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Araştırma

Serum Levels of Leukemia Inhibitory Factor (LIF) in Wheezy Infants and its Relation with Respiratory Syncytial Virus Infection §

Zeynep TAMAY*, Kemal NiŞLi, Nermin GÜLER***, Deniz ÖZÇEKER****, Ülker ÖNEŞ*******

Serum Levels of Leukemia Inhibitory Factor (LIF) in Wheezy Infants and its Relation with Respiratory Syncytial Virus Infection

Objective: *Leukemia inhibitory factor (LIF) is the most pleiotropic member of the interleukin-6 family of cytokines. It utilises a receptor that consists of the LIF receptor β and gp130 and this receptor complex is also used by ciliary neurotrophic growth factor (CNTF), oncostatin M, cardiotrophin1 (CT1) and cardiotrophin-like cytokine (CLC). Evidence is emerging that LIF may play an important role in airway inflammation. Recurrent wheezing is one of the commonest problems of early childhood and respiratory syncytial virus (RSV) is the most frequent cause of wheezy episodes.*

Material and Method: *The aim of this study was to determine whether LIF has a role in the airway inflammation of wheezy children and its relationship with RSV infection.*

Results: *Serum LIF levels, total IgE levels, RSV-IgM and RSV-IgG antibodies were evaluated in wheezy (22F, 18M) and healthy (18F, 20M) infants. RSV-IgM antibodies were detected in 25% of wheezy and 3% of healthy children. RSV-IgG antibodies were detected 35% and 23%, respectively. There was not significant difference in median serum levels of LIF between wheezy and healthy infants. However, median serum LIF level of subjects who were seropositive for RSV-IgM antibodies was significantly higher than those who were seronegative for RSV-IgM antibodies (16 pg/ml vs 10.5 pg/ml; p=0.01).*

Conclusion: *This study suggests that LIF might play a role in early airway inflammation in infants with wheezy RSV infection.*

Keywords: *Leukemia inhibitory factor, wheezy, children, respiratory syncytial virus*

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Hışıltılı Bebeklerde Serum Lösemi İnhibitör Faktör (LIF) Düzeyleri ve Respiratuar Sinsisyal Virüs Enfeksiyonuyla İlişkisi

Amaç: *Lösemi inhibitör faktör sitokinlerin interlökin-6 ailesinin en pleiotropik üyesidir. LIF reseptör β ve gp130'yi içeren bir reseptörü kullanır. Bu reseptör kompleksi aynı zamanda siliyer nötrofilik büyüme faktörü (CTNF),onkostatin M, kardiotrofin e benzer sitokin (CLC) tarafından da kullanılmaktadır. LIF'nin havayolu enflamasyonunda da önemli bir rol oynayabildiğine ilişkin kanıtlar ortaya çıkmaktadır. Erken çocukluk döneminde en çok görülen sorunlardan biri yinelenen hışıltılar ve hışıltı ataklarının en sık görülen nedeni respiratuar sinsisyal virüstür (RSV).*

Gereç ve Yöntem: *Bu çalışmanın amacı LIF'in hışıltılı çocuklarda havayolu enflamasyonunun bir rolü olup olmadığının ve RSV enfeksiyonuyla ilişkisini belirlemekti.*

Bulgular: *Hışıltılı (22K, 18E) ve sağlıklı (18K, 20 E) süt çocuklarında serum LIF, total IgE düzeyleri, RSV-IgM ve RSV-IgG antikorları değerlendirilmiştir. Hışıltılı sütçocuklarının %25 ve sağlıklı çocukların %3'ünde RSV-IgM antikorları saptanmıştır. Çocukların sırasıyla %35 ve %25'inde RSV-IgG antikorları saptanmıştır. Hışıltılı ve sağlıklı süt çocukları arasında ortanca serum LIF düzeyleri arasında anlamlı bir farklılık yoktu. Ancak, RSV-IgM antikorları için seropozitif olan kişilerde ortanca serum LIF düzeyi RSV-IgM antikorlarına karşı seropozitif olan kişilere göre anlamlı derecede daha yüksekti (16 pg/ml'e karşı 10.5 pg/ml; p=0.01).*

