

ORIGINAL INVESTIGATION / ÖZGÜN ARAŞTIRMA

Levels of Cytokines Indicative of T Cell Response in Bronchoalveolar Lavage of Tuberculin Skin Test-Positive Children

Tüberkülin Deri Testi Pozitif Çocuklarda Bronkoalveoler Lavajda T Hücre Yanıtının Göstergesi Sitokinlerin Düzeyleri

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Abstract

Özet

OBJECTIVE: The aim of the study was to evaluate the levels of interleukin (IL)-4, IL-10, transforming growth factor-beta (TGF- β), IL-17, and IL-23 cytokines, which reflect different T lymphocyte responses, in bronchoalveolar lavage (BAL) samples of tuberculin skin test (TST)-positive children.

MATERIAL AND METHODS: Twelve children with TST positivity, who underwent flexible videobronchoscopy (FB) to evaluate airway involvement and to obtain BAL to improve diagnostic yield, and 11 control children with negative TST, who underwent FB for other reasons, were enrolled in this case-control study. BAL samples were obtained from all children during the FB procedure. Levels of IL-4, interferon gamma (IFN- γ), IL-10, TGF- β , IL-17, and IL-23 were measured by the ELISA method.

RESULTS: Mean age of the children enrolled in the TST-positive and -negative groups were 8.6 (4.3) vs. 6.8 (4.5), respectively ($p=0.35$). There was a trend of higher TGF- β levels in TST-positive children compared with TST-negative children [557.9 (515.3) vs. 386.3 (208.1), respectively, $p=0.07$]. Mean levels of IL-23 were 39.2 (29.5) in TST-positive children vs. 46.2 (72.3) in TST-negative children ($p=0.49$). IFN- γ , IL-4, IL-10, and IL-17 levels were not significantly different among the groups ($p>0.05$ for all).

CONCLUSION: Results of this study suggest that TGF- β in BAL fluid of children with TST positivity tends to be higher than that in TST-negative children, which indicates an increased activity of regulatory T lymphocytes that may decrease the Th1 immune response. TGF- β might be studied in future research for its potential as a diagnostic modality and immunomodulatory treatment target.

KEY WORDS: Tuberculin skin test, bronchoalveolar lavage, cytokine, T lymphocyte

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AMAÇ: Çalışmanın amacı tüberkülin deri testi (TDT) pozitif çocuklarda bronkoalveoler lavaj (BAL) örneklerinde farklı T hücre yanıtlarını yansıtan sitokinlerden IL-4, IL-10, TGF- β , IL-17 ve IL-23 düzeylerinin değerlendirilmesidir.

GEREÇ VE YÖNTEMLER: Bu olgu-kontrol çalışmaya, havayolu tutulumunun değerlendirilmesi ve ek tanısal katkıda bulunması açısından BAL alınması için fleksibl videobronkoskopi (FB) yapılan 12 TDT pozitif ve farklı nedenlerle FB yapılan TDT negatif 12 kontrol çocuk dahil edildi. Tüm çocuklarda FB sırasında BAL örnekleri alındı. IL-4, IFN γ , IL-10, TGF- β , IL-17 ve IL-23 düzeyleri ELISA yöntemi ile ölçüldü.

BULGULAR: TDT pozitif ve negatif gruplara dahil edilen çocukların ortalama yaşları sırası ile 8,6 (4,3) ve 6,8 (4,5) bulundu ($p=0,35$). TDT negatif çocuklarla karşılaştırıldığında TDT pozitif çocuklarda TGF beta düzeyleri daha yüksek olma eğilimindeydi [sırasıyla 386,3 (208,1) ve 557,9 (515,3), $p=0,08$]. TDT pozitif çocuklarda ortalama IL-23 düzeyi 39,2 (29,5) iken TDT negatiflerde 46,2 (72,3) saptandı ($p=0,49$). IFN gama, IL-4, IL-10 ve IL-17 düzeyleri gruplar arasında anlamlı farklı değildi (tümünde $p>0,05$).

