의학 석사학위 논문

Serum Specific IgG to Cytokeratin : A Useful Serologic Marker for Toluene Diisocyanate-Induced Asthma

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- ABSTRACT -

Serum Specific IgG to Cytokeratin : A Useful Serologic Marker for Toluene Diisocyanate-Induced Asthma

Purpose: Although specific IgE and IgG to isocyanate-HSA conjugate has been used to predict specific bronchoprovocation test results, they are not sensitive enough for the diagnosis of patients with toluene diisocyanate (TDI)-induced asthma. We evaluate the clinical significance of specific IgG to three major epithelial cytokeratins (CK)-CK8, CK18, and CK19-in 66 patients with TDI-induced asthma (group Ⅰ), compared with three control groups: 169 asymptomatic exposed subjects (group Ⅱ), 64 patients with allergic asthma (group Ⅲ), and 123 unexposed healthy control subjects (group Ⅳ).

Methods: Serum-specific IgG to human recombinant CKs were measured by ELISA, and ELISA inhibition tests were done. The existence of these antibodies was confirmed by IgG immunoblot analysis. Specific IgE and IgG to TDI-HSA conjugates were measured in the same set of the patients by ELISA.

Results: The prevalences of serum specific IgG to CK18 and CK19 in group I were highest (26.2% each), followed by that of CK8 (18.5%) and these were significantly higher than those in the other three groups ($p < 0.0001$). Results of the ELISA inhibition test showed significant inhibition with addition of each CK in a dose-dependent manner. The sensitivity and positive predictive value of specific IgG to CK18 and CK19 were higher than those of specific IgE and IgG antibodies to TDI-HSA conjugate. No significant associations were found between specific IgGs to three CKs and the prevalence of specific IgG and IgE to TDI-HSA conjugate.

Conclusion: These results suggest that serum specific IgG to CK18 and CK19 can be used as a serologic marker for identifying patients with TDI-induced asthma among exposed workers.

Key Words: isocyanate, asthma, cytokeratin, diagnosis

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ABBREVIATION

TDI : toluene diisocyanate

HDI : hexamethylene diisocyanate

CK : cytokeratin

HSA : human serum albumin

ANA : anti-nuclear antibody

MCP-1 : monocyte chemoattractant protein-1

I. INTRODUCTION

Toluene diisocyanates (TDI) are one of the most commonly identified causes of occupational asthma in far-east Asia, with a prevalence of 2.9% ~ 13% in exposed workers (Park et al, 1992; Kim et al, 1994). The pathogenesis of TDI-induced asthma remains not completely understood, and reliable methods of serological testing have not been established.

Long-term follow-up studies have shown that more than 50% of patients with TDI-induced asthma have persistent asthmatic symptoms after complete avoidance of exposure and taking anti-asthmatic medications (Park and Nahm, 1997). Early diagnosis is the best way to prevent TDI-induced asthma. There have been several studies to find a serologic marker to be used for early diagnosis. However, the prevalence of serum-specific IgE to TDI-HSA conjugate was reported to be 0% ~ 50%, and the sensitivity of serum-specific IgG to TDI-HSA conjugate was less than fifty (Park et al, 1999). More studies are needed to determine the standardized methods for detecting specific antibodies. Recent investigations have demonstrated that inhaled hexamethylene diisocyanate could conjugate to the human airway epithelial protein, keratins, which could initiate immune responses (Wisnewski et al, 1999). Also, our recent preliminary data showed that TDI exposure could increase expression of cytokeratin (CK) 19 from human bronchial epithelial cells in serum samples from patients with TDI-induced asthma.

In this study, we extended these findings and evaluated the clinical significance of serum specific IgG to three major epithelial CKs: CK8, CK18, and CK19 in serum samples from patients with TDI-induced asthma, compared with exposed and unexposed control subjects, in order to be applied for identifying for TDI-induced asthma patients.