Sonuç: *Bu çalışma LİF'in hışıltılı RSV enfeksiyonu olan süt çocuklarında erken dönem havayolu enflamasyonunda bir rol oynayabildiğini düşündürtmektedir.*

Anahtar kelimeler: *Lösemi inhibitör faktör, hışıltı, çocuk, respiratory sinsityal virüs*

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*İstanbul Üniversitesi Tıp Fakültesi, Çocuk Alerji Bilim Dalı
** İstanbul Üniversitesi Tıp Fakültesi, Çocuk Kardiyolojisi Bilim Dalı
*** Istanbul Bilim Üniversitesi, Çocuk Sağlığı ve Hastalıkları Bilim Dalı
**** Okmeydanı Eğitim ve Araştırma Hastanesi, Çocuk Sağlığı ve Hastalıkları Bilim Dalı
***** İstanbul Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Bilim Dalı, Emekli öğretim üyesi
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Yazışma adresi: Prof. Dr. Zeynep Tamay, İstanbul Üniversitesi Tıp Fakültesi Çocuk Alerji Bilim Dalı, Çapa / İstanbul
e-posta: eztamay@yahoo.com

INTRODUCTION

Leukemia inhibitory factor (LIF) is the most pleiotropic member of the interleukin-6 family of cytokines. It utilises a receptor that consists of the LIF receptor β and gp130 and this receptor complex is also used by ciliary neurotrophic growth factor (CNTF), oncostatin M, cardiotrophin1 (CT1) and cardiotrophin-like cytokine (CLC) [1]. It can occur in many tissues

with a wide array of actions including differentiation of leukemic cells, proliferation of some hematopoietic cells, blastocyst implantation, neural development, bone formation, inflammation and production of acute-phase proteins [2,3]. The presence of LIF in human bronchial epithelial cells has been demonstrated immunochemically [4]. Expression of LIF by numerous lung cell types suggests a potential role for this cyokine in airway inflammation [5]. It has been also shown that human eosinophils synthesize and store LIF suggesting that LIF has proinflammatory roles in eosinophil-dependent airway disorders [6].

Recurrent wheezing is one of the commonest problems of infancy and early childhood. Respiratory syncytial virus (RSV) is the most frequent cause of wheezy episodes in this age group. Many different cytokines appear to play a role including interferon-gamma, interleukins 8, 10 and 12, and cytokines produced by T helper 1 and T helper 2 cells [7,8]. As to our knowledge, possible role of LIF in wheezy children has not been studied yet. The aim of this study was to determine whether LIF has a role in the airway inflammation of wheezy children and its relationship with RSV infection.

MATERIAL and METHOD

Subjects

Patients

The study was conducted in the Pediatric Allergy Department of University Hospital. The study population consisted of 40 (22 F, 18 M) infants with acute wheezy episodes. Of these, 15 (10 F, 5 M) had experienced the first episode of wheezing, 25 (12 F, 13 M) had recurrent wheezing (parental history that the child had received bronchodilator therapy prescribed by a pediatrician for wheezing on at least 3 occasions) (Table 1). A detailed history of the present episode and the past wheezy episodes, atopy, exposure to cigarette smoke were obtained.. Physical examination and chest X-ray were performed to make a differentiation among atypical causes of wheezing such as congenital anomalies of the respiratory system, gastroesophageal reflux/aspiration, bronchopulmonary dysplasia, congenital cardiac anomalies, cystic fibrosis, foreign body aspiration, immotile cilia syndrome and bronchiolitis obliterans. Those who had a high index of suspicion for these disorders were excluded from the study.

Controls

The control group consisted of 38 (18 F, 20 M) healthy children, who attended the daycare center of the Medical School Hospital and had never wheeze.

Total serum IgE levels

Total IgE levels of subjects were measured in serum samples by radioimmunoassay test (Pharmacia Diagnosis, Sweden).