SONUÇ: Bu çalışmanın bulgularının TDT pozitif çocuklarda BAL sıvısı TGF- β düzeylerinin TDT negatif olanlara göre yüksek olma eğilimine işaret etmesi Th1 immün yanıtı azaltılabilecek artmış regülatuar T lenfosit aktivitesini göstermekte olabilir. Bu açıdan TGF- β ileri araştırmalar ile tanısal modalite ve immunomodülatuar tedavi ajanı olarak potansiyeli açısından incelenmelidir.

ANAHTAR SÖZCÜKLER: Tüberkülin deri testi, bronkoalveoler lavaj, sitokin, T lenfosit

INTRODUCTION

Childhood tuberculosis (TB) is characterised by a paucibacillary nature that precludes the use of microbiological diagnostic techniques used in adults [1]. Moreover, collection of sputum is another challenge to diagnosis in children; therefore, bronchoscopy has been reported as a useful tool for diagnosis of TB in smear-negative patients and may have a place in diagnostic workup [2]. Bronchoalveolar lavage (BAL) may be used for microbiological investigations to increase diagnostic yield. Moreover, it may be used for research purposes to evaluate the immunological response at the infection site.

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The immune response to TB is characterised by a predominance of the T helper (Th)1 response but there are many other T lymphocyte groups that contribute [3,4]. The net result between the balance of these different T lymphocyte subgroups determines the status and immunological outcome of the TB infection [4]. Cytokine products of these cells may be used to evaluate this balance. Interleukin (IL)-4 is a cytokine secreted from Th2 cells and IL-10 is secreted from various cells including regulatory T lymphocytes (Tregs) that inhibit the Th1 type protective immunity against TB [5,6]. Similarly, transforming growth factor beta (TGF- β) plays an inhibitory role in the immune response to TB [5]. There are conflicting reports about the influence of IL-17, which is a product of Th17 cells [7,8]. Immaturity of the immune system in children may have an important impact on this response to TB. Therefore, clarification of the immune response to *Mycobacterium tuberculosis* bacilli in children may aid in the development of novel diagnostic techniques and vaccination methods, as well as immunomodulatory therapies.

The aim of this study was to evaluate the levels of IL-4, IL-10, TGF- β , IL-17, and IL-23 cytokines, which reflect different T lymphocyte responses, in BAL samples of children with tuberculin skin test (TST) positivity and to compare the levels with those of TST-negative children.

MATERIAL AND METHODS

Study Population

Inclusion criteria for the TST-positive group were having a TST induration of above 15 mm and clinical need for flexible videobronchoscopy (FB) to evaluate airway involvement or to obtain BAL samples to improve diagnostic yield. Inclusion criteria for the TST-negative group were having a TST induration of 15 mm or less and clinical need for flexible videobronchoscopy (FB) for recurrent wheezing, suspicion of foreign body aspiration, or bronchiectasis. Exclusion criteria for both groups were presence of active lower respiratory tract infection and immunodeficiency or use of anti-inflammatory treatment during the previous week. All children who met the inclusion criteria were enrolled consecutively.

Study Design and Ethical Approval

This was a case-control study comparing cytokine levels in BAL samples from TST-positive and -negative children. This study was approved by the Institutional Ethics Board of Celal Bayar University (Date: 13.03.2009, number: 0082) and informed consent was obtained from the parents of all children enrolled in the study.

Data Collection

BAL samples were obtained from all children during the FB procedure. A smear for acid-fast bacilli, polymerase chain reaction (PCR), and TB culture were performed in all samples immediately after they were obtained. Samples were stored at -80°C for cytokine measurement. Levels of IL-4, IFN- γ , IL-10, TGF- β , IL-17, and IL-23 were measured by the ELISA method.

Tuberculin Skin Testing

We performed TST by applying 0.1 mL (5 tuberculin units) of the tuberculin solution (PPD-S Tween 80) intradermally to the inner side of the forearm of all children. Induration was

measured in millimetres at the end of 72 hours. TST skin test positivity was defined as induration of more than 15 mm according to the guidelines of the Turkish Ministry of Health.

Statistical Analysis

Statistical analysis in this study was performed using Statistical Package for the Social Sciences (SPSS) 13.0 computer software (Chicago, IL). Demographic characteristics were analysed using descriptive statistics. Gender, BCG vaccination status, and TST results were reported as frequencies. Mann-Whitney U test was used to compare cytokine levels between the groups. Statistical significance was defined as a p value of <0.05.

