating TARC in asthmatics might be a reflection of increased TARC expression at inflammatory sites in the asthmatic airway. Indeed, previous immuno-histochemical studies have demonstrated increased ability of bronchial epithelial cells to express and produce TARC at both the mRNA and protein levels in asthmatic patients as compared to normal subjects²¹.

An important aim of this study was to evaluate plasma TARC in asthmatic children during and after the resolution of an asthma attack. Although plasma TARC concentrations significantly decreased after the resolution of acute asthma when compared to the corresponding value observed during the attack yet, they were still significantly higher than that of the control group. This finding denotes that the airway inflammation is still present in patients with seemingly stable condition. Based on the fact that the elevated circulating TARC in asthmatics may be a reflection of increased TARC expression at inflammatory sites in the asthmatic airway²⁰, it might be suggested that repeated monitoring of plasma TARC levels could help in assessing the degree of allergic inflammation in the asthmatic airway. This would also enable physicians to design the appropriate therapy in terms of dose and duration of treatment, especially among children with quiescent asthma.

In support of our results, Leung and associates²² reported that the circulating TARC concentrations among asthmatic children were significantly lower during the quiescence of asthma symptoms than in asthma exacerbation. However, in their report they did not comment on whether that decrease was comparable with the control values or not. In another study by Leung and co-workers²³, the

plasma TARC levels among patients with stable asthma were significantly higher than the control subjects.

Another goal of this study was to evaluate whether the level of plasma TARC was influenced by the severity of asthma attacks. Our results showed that during exacerbation of asthma the mean plasma TARC level was significantly higher among patients with severe asthma attack (1336.3 pg/ml) as compared with those with moderate asthma (743.8 pg/ml) and patients with mild asthma (437.5 pg/ml). Also, plasma TARC showed an inverse relationship with PEFR measurements during asthma exacerbation. These findings denote that TARC production is positively correlated with the degree of bronchospasm. In this regard, it might be suggested that plasma TARC could be a useful inflammatory marker in assessing asthma exacerbation. On the other hand, after resolution of asthma attacks although the plasma TARC level tended to be higher among patients with moderate persistent asthma (454.6 pg/ml) than in those with mild persistent asthma (378.3 pg/ml) yet, no statistical significance was observed between both groups. Also, PEFR measurements did not significantly correlate with the plasma TARC levels during the quiescence of asthma. In fact, other studies on asthmatic patients reported conflicting results concerning the relationship between plasma TARC concentrations and airflow indices, in one study, no significant relationship between plasma TARC levels and the different degrees of flow limitation on FEV1 measurement was reported²³ while in another study an inverse correlation was found between plasma TARC levels and PEFR during asthma exacerbation²². Perhaps, larger studies involving population cohorts will be needed to confirm the role of plasma TARC concentration in assessing disease severity.

TARC facilitates Th2 cell recruitment into sites of allergic inflammation²⁴. In one study, the nasal epithelial cells derived from allergic individuals released higher concentrations of TARC than those derived from non-allergic subjects¹³. Also, Kakinuma and associates²⁵ found a 10-fold increase of serum TARC level in patients with atopic dermatitis (AD) when compared with levels in healthy adults and patients with psoriasis. The average value of serum TARC of adult patients with AD was 2338.7 pg/ml in that study, which was much higher than the mean value observed in our patients with asthma exacerbation (839.2 pg/ml). This difference would suggest that AD was associated with a much larger end organ of TARC production (skin) compared to that associated with

asthma (lower respiratory tract). Also, a rather convincing explanation of the difference between the above mentioned report and our TARC values rely on the fact that we measured the TARC concentration in plasma and not serum samples. The difference between plasma and serum TARC values was illuminated in the study of Fujisawa and co-workers¹⁵ who found that the average TARC level was 253.2 pg/ml in plasma and 3225.9 in the corresponding serum in AD patients. They concluded that platelets contain high levels of TARC that is released during clotting and thus plasma samples contain lower concentrations of TARC than serum samples.

In fact, we were interested in studying the concentrations of plasma TARC among asthmatic patients who had manifestations of other atopic diseases such as allergic rhinitis and/or skin allergy and to compare these levels with those of asthmatics who had not ever experienced these atopic symptoms. Our results showed that the average plasma TARC concentration among asthmatic children with history of skin and/or nasal allergy (970 pg/ml) seemed higher than in those with no history of atopic diseases (708.3 pg/ml), however, this difference did not reach statistical significance. Also, when plasma TARC levels were re-evaluated after the resolution of asthma attacks, no significant differences were found between these two groups of patients. It should be stressed here that none of our patients had any symptoms of acute nasal or skin allergy during the time of the study. They were presenting with asthma manifestations only. Therefore, the circulating TARC in our patients was mainly influenced by asthma exacerbation and not by the presence of other atopic symptoms or even family history of atopy, since we could not establish any significant difference in plasma TARC levels between patients with family history of allergic diseases and those without family history of atopy.

Analysis of our data did not reveal any significant correlation between plasma TARC levels and either plasma total IgE levels or the eosinophil number in the peripheral blood. Unlike others who reported a weak but significant correlation between plasma total IgE and TARC levels in 60 asthmatic children²³ and a significant correlation between serum TARC levels and eosinophil number in the peripheral blood of 45 patients with AD²⁵. The controversy between our data and those of others has led us to suggest that the circulating TARC level is a more sensitive indicator of asthma exacerbation than plasma total

IgE and eosinophil number in the peripheral blood of our patients.

Glucocorticoids (GCCs) have long been extensively used to treat allergic disorders, with remarkable success²⁶. For patients with moderate and severe asthma, inhaled GCC now represent the first line of medication. Although, not fully proven, inhibition of elaboration of chemokines fundamental to allergic inflammation may be one of the mechanisms by which GCCs exert potent anti-allergic effects²⁷. Sekiya and associates²¹ reported that treatment with various GCCs resulted in attenuation of TARC mRNA expression and concomitant loss of the ability to produce TARC protein. The airway epithelium is the first cell layer to be encountered by inhaled GCCs indicating that bronchial epithelial cells are potential targets for this drug. Since bronchial epithelium-derived TARC potentially contributes to allergic inflammation via a paracrine mechanism, the beneficial effect of inhaled GCCs in bronchial asthma is due, at least in part, to the direct inhibitory effect of GCCs on TARC generation by the bronchial epithelial cells. Our results support this notion since asthmatic patients developed a significant reduction in their plasma TARC levels after being treated with inhaled GCCs drugs. Also, Leung et al²³ found that in patients with stable asthma, plasma TARC levels were significantly lower among patients who received inhaled GCCS than steroid-naïve patients. Nevertheless, our results showed that asthmatic children who were only treated by inhaled and/or oral β 2 agonists also developed a significant reduction in their plasma TARC levels after treatment. It is unclear whether the decrease in plasma TARC levels was due to an inhibitory effect of β 2 agonists on TARC production or whether that was merely due to the resolution of asthma attacks. The effect of inhaled β 2 agonists on TARC production by the epithelial cells is an intriguing question meriting further studies.

In conclusion, plasma TARC is increased in childhood asthma especially during acute attacks and correlates positively with the severity of attacks. These findings add to the increasing evidence that this chemokine may be implicated in the pathogenesis of asthma and support the concept of using inhibitory antibodies and chemokine antagonists that interfere with TARC as a new approach to allergy immuno-therapy.

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