Serum levels of RSV-IgM and RSV-IgG

Determination of RSV-IgM and RSV-IgG were carried out in serum samples of the subjects by using a commercial semi-quantitative enzyme immunoassay (Novum Diagnostica, Germany). Testing was performed according to manufacturer's instructions.

Table 1. Demographic features of the patients with first or recurrent wheezing episodes.

	Patients (First wheezy episode)	Patients (Recurrent wheezing)	Controls	P
Number	15	25	38	
Median age (range) (months)	9 (6-13)	13 (8-23)	12 (6-24)	0.02
Female/ Male n (%)	10/5 (66/34)	12/13 (48/52)	18/20 (48/52)	NS**
Family history of atopy* n (%)	9 (60)	14 (56)	14 (36)	NS**
Passive smoking n (%)	11 (73)	15 (60)	25 (66)	NS**

History of atopy either in one parent or sibling was considered to be positive family history of atopy. NS: not significant*

LIF determination

Determination of LIF levels in the serum samples of the subjects were carried out by using a quantitative "sandwich" enzyme immunoassay technique (Quantikine, R& D Systems, MN, USA) according to manufacturer's instructions. The minimum detectable dose of LIF using a standard curve generated with the manufacurer's "Calibrator Diluent RD5" was 2 pg/ml.

Statistics

Statistical analyses were performed by using the Statistical Package for the Social Sciences software version 12.0 for Windows (SPSS, Inc, Chicago, IL). Non-parametric methods (Mann Whitney U, one-way ANOVA for LIF, IgE levels and age; chi-square tests for family history of atopy, passive smoking, gender) were used for comparisons among groups. The Spearman rank correlation test was used to detect correlations of paired data. P values less than 0.05 considered significant.

RESULTS

Virologic and demographic results

The demographic features of the children are shown in Table 1. Subjects with first wheezy episode were younger than those with recurrent wheezing ($p<0.001$). Control subjects were not significantly different in age than wheezy children. There was no difference in gender, family history of atopy and passive smoking between wheezy and healthy children.

RSV IgM and RSV IgG antibodies were detected in 25% (10/40) and 35% (14/40) of wheezy children, respectively. Among the wheezy children, RSV-IgM antibodies were detected in 46% (7/15) of the patients with the first episode and 12% (3/25) of the patients with recurrent wheezing whereas RSV-IgG antibodies were detected in 7% (1/15) and 52% (13/25) of the patients, respectively. RSV-IgM and

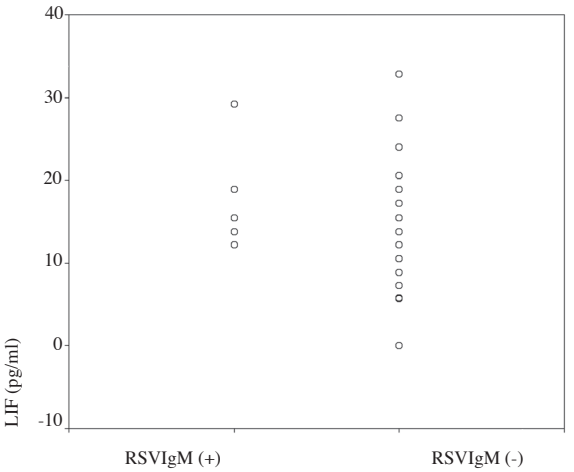

Figure 1. Serum LIF levels of children according to RSV IgM antibodies.

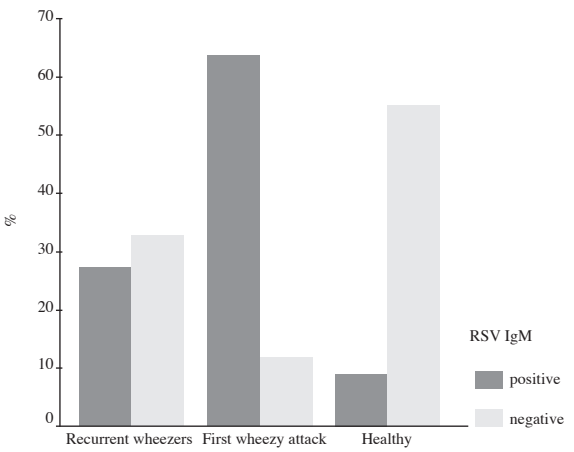

Figure 2. Percentage of presence and absence of RSV IgM antibodies in children.