II. MATERIALS AND METHODS

A. Materials

1. Subjects

Sixty-six patients with TDI-induced asthma confirmed by positive responses to TDI bronchoprovocation tests were enrolled and classified as group Ⅰ. As control subjects, 169 exposed asymptomatic workers (group Ⅱ), 64 patients with allergic asthma sensitive to house dust-mite allergen (group Ⅲ), and 123 unexposed healthy control subjects (group Ⅳ) were enrolled. Atopy was determined by a positive skin-test results to at least one common inhalant allergen, including house dust mite, tree and pollen mixtures, mugwort, and ragweed pollens, Alternaria (Bencard, Brentford, U.K.). The demographic data of the four study groups are compared in Table 1. Serum samples from group Ⅰ and Ⅲ subjects were collected before the treatments; all of them stopped using inhaled or oral steroids for 4 weeks before the study. Subjects underwent an interview, chest radiography, skin-prick test with common inhalant allergens, lung function measurement, and inhalation challenge with methacholine. All subjects gave their informed consent, which was regulated by the Institutional Review of Board of Ajou Medical Center, Suwon, Korea.

B. Methods

1. Bronchial challenge test with methacholine and TDI

The methacholine bronchial challenge test was done for subjects in groups Ⅰand Ⅲ, according to methods previously described (Park et al, 1999). Briefly, aerosols were generated by a DeVilbiss 646 nebulizer connected to a Devilbiss dosimeter driven by compressed air (Devilbis Co., Doylestown, PA). Five inhalations of normal saline at 5-min intervals were administered, followed by a series of successively doubled doses of methacholine (0.075 ~ 25 mg/ml) until a 20% decrease in FEV1 was observed or the maximum dose was given. FEV1 was

measured 5 min after the beginning of each set of inhalations of aerosolized methacholine. The methacholine PC20 level was determined by interpolation from the dose-response curve. The TDI bronchial challenge test was performed according to the protocol described in a previous report (Park et al, 1999).

2. Preparation of TDI-HSA conjugate

2,4-TDI-HSA conjugates were prepared as described previously by using a modification of Tse and Pesce's method (Park et al, 1999). 2,4-TDI was added to 90 ml of 1% HSA in PBS with constant stirring. Aliquots were taken 5, 10, 20, 30, and 40 min after the reaction. Ten milliliters of 1% HSA solution was used as an unconjugated control. Ammonium carbonate (2 mol/L) was added to each aliquot to terminate reactions. All reactive samples were centrifuged at 3,000 g for 40 min at 4°C, to remove unreactive TDI, extensively dialyzed for 3 days against 0.1 mol/L ammonium carbonate, precipitated with an equal volume of 20% trichloroacetic acid, redissolved in 1 mol/L sodium hydroxide, and then dialyzed with 4 L of deionized water for 1 day. When the degrees of substitution were determined by modified Gutmann assay, the molar ratio was 5. The protein content of a conjugated sample was determined by Lowery method. All reagents used for the preparation of TDI-HSA conjugates were purchased from Sigma Chemical Co. (St Louis, MO).

3. Specific IgE and IgG antibody to TDI-HSA conjugate by ELISA

The serum specific IgG level was detected by ELISA, as described previously (Park et al, 1999). In brief, after 1 μg of TDI-HSA conjugate or mock conjugate was dissolved in sodium bicarbonate buffer (pH 9.5), it was coated onto an ELISA plate individually (Corning, New York, NY) at 4°C. After washing with PBS-Tween 20 (PBS-T), they were blocked with 350 μL of blocking buffer (PBS that contained 1% BSA and 0.1% Tween 20). Diluted serum (50 μL) from patient or control subjects at a concentration of 1:50 for specific IgG and 1:2 for specific IgE in the preliminary

experiments was incubated for 1 hr at 37°C in both TDI-HSA- and HSA-coated wells and washed three times. Peroxidase-conjugated anti-IgG (1:2000 v/v, 50 μL; Sigma) or biotin-conjugate anti-IgE antibody (1:200 v/v, Sigma) was added into each well, and incubated for 1 hr at room temperature. Then, 50 μL of substrate solution dissolved in substrate buffer (Sigma) was added. After 15 min of incubation at 37°C, absorbance values were read using an ELISA reader at 405/450 nm. The final absorbance value of each individual was decided after subtraction of the HSA-coated value from that of the TDI-HSA-coated value. The positive cutoff value (0.18 for IgG, and 0.13 for IgE) was determined as mean plus 2-folds SD of the absorbance value from 123 unexposed healthy control subjects.