RESULTS

Sociodemographic and Disease Characteristics of the Study Population

Mean age of the children enrolled in the TST-positive (n=12) and -negative groups (n=11) were 8.6 (4.3) vs. 6.8 (4.5) years, respectively (p=0.35). All of the patients had been vaccinated once in their lifetime. Mean TST measurement of the TST-positive group was 18.4 (3.1) mm, compared with 2.3 (2.7) mm in the TST-negative group (Table 1). All children had at least one BCG vaccine scar.

Bronchoalveolar Lavage Cytokine Levels

There was a trend of higher TGF- β levels in TST-positive children compared with TST-negative children [557.9 (515.3) pg/mL vs. 386.3 (208.1) pg/mL, respectively, p=0.07] (Figure 1). Mean levels of IL-23 in TST-positive children were 39.2 (29.5) pg/mL vs. 46.2 (72.3) pg/mL in TST-negative children (p=0.49). IFN- γ levels were not significantly different between TST-positive and -negative children [47.1 (59.6) pg/mL vs. 37.4 (24.5) pg/mL, respectively, p=0.42]. Similarly, there was no significant difference in IL-4, IL-10, or IL-17 levels between the two groups (p>0.05) (Table 2).

DISCUSSION

The results of our present study demonstrated a trend of increase in TGF- β levels in local immune response in TST-positive cases but failed to detect a significant change in IFN- γ , IL-4, IL-10, IL-17, and IL-23, indicative of Th1 and Th2 immune responses as well as Th17. The trend of higher

Table 1. Sociodemographic and disease characteristics of the study population

	TST (+) Group	TST (-) Group	p
Age (years)*	8.6 (4.3)	6.8 (4.5)	0.35 [§]
TST (mm)*	18.4 (3.1)	2.3 (2.7)	<0.001 [§]
Gender			
Male (%)	41.7	54.5	0.54 [£]
Female (%)	58.3	45.5	
Female (%)	58.3	45.5	

TST: Tuberculin skin test

*Expressed as mean (standard deviation)

[§]Mann-Whitney U test

[£]Chi square test

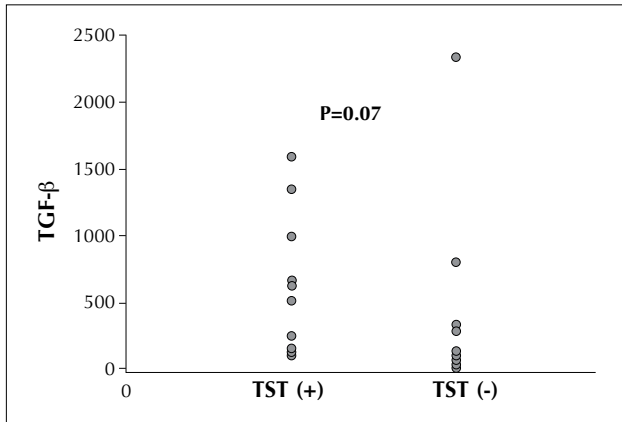


Figure 1. Distribution of TGF- β levels in TST (+) and TST (-) g

TGF- β levels in TST-positive cases might indicate higher Treg function in these children.

BAL might be an important tool for diagnosis of sputum-negative TB despite conflicting reports, and it yields improved sensitivity of culture and ELISPOT results in these patients [9,10]. These findings point out that BAL may also be used as a valuable tool to investigate immune response to TB. That paediatric TB is mostly smear-negative increases the value of BAL as a sample to investigate immune response [1]. Moreover, cytokine release from bronchoalveolar cell cultures is more significantly different between TB and non-TB cases compared with peripheral blood mononuclear cell cultures, indicating the concentration of immune response locally [11]. Therefore, BAL was chosen as a sample in our study to investigate the immune response in paediatric TST-positive cases.