Table 2. Median serum LIF and IgE levels of children.

Children n=78	LIF (pg/ml) Median (quartiles)	IgE (IU/L) Median (quartiles)
Patients with first wheezy episode (n=15)	10.5 (0-13.8)	10 (6-15)
Patients with recurrent wheezing (n=25)	13.8 (6.5-16.4)	14 (10-17)
Patients-wheezy children (n=40)	12.2 (0-15.5)	13 (8-16)
Controls (n=38)	11.4 (0-17.2)	8 (6-12)
Children with positive serology for RSV-IgM (n=11)	15.5 (13.8-18.9)	14 (6-23)
Children with negative serology for RSV-IgM (n=67)	10.5 (0-15.5)	10 (7-14)
Children with positive serology for RSV-IgG (n=23)	10.5 (0-15.5)	14 (8-16)
Children with negative serology for RSV-IgG (n=55)	12.2 (0-15.5)	10 (6-12)

RSV-IgG antibodies were documented in 3% (1/38) and 23% (9/38) of the healthy children, respectively (Figure 2 and Figure 3).

Serum IgE levels

Median serum IgE levels of children were given in Table 2. There was no child with serum IgE level above 100 IU/L. Children who had positive family history for atopy had significantly higher serum IgE levels than others (p=0.002). The median serum IgE level was significantly higher in wheezy infants than healthy controls (13 IU/L vs 8 IU/L; p=0.004). Median serum IgE level did not significantly differ between recurrent wheezers and children with first wheezy episodes. Serum IgE level was significantly higher in patients who were seropositive for RSV IgG antibodies than those who were not (p=0.025). There was no relation between serum IgE and RSV-IgM antibodies.

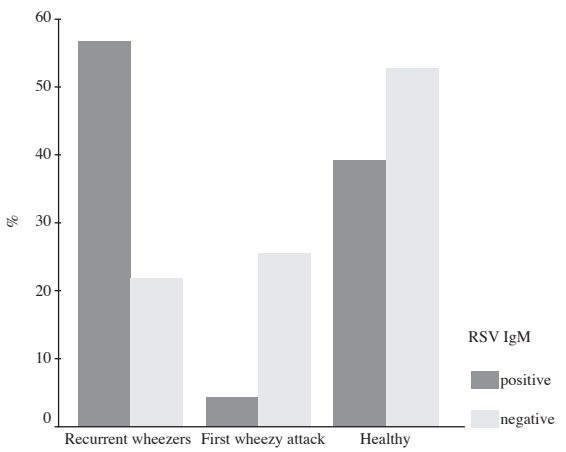

Figure 3. Percentage of presence and absence of RSV IgG antibodies in children.

Serum LIF levels

The median serum levels of LIF in wheezy infants and healthy children are given in Table 2. There was not a significant difference for serum LIF levels between wheezy and healthy children. Although the median serum LIF level of recurrent wheezers was slightly higher than those who had first wheezy episode, the difference was not statistically significant. However, median serum LIF level of subjects who were seropositive for RSV-IgM antibodies was significantly higher than that of the subjects who were seronegative for RSV-IgM antibodies (15.5 pg/ml vs 10.5 pg/ml; p=0.01) (Figure 1). There was no significant difference in demographic features between children who were seropositive or seronegative for RSV-IgM antibodies (Table 3). Additionally, children with undetectable serum LIF levels were seronegative for RSV-IgM. Also, neither of the children with positive serology for RSV-IgM had undetectable serum LIF values. There was a significant correlation between LIF and RSV-IgM antibodies (p=0.009, r=0.296). Such a relationship between LIF and RSV-IgG antibodies was not observed. Serum IgE levels correlated weakly with serum LIF levels (p<0.001, r=0.478). Presence of family history of atopy or passive smoking did not significantly influence serum LIF levels.

