Tumour necrosis factor-alpha concentration in severely asthmatic children

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تركيز عامل نخر الورم ألفا، في الأطفال المصابين بالربو الوخيم

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خلاصة: قمنا بتقدير تركيزات عامل نخر الورم ألفا لدى ثمانين طفلاً مصاباً بالربو، وكان لدى 26 منهم ربو وخيم في مرحلة التفاعل المبكرة وكان 26 آخرون في مرحلة التفاعل المتأخرة. بينما كان 28 طفلاً مصاباً بربو وخيم تحت السيطرة فيما بين النوبات عن طريق استعمال البريدنيزون الفموي. وتشكّلت المجموعة الشاهدة من 20 طفلاً من ذوي الصفات المشابهة للمرضى المدروسين. وقد تم قياس عامل نخر الورم ألفا في بلازما المرضى وفي طفاوة كريات الدم المحيطية وحيدات النواة بعد تنبيهها بعديد السكريد الشحمي. وتبيّن أن تركيزات عامل نخر الأورام ألفا، في البلازما وكريات الدم الطافية بعد التنبيه، تبدي ارتباطاً إيجابياً. كما ارتبط التركيز إيجابياً كذلك مع المدة الفاصلة بين بداية نوبة الربو وبين وقت أخذ عينة الدم. وكان تركيز عامل نخر الورم ألفا، أعلى بدرجة يعتد بها إحصائياً في مجموعة تفاعل المرحلة المتأخرة بالمقارنة مع المجموعات الأخرى، الأمر الذي يشير إلى ضرورة منع انطلاقه، أو مقاومة تأثيراته في وقت مبكر، في المصابين بالربو، أو كليهما معاً.

ABSTRACT We assessed tumour necrosis factor-alpha (TNF-α) concentrations in 80 asthmatic children, 26 with severe asthma in early-phase reaction, 26 with severe asthma in late-phase reaction, 28 with severe asthma controlled in between attacks with oral prednisone and 20 matched control children. TNF-α was measured in patients' plasma and in a supernatant of lipopolysaccharide-stimulated (LPS) peripheral blood mononuclear (PBM) cells. TNF-α concentrations in plasma and the supernatant of LPS-stimulated cells were positively correlated and the concentration also correlated positively with the time lapse between the start of the asthma attack and the time of blood sampling. TNF-α concentration was significantly higher in the late-phase reaction group compared to the other groups, indicating a need to counteract its release and/or effects early in asthma patients.

La concentration du facteur nécrosant des tumeurs alpha chez les enfants asthmatiques graves
RESUME Nous avons évalué les concentrations du facteur nécrosant des tumeurs alpha (TNF-α) chez 80 enfants asthmatiques, 26 souffrant d'asthme sévère en phase précoce de réaction, 26 d'asthme sévère en phase tardive de réaction, 28 d'asthme sévère contrôlé entre les crises par prednisone orale et 20 enfants témoins appariés. Le TNF-α a été mesuré dans le plasma des patients et dans un surnageant de cellules mononuclées du sang périphérique stimulées par lipopolysaccharide (LPS). Il y avait une corrélation positive entre les concentrations du TNF-α dans le plasma et le surnageant de cellules stimulées par LPS ainsi qu'entre la concentration et le temps écoulé depuis le début de la crise d'asthme jusqu'au moment du prélèvement sanguin. La concentration du TNF-α était considérablement plus élevée dans le groupe en phase de réaction tardive par comparaison avec les autres groupes, ce qui indique une nécessité de neutraliser sa libération et/ou ses effets à un stade précoce chez les patients asthmatiques.

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Introduction

Bronchial asthma is a chronic inflammatory disease that causes widespread narrowing of the tracheobronchial tree [*1,2*]. It affects approximately 5%–10% of children in the United States of America and a recent study indicated that the prevalence rate in Egypt was 3.25% [*3,4*]. The pathogenesis of asthma, particularly in non-atopic patients, is not totally clear [*5*]. The inflammation triggers two phases of reactions, an early-phase reaction (EPR) and, 3–12 hours after it, a late-phase reaction (LPR) [*6*]. Various mediators and cytokines are produced during the early and late phases, including interleukins such as IL-1 beta, IL-2, IL-6, IL-8, IL-10, as well as tumour necrosis factor-alpha (TNF-α). They act in different ways to modify the inflammatory process. These cytokine-bronchial epithelial interactions represent an important mechanism by which local inflammatory events in the airway microenvironment can be regulated [*7*]. However, events in bronchial asthma during EPR and LPR are still like pieces of a puzzle, TNF-α being an important piece that has multiple sites of action [*6,8*].

The aim of this study was to examine the TNF-α concentration in children suffering from severe bronchial asthma and to assess its clinical relevance.

Patients and methods

The study included 80 asthmatic children aged 1–7 years and 20 matched control children. Informed parental consent was obtained for all children before their inclusion in the study, and the Ethics Committee of the University of Alexandria approved the protocol of the study.