Imbalance between Th1 and Th2 lymphocyte responses constitutes one of the major components of TB immunopathogenesis [3,4]. IFN- γ is a marker of the Th1 response and IL-4 is of the Th2 response [3,4]. Release of both IFN- γ and IL-4 from bronchoalveolar lavage cells was found to be increased in TB cases compared with non-TB controls [11]. However, our results failed to demonstrate a difference between direct measurements of these cytokines in BAL fluids of children with positive and negative TST. This may be attributed to the lack of culture preparation from cells and antigenic stimulation of these cultures to measure cytokine levels. Another reason may be the primary TB characteristics of the paediatric TST-positive cases enrolled in our study. These may have weakened the local immune response. Similarly, IL-10, which is also a cytokine that inhibits proinflammatory cells, was not found to be significantly different between the groups of our study, despite previous studies performed by measuring cellular IL-10 response that revealed high levels in TB cases compared with non-TB patients [12]. The discrepancy between our study and this cellular culture-based measurement of IL-10 also supports that direct measurement of cytokine levels in BAL fluid may be inadequate to detect differences among TB and non-TB cases, weakening the possibility of using it as a diagnostic technique.

Th17 cells are a relatively new T lymphocyte subgroup that secretes mainly IL-17 and IL-23 is an important regulator of

Table 2. Cytokine concentrations in the bronchoalveolar lavage fluid of the study groups

	TST (+)	TST (-)	p ^s
IL-4 pg/mL*	7.0 (6.1)	6.4 (4.9)	0.85
IL-10 pg/mL*	2.7 (3.3)	2.0 (0.9)	0.27
IFN- γ pg/mL*	47.1 (59.6)	37.4 (24.5)	0.42
TGF- β pg/mL*	557.9 (515.3)	386.3 (690.3)	0.07
IL-17 pg/mL*	115.0 (106.9)	108.6 (97.6)	0.85
IL-23 pg/mL*	39.2 (29.5)	46.2 (72.3)	0.49

IL: Interleukin, IFN: Interferon, TGF: Transforming growth factor

TST: Tuberculin skin test

*Expressed as mean (standard deviation)

^sMann-Whitney U test

IL-17 secretion [13-15]. IL-23 was proposed to participate in immunity against TB by induction of IL-17 [15]. IL-17 is an important pro-inflammatory cytokine that plays role in TB immunopathogenesis, as a regulator of the Th1 response [14,16]. The results of our study failed to demonstrate a significant difference of IL-17 levels in BAL fluid of TST-positive and -negative children. This failure to detect a significant difference despite previous research indicating an important role of IL-17 in TB immunity suggests that direct IL-17 measurement in BAL may not be suitable for diagnostic purposes, similar to other cytokines we have measured in this study except TGF- β .

Tregs are a subset of T lymphocytes that suppress effector T lymphocytes via cytokines such as TGF- β [17,18]. Therefore, increased numbers of Tregs may lead to suppression of the Th1 response via TGF- β secretion [17]. Similar to our results, a previous study reported insignificantly higher levels of TGF- β in BAL fluids of adult cases with active TB compared with healthy controls [11]. In the same study, other cytokines measured, including IL-10 and IL-9, were not significantly different between the groups [11]. These findings suggest that cytokine levels in BAL fluid may be too low to detect a significant difference between TB and non-TB groups. However, the increase of TGF- β in cases with TB compared with non-TB cases was more prominent than that of other cytokines that are thought to have a role in TB immunopathogenesis. Further studies on direct measurement of TGF- β levels in BAL fluid or in cell cultures obtained from BAL may provide more information about this issue, clarifying the potential of TGF- β in BAL as a diagnostic tool and the possibility of TGF- β -inhibiting immunomodulatory therapies.

The major limitation of this study was the small size of the study sample. However, bronchoscopy is an invasive technique that is not performed in every paediatric case of suspected TB. Therefore, this study was performed as a preliminary one to plan future research on the utility of BAL cytokine measurements as a diagnostic tool as well as a research tool for TB immunology.

In conclusion, the results of this study suggest that TGF- β in BAL fluid of children with TST positivity tends to be higher than that of TST-negative children, which indicates an

increased activity of Tregs that may decrease the Th1 immune response. However, the low number of cases enrolled precludes any direct conclusions. If this difference in TGF- β levels can be supported by future research, TGF- β might be a future diagnostic modality and immunomodulatory treatment target.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Celal Bayar University School of Medicine (13.03.2009, No: 0082).

Informed Consent: Written informed consent was obtained from the parents of the patient who participated in this study.

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