DISCUSSION

RSV is the most important viral respiratory pathogen of infancy. Although it can cause upper respiratory infections with mild symptoms, one third of the children with RSV infection presents with lower respiratory infection and wheeze [9]. We detected RSV-IgM antibodies in 25% of wheezy children. Nearly

Table 3. Demographic features of children who were seropositive or seronegative for RSV IgM.

	Children with RSV-IGM (+)	Children with RSV-IgM (-)	P
Number	11	67	
Median age (range) (months)	9 (6-15)	11 (6-24)	NS**
Female/ Male n (%)	6/5 (54/46)	34/33 (51/49)	NS**
Family history of atopy* n (%)	5 (46)	34 (51)	NS**
Passive smoking n (%)	8 (73)	43 (65)	NS**

History of atopy either in one parent or sibling was considered to be positive family history of atopy, NS:not significant*

half of the children with first wheezy episode had detectable RSV-IgM antibodies, which was expected since the children who experienced first wheezy episode had greater chance to have acute RSV infection. The prevalence rate for RSV-IgG antibodies was 35% in wheezy children. Among wheezy children nearly half of the recurrent wheezers had detectable RSV-IgG antibodies. Frequent detection of RSV-IgG antibodies in recurrent wheezers was also expected since symptomatic children with the involvement of lower airways had the higher chance of RSV infection and re-infection resulting from long-lived serum antibody titres [15]. We detected RSV-IgM and RSV-IgG antibodies in healthy children with the frequency of 3% and 23%, respectively. The median age of the healthy children was 12 months (quartiles 6-16 months). Acute RSV infection was not common in healthy children as previously reported [9]. The seroprevalence of IgG antibodies in 6 to 12 month- old healthy children was found to be 12% to 27%, which were compatible with our results [10,11].

In this study we found that children who were seropositive for RSV-IgM antibodies had higher LIF levels than children who were not. But only one child in the healthy group had detectable RSV-IgM antibodies, so we could not comment precisely whether there was a difference in LIF production in response to acute RSV infection between wheezy and healthy children. As to our knowledge, this is the first study evaluating LIF in wheezy infants. Previous studies have shown that IL-6 and IL-11 production, which are the members of the same IL-6 family of cytokines with LIF, are enhanced in RSV infection [12-14]. Since IL-6 was shown to release LIF in lung fibroblasts in the study conducted by Knight et al. [5], one can speculate that some biological effects previously attributed to IL-6 may in fact be due to LIF.

We couldn't find a significant difference in LIF levels between wheezy and healthy children. Zheng et al. [6] reported higher serum LIF levels in mild asthmatics than healthy controls. Wheezing in early life is a heterogeneous condition due to atopic and non-atopic status, but not purely based on eosinophilic inflammation as in adult asthma. We couldn't classify wheezy children as atopic and non-atopic, since there was no child with serum IgE levels above 100 IU/L, which can be a marker for atopy. There might be two possible explanations. One potential scenario is that, all of them were true non-atopics. Another scenario is that adequate sensitization to allergens did not occur in wheezy infants to mount high IgE response. Relatively higher serum IgE levels in wheezy infants than healthy controls and positive relation between serum IgE level and family history for atopy supported the assertion that some of them were atopic. Wheezy children, who had genetic susceptibility to RSV infection, might also produce IgE-type immune response against re-infection, hence had high serum IgE levels with detectable RSV-IgG antibodies. As we couldn't identify atopic and non-atopic wheezers clearly, we couldn't compare LIF levels in these subsets. However serum LIF levels of children with positive family history of atopy did not significantly differ from children without such a family history, which might indirectly show irrelevance of atopic status with serum LIF levels.

In conclusion, this study suggests that LIF might have a role in early airway inflammation in wheezy infants with RSV infection. Further investigations are needed to evaluate its precise role in relation with RSV infection in wheezy children.

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