Asthmatic severity was specified according to the classification of the American National Asthma Education Program, 1991 [*9*]. Patients were divided into three groups: group 1 had 26 patients with severe asthma in EPR, group 2 had 26 patients with severe asthma in LPR and group 3 had 28 patients with severe asthma that was controlled in between attacks. Groups 1 and 2 were not on treatment in between at-

Table 1 **Anthropometric data and history of allergy in asthmatic and control children**

Factor	Control group		Group 1		Group 2		Group 3		Statistical test
	No.	%	No.	%	No.	%	No.	%	
Sex									
Male	12	60.0	14	53.8	10	38.5	22	78.6	$\chi^2 = 9.16$
Female	8	40.0	12	46.2	16	61.5	6	21.4	$P < 0.05$
Family history									
Bronchial asthma	0	0.0	8	30.8	6	23.1	6	21.4	Not valid
No bronchial asthma	20	100.0	18	69.2	18	69.2	16	57.2	
Allergic disease	0	0.0	0	0.0	2	7.7	6	21.4	
Age (years) ($x \pm s$)	4.20 ±1.9889		3.94 ± 1.6713		4.94 ± 1.4074		4.64 ± 1.0818		$F = 1.06$
Weight (kg) ($x \pm s$)	16.9	± 4.123	15.19 ± 4.299		16.35 ± 3.145		17.00 ± 2.228		$F = 0.86$

s = *standard deviation*

tacks, while patients in group 3 were corticosteroid-dependent, receiving only oral prednisone (5–15 mg) every other day for at least the previous 3 months.

A detailed history was recorded for all patients with emphasis on any family history of asthma or other allergic diseases, severity of attacks, possible predisposing factors, associated manifestations and drug intake during and in between asthmatic attacks. Anthropometric data are given in Table 1.

The time lapse from the start of the asthmatic attack until hospital admission and blood sampling was recorded for groups 1 and 2. A full clinical examination and chest radiography were obtained for all patients. A blood sample was taken to obtain a complete blood picture and plasma separated in order to estimate the TNF-α concentration in plasma and the TNF-α concentration in a supernatant of lipopolysaccharide-stimulated (LPS) cultured peripheral blood mononuclear (PBM) cells using kits supplied by Medgenix Diagnostics, Belgium [*10*]. The Medgenix TNF-α is a solid-phase enzyme-linked amplified sensitivity immunoassay performed on a microtitre plate. The minimum detectable concentration is estimated at 3 pg/mL. Samples were obtained from group 1 during EPR and group 2 during LPR—before any treatment was given. Blood samples for group 3 were obtained during the patient's regular visits to the asthma clinic.

Statistical analysis was performed using the chi-squared test, Student *t*-test and one-way analysis of variance, as well as the Pearson correlation coefficient. The level of significance accepted was $P < 0.05$.

Table 2 **Concentration of plasma and culture TNF-α in asthmatic and control children**

Group	Plasma TNF-α level (pg/mL)	Culture TNF-α level (pg/mL)
Controls (n = 20)		
Mean	80.56	99.14
s	24.7655	18.2122
Group 1 (n = 26)		
Mean	111.87	114.07
s	15.2203	20.0098
Group 2 (n = 26)		
Mean	381.89[a]	457.75[a]
s	58.4074	59.1329
Group 3 (n = 28)		
Mean	113.81	131.30
s	17.7381	2.4757
F-test	215.5818	303.379

[a] *Significantly higher compared with the other groups (P < 0.05)*
TNF-α = tumour necrosis factor-alpha
s = standard deviation

Results

Samples for group 1 (obtained in EPR) showed a mean ± standard deviation of 2.4 ± 1.6 hours between the beginning of the attack and sampling, while group 2 samples (obtained in the LPR) showed a mean of 7.5 ± 1.6 hours. The concentration of TNF-α in plasma and in the LPS-stimulated PBM culture supernatant correlated positively with the time lapse between the start of the attack and blood sampling (r = 0.5342 and 0.5376 respectively). Plasma and LPS-stimulated PBM culture supernatant concentrations of TNF-α were positively correlated (r = 0.947). Plasma and culture TNF-α concentrations were significantly higher in group 2 compared to asthmatic groups 1 and 3 and the control group (F = 303.379) (Table 2).

Discussion

TNF-α is an important mediator in initiating airway inflammation and a potent mod-

ulator of immune and inflammatory responses [*11,12*]. Our results showed that, for group 1 (untreated severe asthma in EPR), the plasma concentration of TNF-α as well as its concentration in LPS-stimulated PBM culture supernatant, while higher, was not significantly higher than the control group. This non-significant elevation of TNF-α in severe cases in EPR could be explained by the early suppression of its secretion by several mechanisms. It is known that histamine liberated from mast cells down-regulates TNF-α release from the same mast cell population within 10 minutes, with histamine acting as an autocrine regulator [*13*]. Moreover, IL-10 released from mast cells inhibits TNF-α release without affecting histamine release [*14*]. Interleukin-12 can also suppress antigen-induced airway changes despite the presence of specific IgE, thereby suppressing mast cell activation and degeneration action [*15*].

The concentrations of TNF-α in plasma and in the LPS-stimulated PBM culture for group 2 (untreated severe asthma in LPR) were significantly higher than for groups 1 and 3. This is in accordance with findings in other studies, indicating that in LPR, various recruited cells such as eosinophils, macrophages and lymphocytes secrete TNF-α [*16–18*]. In our study the concentrations of TNF-α in plasma and supernatant of LPS-stimulated PBM cultured cells correlated positively with the time lapse from the beginning of the asthmatic attack (r = 0.5342 and 0.5376 respectively). The plasma concentration of TNF-α in group 3 (corticosteroid-dependent) showed no significant difference when compared to results for the control group and group 1. This means that oral prednisone (every other day) can suppress TNF-α release. In support of this view, it was found that TNF-α has an inverse relation to the plasma cortisol level [*19*]. Corticosteroids may prevent airway inflammation by down-regulating the synthesis and/or release of proinflammatory mediators, especially TNF-α, through inhibition of transcription factors that regulate cytokine synthesis [*20–22*].

In summary, while TNF-α is released in EPR asthmatic attacks it is even higher in LPR attacks. If its release in EPR is autoregulated and suppressed by various intrinsic mechanisms, it seems logical that we should counteract its release and/or effects in LPR through the proper and early use of corticosteroids or other anti-inflammatory drugs.

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