4. Specific IgG antibodies to three cytokeratins by ELISA

The serum specific IgG level was detected by ELISA using 50 μL of diluted (1:20) patient serum or negative control serum. This was determined to be the optimal concentration in the preliminary experiment and was added to each well of ELISA plates that had been coated with 100 ng/well of CK8, 18, and 19 (Research Diagnostics, Flanders, NJ) dissolved in PBS and blocked with 200 μL of blocking buffer (PBS that contained 10% FBS). After 1 hour of incubation at room temperature, the plate was washed three times with PBS-T. Alkaline phosphatase-conjugated anti-human IgG (100 μL; Sigma), diluted to 1:10000 v/v with 10% FBS-PBS was incubated for 1 hour at 37°C and washed with PBS-T and PNPP solution (p-nitro-phenyl phosphate; Sigma) was added as the substrate solution. Reactions were stopped with the addition of 1N NaOH. The optical density of the solution was determined at 405nm by using an ELISA reader. The final absorbance value was determined from the subtraction of the absorbance value of the uncoated well. Each absorbance value was presented in arbitrary units based on the standard curve, which was derived from serial dilutions of the pooled serum samples with high specific IgG levels to CK8, 18, and 19, respectively. The positive cutoff value was determined as

the mean plus 2-folds SD of the absorbance values of serum samples from 80 healthy control subjects.

5. ELISA inhibition tests with three cytokeratins

Increasing amounts (0.1~100 μg/ml) of each CK as test inhibitor dissolved in blocking solution (PBS that contained 0.005% Tween 20 and 0.5% nonfat dry milk) were incubated with the pooled serum sample from a patient TDI-induced asthma that had a high IgG level to each CK for 1 hour; then they were added to the wells of antigen-coated microtiter plates, and ELISA experiments were repeated as described above. The percentage of inhibition was calculated as follows: % inhibition = 100 × (1 - absorbance with inhibitor) / (absorbance without inhibitor)

6. IgG immunoblot on three cytokeratins

Recombinant cytokeratins were containing 30mM TRIS hydrochloride, 2mM EDTA, 9.5M urea, 2mM DTT, and 10mM methylammonium chloride (pH 8.0) and separated by discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) using a 4~20% Tris-glycine gradient gel (Invitrogen, Carlsbad, CA). After electrophoresis, proteins were transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA) and blocked with 5% nonfat milk. After the transfer, the membrane strips were probed with monoclonal antibody to CK8, CK18, and CK19 (Sigma) at 1 in 20 dilution for 3 hours at room temperature. After washes, the membrane was incubated with alkaline phosphatase-conjugated goat anti-human IgG (Sigma) for 1 hour at room temperature. After final washing, the membrane was stained with a substrate solution; nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl phosphate (Sigma), and the intensity was measured using a densitometer and presented as the relative intensity.

7. Measurement of ANA

To evaluate the presence of autoantibodies, serum ANA level was detected by ELISA method using Hep Scan ANA in the same set of serum samples from patients with TDI-induced asthma, using diagnostic kits (BL Diagnostika, Hamburg, Germany) according to the instruction manual.

8. Statistical analysis

Student's t-test was applied to compare the mean level of specific IgG to CK8, CK18, and CK19 among the four study groups. The Pearson's chi-square test and Fisher's exact test were applied to compare the prevalences of specific IgG to three CKs with specific antibodies to TDI-HSA conjugate or with laboratory findings. The ANOVA test was applied to compare demographic data among the four study groups. A *P* value of 0.05 or less was regarded as significant.

