

(Panonychus citri)

Panonychus citri induced allergy among Yuzu farm workers in Koheung area

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Background : Citrus red mite (CRM; *Panonychus citri*), which damages the peels and leaves of Citrus, has been reported as an important occupational allergen in Citrus farm workers. Yuzu belongs to the Citrus genus and its peels are the favorite material for tea in Korea.

Aims : We evaluated the prevalence of the sensitization to CRM in Yuzu farm workers in Koheung area (Southern provinces of Korean peninsula) and analyzed the allergenicity of CRM and the cross-reactivity between CRM and *Dermatophagoides pteronyssinus* (DP) with the sera of the workers and urbanites.

Methods : One hundred and eleven Yuzu farm workers and 36 respiratory allergic urban patients were enrolled in this study. Skin prick test (SPT) to CRM and DP was performed. CRM specific IgE was detected by ELISA and immunoblotting method. Cross-reactivity was investigated through inhibition ELISA and inhibition immunoblotting.

Results : About 39% of the workers showed positive response to CRM in SPT. Prevalence of allergic disease was 66%. Symptoms were associated with their work in 53% of patients. CRM-specific IgE from workers was not inhibited by DP crude antigen but CRM-specific IgE from urban patients was inhibited by DP crude antigen by an average of 70%. With immunoblotting, we could identify 14.5, 15, 17, 18, 21, 22, 32, 33, 49.5 kDa CRM allergens with the sera of the worker.

Conclusions : These results suggest that CRM is one of the unique occupational allergens to the Yuzu farm workers. Cross-reactivity between CRM and DP was found in urban patients. (J Asthma Allergy Clin Immunol 21: 525-534, 2001)

Key words : *Panonychus citri*, *Dermatophagoides pteronyssinus*, cross allergenicity, allergen

Arthropoda), (Class Arachnida),
(Phylum (Order Acarina), (Sub-

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order Prostigmata), (Family Tetranychidae)^{5,6)},
*Panonychus citri*¹⁾.

,
 ,
 ,⁸⁾ IgE

²⁻³⁾ .
 ,
 111

가
 .
 (citrus red mite, CRM)
 5~11

7~8 10 ²⁻⁴⁾ (Fig. 1).
 (house dust mite, HDM)
 가

,
 .
⁵⁻¹³⁾ 1.

111
 62.1 (32~
 81) : = 1 : 1.8
 15.1±8.0 .

36
 3+
 28.2 (14~38)
 : = 1 : 0.4 .

Fig. 1. *Panonychus citri* (Adult Lt : female, Rt : male)

10 .
 2.
 , , ,
 가 ⁵⁻⁶⁾ .
 12.1%, 19.3%가
 .
 3
 , 2
 (Table 1).

3. DP histamine (1 mg/ml)
15 histamine

DP

ethylether 가 1 가 21 mm

4 1:200 W/V phosphate buffered saline(PBS) 24 1+, 2+, 3+, 4+
15 12,000 rpm (pore size - 10 kDa) 24 4 PBS 5. ELISA IgE assay kit Biorad (USA) protein 1:100 SA IgE ELI-carbo-

W/V glycerin nate (pH 9.6) 10 µg/ml 96 well microtiter plate (Costar, Cambridge, MA, USA) well

4. 4 18 Tween phosphate buffered saline(PBST) 2 (1:100 w/v), 6 0.1% bovine serum albumin (BSA)-PBS 200 µl well 1 3

, *Dermatophagoides pteronyssinus* (DP), *Blattella germanica*, Cat, Sagebrush, Ryegrass, Oak 50 µl

Table 1. Questionnaire for allergic diseases and related symptoms

Diseases	Questions
Asthma	Chest tightness Dyspnea /short of breath Paroxysmal cough (often night time) Expiratory wheezing
Allergic rhinoconjunctivitis	Frequent sneezing Watery rhinorrhea Frequent common cold symptom Nasal obstruction Conjunctival injection
Urticaria	Itching sensation on both eyes Itching sensation Erythematous lesion
Relation to work	Aggravated by working Newly developed by working

1:500 biotinylated anti-human IgE (Vector, Burlingame, CA, USA) 50 µl
 1:1000 streptavidinperoxidase (Sigma, St Louis, MO, USA) 50 µl
 30 PBS-T 4
 . ABTS (2,2'-azinobis-3-ethylbenzthiazoline sulfuric acid citrate phosphate buffer) 100 µl
 5 2mM NaN₃ 100 µl
 405 nm

6. ELISA

ELISA

100 µl
 DP 10 µg/ml, 1.0 µg/ml, 0.1 µg/ml, 0.01 µg/ml, 0.001 µg/ml 가 24
 4 .

7. SDS-PAGE immunoblotting

13.5% SDS-polyacrylamide gel
 . 150 µg reducing buffer (60 mM Tris-HCl, 25% glycerol, 2% SDS, 14.4 mM mercaptoethanol, 0.1% bromphenol blue) stacking gel
 50V 30 180V 1
 30 (Small mi-ghty, Hoeffler, San Fransisco, CA, USA).

SDS-PAGE gel nitrocellulose membrane (pore size - 0.45 µm, Amersham, Buckinghamshir, UK) 2

4 mm

5%

. 1:4

24

, 1:2000 alka-

line phosphatase가 goat antihuman IgE (Sigma, St. Louis, MO, USA) 2
 . PBS-T 10 3 , TBS 10
 1 , BCIP-NBT (Promega, Madison, WI, USA) 15

8. Immunoblotting

DP 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml
 가 24 .

1.

가 43 (38.7%) 가
 28 (25.2%),
 18 (16.2%) (Table 2).
 111 73 (65.8%)
 가
 39 (53.4%)
 . (Table 3).
 (22), 가
 (22), 가 (20), (17
), (13), 가 (13)

Table 2. Antigen sensitization of Koheung Yuzu farm workers

	Sensitized people	Percentage(%)
<i>Panonychus citri</i>	43	38.7
DP	28	25.2
<i>Blatella germanica</i>	18	16.2
Oak	10	9.0
Sagebrush	7	6.3
Cat	6	5.4
Ryegrass	3	2.7

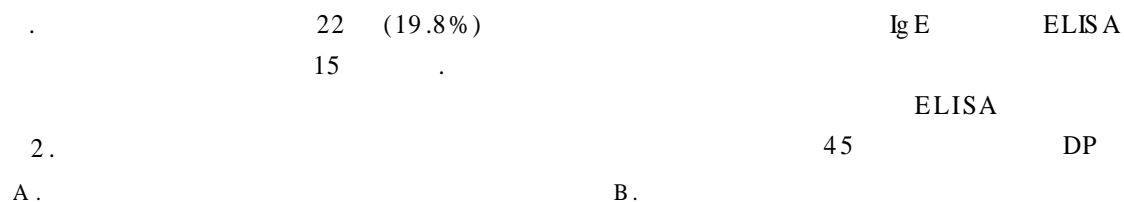


Fig. 2. Distribution of ELISA absorbances of DP vs CRM in 111 Koheung yuzu farm workers(A) and in 36 urban respiratory allergic patients(B).

Table 3. Prevalence of allergic diseases in Koheung Yuzu farm workers and their work relationship

Allergic diseases	Total		Work relationship	
	n (total = 111)	prevalence (%)	n (total = 73)	prevalence (%)
Asthma	19	17.1	10	52.6
Allergic rhinitis	32	28.8	13	40.6
Allergic conjunctivitis	43	38.7	22	51.2
Urticaria	24	21.6	9	37.5
Overall disease	73	65.8	39	53.4

Table 4. Distribution of *P. citri* & DP -specific IgE ELISA absorbances in Koheung Yuzu farm workers and urban respiratory allergic patients

	Koheung Yuzu farm workers (111 people)		Urban allergic patients (36 people)		
	<i>P. citri</i> **		<i>P. citri</i>		
	+	-	+	-	
DP*	+	23	6	28	3
	-	45	37	4	1

*DP cut-off value : Koheung -0.024 / urban -0.006

***P. citri* cut-off value : Koheung 0.016 / urban 0.019

23 . 가 90% , DP
 4 , . DP
 DP 28 (Fig. 2,
 Table 4).
 DP
 IgE ELISA
 2.5 µg/ml 50% ,
 10.0 µg/ml IgE ELISA
 A.

6.0 µg/ml 10.0 µg/ml
 IgE가 50% , 80% ,
 DP 7.5 µg/ml 10.0 µg/ml
 IgE가 50% 65%
 (Fig. 3).

Fig. 4. Analysis of protein bands found in immunoblotting using 20 Koheung yuzu workers' individual sera

B.

Fig. 3. ELISA-inhibition using pooled sera of 4 yuzu workers sensitized only to CRM. CRM is the solid phase, with CRM() and DP() as fluid phase (DP = *D. pteronyssinus*, CRM = *P. citri*) (A) and ELISA-inhibition using pooled sera of 4 urbaners sensitized to both CRM and DP. CRM is the solid phase, with CRM() and DP() as fluid phase (DP = *D. pteronyssinus*, CRM = *P. citri*) (B).

Fig. 5. Immunoblot of *P. citri* allergenic extracts probed with each sera of individuals in urban area (Lane S1-4).

3 .
 blotting
 ELISA
 20
 IgE immu-
 nblotting (Fig. 4).
 14.5, 15, 17, 18, 21, 22, 32, 33, 49.5
 kDa
 IgE가
 , 22 kDa, 18 kDa, 32 kDa
 80%, 70%, 60%
 IgE가 (Table 5).
 DP 4
 IgE immunoblotting
 immunoblotting
 IgE immu-
 noblotting
 가 ,
 (Fig. 5).
 ELISA
 DP
 IgE
 IgE immunoblotting
 IgE

Fig. 6. (A): Inhibition immunoblot of *P. citri* allergenic extracts probed with mixture of pooled sera and 10 fold diluted *P. citri* antigen ($\mu\text{g/ml}$) (NI=no inhibitor).
 (B): probed with mixture of pooled sera and $10\mu\text{g/ml}$ DP antigen (Kh= Koheung Yuzu farm workers, Ub = urban allergic patients).

Table 5. Prevalence of *P. citri* allergens in Koheung Yuzu farm workers

Lane no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	no.	% binding
Protein band (kDa)	49.5	•	•	•		•	•		•					•	•	•	•				10	50
	33	•	•	•		•						•		•	•	•	•				9	45
	32	•	•	•		•	•		•		•			•	•	•	•				12	60
	22	•	•	•	•	•	•		•	•	•	•		•	•	•	•		•		16	80
	21	•	•	•		•	•		•	•	•			•	•		•				11	55
	18	•	•	•	•	•	•		•	•	•		•		•	•		•		•	14	70
	17	•					•								•	•					4	20
	15	•	•	•			•		•						•	•	•	•			11	55
	14.5	•	•	•			•		•						•	•		•			8	40

DP
 DP 10 µg/ml
 immunoblotting
 IgE가 (Fig. 6).
 38.7%
 가
 , 가
 가
 53.4%
 65.8%
 5)
 15.1
 가
 가
 DP
 ELISA
 DP
 Burches DP
 50%
 8)
 가
 ELISA
 IgE
 DP

가

14) .
 , 가
 5-16) .
 가
 가
 가
 immunoblotting
 ,
 22 (24) kDa 32 (34)
 kDa 48.5 kDa, 21
 kDa, 18 kDa
 22, 18, 32 kDa
 80, 70, 60%
 ,
 가
 .
 IgE
 22, 32 kDa 가
 18 kDa

- 1) , , : II.
 (:)
 3: 95-116, 1987
- 2) Choi DS, Kim KC: Population fluctuation, developmental character of *Panonychus citri* and damage degree as its control density on young Yuzu. Kor J Appl Entomol 32: 193-8, 1998
- 3) : (*Panonychus citri*)
 .
 10-8, 1997
- 4) :
 . 6-7,
 1997
- 5) Kim YK, Son JW, Kim HY, Park HS, Lee MH, Cho SH et al: New occupational allergen in citrus farmers: citrus red mite (*Panonychus citri*). Ann Allergy Asthma Immunol 82: 223-8, 1999
- 6) Kim YK, Son JW, Kim HY, Park HS, Lee MH, Cho SH et al: Citrus red mite (*Panonychus citri*) is the most common sensitizing allergen of asthma and rhinitis in citrus farmers. Clin Exp Allergy 29: 1102-9, 1999
- 7) Lee MH, Cho SH, Park HS, Bahn JW, Lee BJ, Son JW et al: Citrus red mite (*Panonychus citri*) is a common sensitizing allergen among children living around citrus orchards. Ann Allergy Asthma Immunol 85: 200-4, 2000
- 8) Burches E, Pelaez A, Morales C, Braso JV, Rochina A, Lopez S et al: Occupational allergy due to spider mites: *Tetranychus urticae*(Koch) and *Panonychus citri*(Koch). Clinl Exp Allergy 13: 383-8, 1983
- 9) Kroidl R, Maasch HJ, Wahl R: Respiratory allergies(bronchial asthma and rhinitis) due to sensitization of type I allergy to red

- spider mite (*Panonychus ulmi* Koch). Clin Exp Allergy 22: 958-62, 1992
- 10) Delgado J, