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SHORT COMMUNICATION

Serum cytokines markers in toluene diisocyanate-induced asthma☆

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Summary
Background: Toluene diisocyante-induced occupational asthma (TDI-OA) is an inflammatory disease of airway, composing inflammatory cells and cytokines associated with airway remodeling. Majority of the patients with TDI-OA presented persistent asthma. Therefore, early diagnosis is essential for the favorable prognosis. We investigated to identify serologic markers for early diagnosis of TDI-OA.
Methods: We enrolled 69 patients with TDI-OA and 95 asymptomatic exposed controls (AECs). Neutrophil-related cytokines, including myeloperoxidase (MPO) and interleukin-8 (IL-8), as well as airway remodeling-related cytokines, including transforming growth factor-β1 (TGF-β1), metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), and vascular endothelial growth factor (VEGF), were measured by ELISA in the sera of each subject. To evaluate the validity of the each cytokine and combined cytokines for discriminating between TDI-OA and AEC, Receiver operating characteristic (ROC) curve was used.
Results: There were significant differences in the serum levels of MMP-9 and VEGF between two groups ($p < 0.05$). Using the optimal cutoff value of MMP-9 (182.96 ng/mL), the sensitivity and specificity were 79.7% and 80.0%, respectively, with the area under the curve (AUC) of 0.815. When the cytokines were combined to improve sensitivity, the combined values of MMP-9, VEGF, and IL-8 comprised the best set, for which the AUC increased to 0.822 and the sensitivity increased to 82.6% but specificity decreased to 75.8%.

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Conclusions: The single serum cytokine MMP-9 level or the combined cytokines MMP-9, VEGF, and IL-8 can be used as meaningful serologic markers for identifying patients with TDI-OA among exposed workers.
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Introduction

Toluene diisocyanate-induced occupational asthma (TDI-OA) is the most common occupational asthma, which is characterized by hyperresponsiveness, inflammation, and remodeling of the airways.[1,2] This inflammation is associated with the infiltration of various inflammatory cells and chemical mediators.[3] Neutrophils in the lung may contribute to TDI-induced bronchoconstriction, which may involve myeloperoxidase (MPO) and interleukin-8 (IL-8).[4,5] Increased production of matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1),[6,7] vascular endothelial growth factor (VEGF), and transforming growth factor-β1 (TGF-β1) have been detected in the bronchoalveolar lavage (BAL), sputum, or lung tissue of patients or mouse models of TDI-induced asthma.[8,9] Long-term follow-up studies have revealed that more than 50% of patients with TDI-OA experience persistent asthmatic symptoms.[9] Many studies have focused on developing tools for the early diagnosis of TDI-OA in exposed workers. The use of TDI-specific antibodies or IgG antibodies to cytokeratin 19 as biologic markers for TDI-OA has been evaluated; however, the sensitivities were too low to be applied.[10–14] Therefore, we measured the levels of serum cytokines in patients with TDI-OA and compared them with those of the AEC group, and sought to identify a new serologic marker for screening potential asthmatic subjects among TDI-exposed individuals.

Materials and methods

Sixty-nine patients with TDI-OA, confirmed by positive response to a TDI bronchoprovocation test,[4] and 95 asymptomatic exposed workers, as asymptomatic exposed controls (AEC), were enrolled. All the subjects remained in exposed state at their working environment during the study period. Demographic data of the study subjects were summarized in Table 1. Serum samples from the subjects were collected before the treatments; TDI-OA asthmatics stopped using anti-leukotrines and anti-inflammatory agents including inhaled or oral steroids for one week before the study. Serum samples were stored at −70 °C until cytokine and immunological assay. The cytokine levels were measured using a commercially available ELISA kit (MPO: BioCheck Inc, Foster City, CA; MMP-9, IL-8, TGF-β1, and VEGF: R&D Systems Inc., Minneapolis, MN; TIMP-1: cat. no. RPN 2618; Amersham Pharmacia Biotech, Amersham, U.K.). 2, 4-TDI-human serum albumin (HSA) conjugates were prepared using a modified version of Tse and Pesce's method.[11] Serum-specific IgG and IgE levels against the TDI-HSA conjugate and serum-specific IgG levels against CK 19 were measured by ELISA, as described previously.[11,15] The positive cutoff value was determined as the mean absorbance of 120 unexposed healthy control subjects plus 2 SD. All subjects gave their informed consent, which was regulated by the Institutional Review Board of Ajou Medical Center, Suwon, Korea.

Mann–Whitney U test was used to compare the serum cytokines between the two groups. Logistic regression analysis was applied to control for age, sex, smoking status and exposure duration as covariables. p - value of ≤ 0.05 was considered to be significant. Receiver operating characteristic (ROC) curves were used to evaluate the validity of the serum cytokines for discriminating between TDI-OA and AEC, and the area under the curve (AUC) with a 95% CI was computed. Sensitivity and specificity were calculated according to the identified optimal cutoffs. Bonferroni's test was used for multiple comparisons.

Table 1 Clinical features of the study subjects.

	TDI-OA ($n = 69$)	AEC ($n = 95$)	p-value
Sex (male/female)	40/29	68/27	0.428
Age (years)	42.6 ± 9.9	41.1 ± 8.2	0.288
Atopy (%)	44.8	ND	NA
Smoker (%)[a]	34.4	53.6	0.020
FEV1 (%)[b]	83.1 ± 23.3	ND	NA
PC20 (mg/mL)	6.5 ± 9.6	ND	NA
Duration of exposure (years)	6.7 ± 0.8	11.2 ± 1.8	0.016
Total IgE (IU/mL)	265.8 ± 48.7	157.2 ± 38.2	0.037
IgG to CK19 (%)	25	5.3	< 0.001
sIgG to TDI-HSA (%)	22.7	9.5	< 0.001
sIgE to TDI-HSA (%)	25.4	3.2	< 0.001

All values are presented as mean ± SD. TDI-OA, Toluene diisocyanate - induced occupational asthma; AEC, asymptomatic exposed control; NA, not applicable; ND, not done; CK, cytokeratin; sIgE, serum-specific IgE antibodies; TDI-HSA, toluene diisocyanate-human serum albumin; sIgG, serum-specific IgG antibodies.

[a] Current and ex-smoker.

[b] Performed before the methacholine challenge test.

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Table 2 Profile of serum cytokines in TDI-OA and AEC.

	TDI-OA ($n = 69$)	AEC ($n = 95$)	Unadjusted p-value	Adjusted p-value
MMP-9 (ng/mL)	94.00 (127.81)	288.05 (209.85)	<0.001	<0.001
MMP-9/TIMP-1	1.41 (2.09)	4.06 (3.74)	<0.001	0.072
IL-8 (pg/mL)	15.37 (25.38)	39.42 (47.39)	<0.001	0.648
MPO (ng/mL)	93.88 (161.19)	155.33 (169.39)	0.001	0.177
VEGF (pg/mL)	269.00 (339.92)	211.07 (177.65)	0.015	0.017
TGF-β1 (ng/mL)	39.10 (18.47)	42.73 (13.55)	0.181	0.417
TIMP-1 (ng/mL)	68.00 (37.23)	72.84 (18.87)	0.470	0.473

All data are presented as median (interquantile range). Logistic regression analysis was applied to control for age, sex, smoking status and exposure duration as covariables. TDI-OA, Toluene diisocyanate - induced occupational asthma; AEC, asymptomatic exposed control; MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor; TIMP-1, tissue inhibitor of metalloproteinase-1; IL-8, interleukin-8; TGF-β1, transforming growth factor-beta1; MPO, myeloperoxidase.

Table 3 Sensitivity, specificity, and values of area under the ROC curve with 95% CI and p-values of various combinations of cytokines to identify TDI-OA among exposed workers.

Rank	Variables	Sensitivity (%)	Specificity (%)	AUC	p-value
1	MMP-9 & VEGF & IL-8	82.6	75.8	0.822	<0.001
2	MMP-9/TMIP-1 & VEGF & IL-8	82.6	71.6	0.801	<0.001
3	MMP-9 & VEGF & TGF-β1	81.2	76.8	0.809	<0.001
4	MMP-9 & VEGF & MMP-9/TIMP-1	81.2	75.8	0.803	<0.001
5	MMP-9	79.7	80.0	0.815	<0.001
6	MMP-9/TIMP-1	73.9	69.5	0.734	<0.001
7	IL-8	71.0	71.6	0.745	<0.001
8	VEGF	69.6	49.5	0.596	0.052

TDI-OA, Toluene diisocyanate - induced occupational asthma; AEC, asymptomatic exposed control; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; IL-8, interleukin-8; MPO, myeloperoxidase; VEGF, vascular endothelial growth factor; TGF-β1, transforming growth factor-beta1.

Results

Table 1 listed the baseline clinical features of the study subjects. The mean FEV1 and PC20 in TDI-OA were 83.1 ± 23.3% and 6.5 ± 9.6 mg/mL, respectively (Table 1). The serum level of MMP-9 was significantly lower in TDI-OA than in AEC, whereas the serum level of VEGF was significantly higher in TDI-OA (Table 2). We evaluated the diagnostic performance of each cytokine in discriminating between subjects in TDI-OA and AEC by analyzing the AUC that was computed from the ROC curve. AUC values were found to be 0.872 for MMP-9, 0.794 for MMP-9/TIMP-1, 0.744 for IL-8 and 0.016 for VEGF with statistical significance ($p < 0.001$). The AUC for MMP-9 was the highest and then, we selected the appropriate cutoff values for each ROC curve. Using the cutoff for MMP-9 (182.96 ng/mL), the sensitivity and specificity were 79.7% and 80.0%, respectively, with an AUC value of 0.815 (95% CI, 0.74—0.89; $p < 0.001$). The other cytokines—MMP-9/TIMP-1, IL-8, and VEGF—having displayed statistical differences between the two groups, were analyzed by the same method (Table 3). When each cytokine was combined to improve sensitivity and specificity, the combination of MMP-9, VEGF, and IL-8 was the best, with the AUC value increasing to 0.822 (95% CI, 0.752—0.894; $p < 0.001$) and the sensitivity increasing to 82.6% but specificity decreasing to 75.8%, respectively.

Discussion

In this study, we found significant differences in the serum levels of cytokines MMP-9 and VEGF between TDI-OA and AEC. Among them, MMP-9 is the most prominent cytokine, displaying high sensitivity and specificity using the specific cutoff value. Elevation in the levels of MMP-9 in bronchial mucosal biopsies, BAL, and in induced sputum specimens have been found in TDI-OA.[6,16,17] In recent, a longitudinal study of patients with diisocyanate-induced asthma showed that inhaled steroid medication increased BAL levels of MMP-9, which were correlated inversely with Th-2 type inflammation.[17] In the animal experiment, MMP-9 depleted mice showed increased inflammatory cells aberrantly and fewer eosinophils and neutrophils in their lungs after allergen challenge. These aberrant cellular trafficking patterns were caused by disruption of transepithelial chemokine gradients, indicating that MMP-9 plays a role in the resolution of airway inflammation.[18,19] From this study, serum level of MMP-9 was significantly lower in the patients with TDI-OA than AEC, which may be associated with the protective role of MMP-9. Decreased serum MMP-9 level in TDI-OA in our study may be due to compartmentalization of inflammatory cells. However, further studies to clarity the relationship MMP-9 levels from serum and lung and degree of airway remodeling will be needed.

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VEGF contributed to airway obstruction and migration of inflammatory cells in a mouse model of TDI asthma.[20] Serum VEGF was significantly higher in TDI-OA than in AEC in this study, which are similar results in the patients with allergic asthma.[21]

This study is the first to evaluate the serum levels of various cytokines related to the pathogenesis of TDI-OA compared to an AEC group. Significant differences were noted in the serum levels of MMP-9 and VEGF between TDI-OA and AEC. Considering the cost—benefit and applicability in clinical practice, MMP-9 can be the better serologic marker for predicting TDI-OA.

Conflict of interest

The authors in this article don't have any significant conflicts of interest with any companies/organizations.

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