III. RESULTS

A. ELISA results for specific IgG to CK8, CK18, and CK19

Figure 1 shows the specific IgG levels to CK8 (Fig. 1A), CK18 (Fig. 1B), and CK19 (Fig. 1C) by ELISA in the four study groups. When the serum specific IgG level to the three CKs were compared, there were significant differences among the four groups. The specific IgG level to the three CKs were significantly higher in group I than in those of three control groups ($P < 0.05$, respectively; Table 1). In group I, twelve patients (18.5%) had specific IgG to CK8, and specific IgG to CK18 and CK19 were detected in 26.2% of patients, respectively (Table 2). The prevalences of specific IgG to three CKs in group I were significantly higher than those of group Ⅱ, Ⅲ, and group Ⅳ ($P < 0.05$, respectively; Table 2).

B. Association of specific IgG to CKs with specific antibodies to TDI-HSA conjugates and clinical parameters

The prevalences of specific IgG to the three CKs were not significantly associated with those of specific antibodies to TDI-HSA conjugates and clinical parameters such as sex, atopy, smoking status, and ANA in patients with TDI-induced asthma ($P > 0.05$, respectively, Tables 3, 4). Among the subjects in group I, a significant association was noted between the prevalence of specific IgG to CK19 and those of CK8 and CK18 ($P < 0.0001$, respectively; data not shown).

C. ELISA inhibition tests for three CKs

Figure 2 shows the results of the ELISA inhibition test when serum samples from subjects showing isolated positive results to each CK on IgG-ELISA were used. Significant inhibitions were noted with additions of three CKs in a dose-dependent manner.

D. IgG immunoblot analysis of specific IgG to three CKs

Figure 3 shows an IgG immunoblot analysis of three CKs reacting with serum samples from patients with TDI-induced asthma, unexposed healthy control subjects, and buffer controls, which were compared with those of monoclonal antibodies to the three CKs. Specific IgG bindings to three CKs were confirmed on immunoblot analysis.

E. Sensitivity and specificity of IgG to three CKs in TDI-exposed workers

Table 4 demonstrated the sensitivity, specificity, test efficiency, positive and negative predictive values of specific IgG to three CKs and serum specific IgE and IgG antibodies to TDI-HSA conjugate in the study subjects. The sensitivity and positive predictive value of specific IgG to CK18 and CK19 were higher than those of specific IgE and IgG antibodies to TDI-HSA conjugate. Specificity, negative predictive value, and test efficiency were similar to those of specific antibodies.

IV. DISCUSSION

In this study, higher levels of specific IgG to three epithelial CKs; CK8, CK18, and CK19, were detected in serum samples from patients with TDI-induced asthma than in asymptomatic exposed workers or in patients with allergic asthma and healthy unexposed control subjects. Among the three antibodies, those to CK18 and 19 showed the highest sensitivities. Furthermore, the prevalences of specific IgG to CK18 and 19 were significantly higher in patients with TDI-induced asthma than those of asymptomatic exposed workers, and their binding specificities were confirmed by ELISA inhibition tests. Comparing to prevalences of specific IgE and IgG to TDI-HSA conjugate in this study, we can suggest that serum specific IgG to CK18 and 19 might be used as a serological marker for identifying patients with TDI-induced asthma among exposed workers, although the sensitivity was not high enough (26.2%).

Previous studies of the long-term prognosis of TDI-induced asthma suggested that the early diagnosis of sensitized patients and their immediate removal from the exposure could increase the likelihood of remission. However, an appreciable number of patients with TDI-induced asthma do not recover completely, even after avoidance and treatment (Banks et al, 1990; Park and Nahm, 1997), and to develop an early diagnostic marker based on serologic tests is essential in TDI-induced asthma. Although MCP-1 could be a sensitive test to identify diisocyanate (TDI, MDI or HDI) induced asthma (Bernstein et al, 2002), this is not easy to apply in a larger sample repeatedly. Several investigators have detected specific IgE and IgG antibodies to TDI-HSA conjugate in serum samples from patients with TDI-induced asthma using ELISA or RAST. The prevalence of specific IgE to TDI-HSA conjugates has proved quite variable between laboratories, and sensitivity to IgG was not high enough (Park et al, 1999; Park et al, 2002). This study is the first to evaluate whether autoantibodies to major bronchial epithelial CKs can be applied for

identifying asthma patients in a single kind of isocyanate exposed group (patients with TDI-induced asthma. *vs* TDI-exposed, but asymptomatic workers) and sensitivity, specificity, positive and negative predictive value were compared with those of specific IgE and IgG antibodies to TDI-HSA conjugates. The sensitivity and positive predictive value of specific IgG to CK18 and CK19 were higher than those of specific IgE and IgG antibodies to TDI-HSA conjugates with similar specificity, negative predictive value and test efficiency. Significant associations were not found between the prevalences of specific IgG to CKs and those of specific IgE and IgG to TDI-HSA conjugate. These findings suggest that specific IgG to CK18 and CK19 may be used as a supplementary serologic marker to increase diagnostic sensitivities.

Several airway epithelial cell proteins have been identified to be conjugated to diisocynates according to the results of in vitro and in vivo studies (Wisnewski et al, 1999; Lange et al, 1999 CKs; Lange et al, 1999; Wisnewski et al, 2000). CK, a major intracytoplasmic cytoskeleton, would be one of the potential self-antigens and has categorized among a total of 20 human epithelial keratins on the basis of their molecular weights and isoelectric points. Pairs of CKs seem to be consistently coexpressed in different types of epithelial cells (Chu and weiss, 2000). The three CKs applied in this study have been found in both bronchial and lung alveolar epithelial cells, which are the major target cells of inflammatory response in bronchial asthma.

CKs are normally located in the intracellular space and could gain access to the immune system after epithelial damage or cell death. The precise mechanism of their generation is still unknown, although several studies have suggested that CKs are proteolyzed during cell apoptosis can leak into the circulation as a soluble form, and may follow the formation of antibodies against new epitopes (Leers et al, 1999; Dobashi et al, 2000; Murota et al, 2001; Nahm et al, 2002). In this study, high level of specific IgGs to these CKs were detected only in patients with TDI-induced

asthma, not in those with allergic asthma. Therefore, these findings may not be derived from simply epithelial damage found in asthmatic airway. Among the patients with TDI-induced asthma, those who are more susceptible to epithelial damage by TDI exposure may develop serum specific IgG to CKs. Because there is no association between these antibodies and clinical characteristics of TDI-induced asthma, including airway hyper-responsiveness to methacholine, asthma symptoms, and duration of exposure, we could not speculate who would be susceptible patients. Further studies will be needed to investigate genetically susceptible markers for developing specific IgGs to CKs among workers exposed to TDI. The mechanism how the interaction between diisocyantes and human airway epithelial cells can be involved in airway inflammation has not been studied fully (Wisnewski and Redlich, 2001). It has been demonstrated that HDI conjugated with bronchial CK18 could induce T cell proliferation and cytokine production in patients with diisocyanate asthma (Wisnewski et al, 2000; Wisnewski and Redlich, 2001). Further studies will be needed to confirm that specific IgGs to CKs is an epiphenomenon of chronic airway damage occurring in patients with TDI-induced asthma or whether pathogenic or autoantibody-mediated mechanisms are involved.

In conclusion, we demonstrated circulating specific IgG to three bronchial epithelial CKs; CK8, CK18, and CK19 in serum samples from patients with TDI-induced asthma. Among these, IgG to CK18 and CK19 may be used as a serological marker for the detection of TDI-induced asthma among the high risk exposed workers. Further studies will be needed to investigate how these specific IgG to CKs may be involved in the chronic persistent airway inflammation found in TDI-induced asthma.

V. CONCLUSION

Our study suggest that specific IgG to CK18 and CK19 may be used as a suppplementary serologic marker for identification of TDI-induced asthma among exposed workers.

Table 1. Demographic data and levels of serum specific IgG to cytokeratins (CK); CK8, CK18, and CK19

	TDI-induced asthma (n=66)	Asymptomatic exposed workers (n=169)	Allergic asthma (n=64)	Normal control (n=123)
Age (yr)	42.8 ± 10.3	41.0 ± 8.4	31.0 ± 11.7	37.2 ± 11.2
Sex (M/F)	46/21	125/44	34/30	70/53
IgG to				
CK8*	116 ± 61.6	52.6 ± 45.1	76.1 ± 35.7	76.3 ± 39.7
CK18*	132.1 ± 11.22	62.3 ± 57.3	58.3 ± 68.1	72.7 ± 40.2
CK19*	107.7 ± 30.7	55.2 ± 56.0	72.7 ± 40.2	61.6 ± 37.4

All values are presented as mean ± SD.

*$P < 0.05$, significant differences in mean specific IgG levels between subjects with TDI-induced asthma and other three groups.

Table 2. Prevalence of specific IgG to cytokeratin (CK)8, CK18, and CK19 in serum.

	TDI-induced asthma (n=66)	Asymptomatic exposed worker (n=169)	Allergic asthma (n=46)	Normal controls (n=123)	P^*
Specific IgG to					
CK 8	12 (18.5%)[a]	8 (4.7%)[b]	2 (3.1%)[b]	4 (3.3%)[b]	<0.0001
CK 18	17 (26.2%)[c]	11 (6.5%)[d]	3 (4.7%)[d]	4 (3.3%)[d]	<0.0001
CK 19	17 (26.2%)[e]	10 (5.9%)[f]	2 (3.1%)[f]	4 (3.3%)[f]	<0.0001
Specific IgG to TDI-HSA	10 (15.2%)	9 (5.3%)	0 (0%)	1 (1.9%)	<0.0001
Specific IgE to TDI-HSA	5 (7.6%)	1 (0.6%)	0 (0%)	0 (0%)	0.002

* Statistical significance were evaluated by Pearson's chi-square test between patients with TDI-induced asthma and asymptomatic exposed workers.

[a-f] The same letters indicate non-significnat difference between groups based on Pearson's chi-square test and Fisher's exact test.

Table 3. Association of specific IgG to three cytokeratins (CK) with clinical parameters and IgG and IgE antibodies to TDI-HSA conjugate in 66 patients with TDI-induced asthma.

	CK 8		CK 18		CK 19	
Parameters	Positive	Negative	Positive	Negative	Positive	Negative
ANA(n=53)						
Positive	2	3	1	4	1	4
Negative	9	39	15	33	13	35
Smoker (n=66)						
Current	6	17	5	18	6	17
Ex/Non	6	37	12	30	11	32
Sex (n=66)						
Male	2	34	2	34	14	32
Female	1	19	6	14	4	16
Atopy (n=64)						
Present	4	19	5	18	5	18
Absent	7	28	11	24	11	24
IgG to TDI-HSA (n=66)						
Positive	2	9	2	9	1	10
Negative	11	44	16	39	16	39
IgE to TDI-HSA (n=66)						
Positive	2	3	2	3	0	5
Negative	11	50	16	45	17	44

Table 4. Sensitivity and specificity of IgG to cytokeratin (CK) 8, CK 18 and CK 19 for diagnosis of TDI-induced asthma based on the results of bronchoprovocation test in 235 TDI exposed workers.

	TP	TN	FN	FP	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	TE (%)
IgG to									
CK 8	12	161	54	8	18.2	95.2	74.9	60.0	73.6
CK 18	17	158	49	11	26.2	93.5	76.3	60.7	74.5
CK 19	17	159	49	10	26.2	93.5	76.4	62.9	74.9
Specific IgE to TDI-HSA	5	168	61	1	7.6	99.4	73.3	45.4	73.6
Specific IgG to TDI-HSA	10	160	56	9	15.2	94.6	74.1	52.6	72.3

TP: true positive, TN: true negative, FN: false negative, FP: false positive

NPV: negative predicitive value, PPV: positive predicitve value, TE: test efficiency

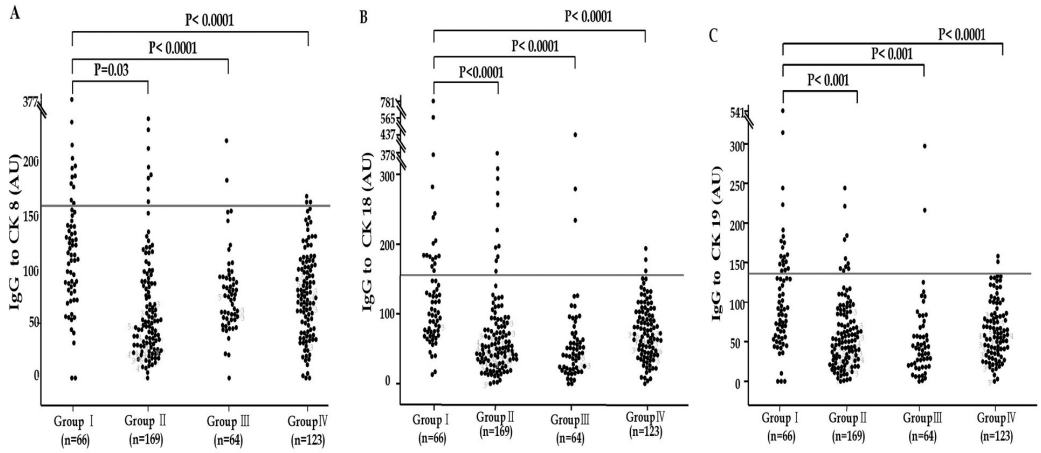

Fig. 1. Specific IgG to cytokeatrain (CK)8 (A), CK18 (B), and CK19 (C) by ELISA in serum samples from the four groups of study subjects. Horizontal bars indicate positive cutoff values.

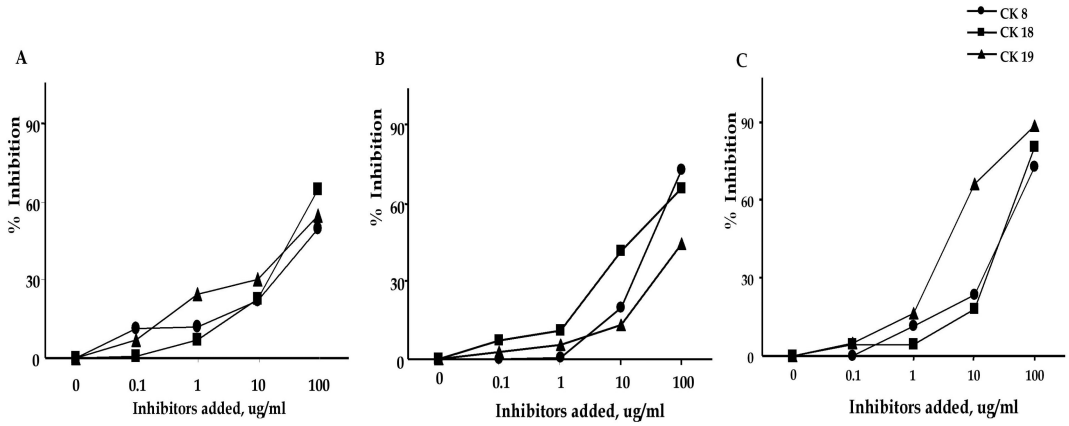

Fig. 2. ELISA inhibition test results for three cytokeratins (CK) : CK8 (A), CK18 (B), and CK19 (C), with serial addition of each cytokeratin (● CK 8, ■ CK18, ▲ CK 19).

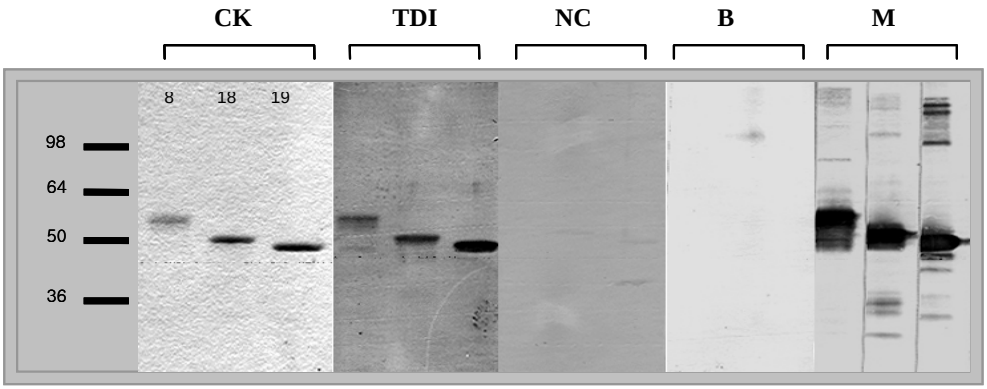

Fig 3. Recombinant cytokeratins on SDS-PAGE (CK) and IgG immunoblot analysis of three cytokeratins using the serum samples from TDI-induced patients with asthma (TDI), unexposed healthy control subjects (NC), buffer control samples (B) and monoclonal antibodies (M) to three cytokeratins.

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- 국문요약 -

TDI 천식에서 사이토케라틴에 대한 특이 IgG 항체의 진단적 유용성

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목적: TDI 천식은 국내에서 가장 흔한 직업성 천식으로 유병율이 2~13%에 달한다. 그러나 많은 연구에도 불구하고 아직까지 정확한 병인기전이 밝혀지지 않았으며, 병력청취와 천식유발시험 외에 혈청학적인 진단의 지표가 확립되지 않은 상태이다. 이에 저자들은 TDI 천식의 진단에 있어 기도 상피세포에 존재하는 사이토케라틴 (CK8, CK18, CK19)에 대한 특이 IgG 항체가 지니는 진단적 유용성을 확인하고자 하였다.

대상 및 방법: TDI 천식 환자 66명과 TDI에 노출되었으나 증상이 없는 근로자 169명, 집먼지진드기에 감수성인 알레르기 천식 환자 64명, 그리고 정상 대조군 123명을 대상으로 하였다. TDI 천식은 TDI 기도유발시험으로 진단하였다. 세 사이토케라틴 (CK8, CK18, CK19)에 대한 특이 IgG 항체를 효소면역법 (ELISA)으로 측정하였고, 사이토케라틴에 대한 특이 IgG 항체를 각각에 대한 효소면역 억제시험 (ELISA inhibition test)과 면역블롯법 (immunoblot)으로 확인하였다.

결과: TDI 천식 환자군에서 CK18과 CK19에 대한 특이 IgG 항체의

양성율이 각각 26.2%로, CK8에 대한 특이 IgG 항체의 양성율 18.5%에 비해 높았다 ($p < 0.0001$). 정상 대조군 뿐만 아니라 알레르기 천식군 및 무증상 TDI 노출군에 비해서 TDI 천식 환자군에서 세 사이토케라틴에 대한 특이 IgG 항체가와 양성율이 통계적으로 유의하게 높았다. TDI 천식 환자에서 사이토케라틴에 대한 특이 IgG 항체의 양성 유무와 TDI 인혈청 알부민 접합체에 대한 특이 항체 (IgE와 IgG) 및 항핵항체, 흡연, 아토피의 유무 등과는 유의한 상관관계가 없었으며, CK18과 CK19에 대한 특이 IgG 항체의 민감도 및 양성예측치가 TDI 인혈청 알부민 접합체에 대한 특이 항체의 것 보다 높게 나타났다.

결론: CK18과 CK19에 대한 혈청 특이 IgG 항체가 TDI에 노출 된 근로자 중에서 TDI 천식 환자를 확인하는데 유용한 혈청학적 지표로 이용 될 수 있을 것으로 생각되며, 향후 이들 사이토케라틴에 대한 특이 IgG 항체가 TDI 천식의 병인기전에 어떻게 관여하는지에 관한 연구가 필요하다.

핵심어: TDI, 천식, 사이토케라틴, 진단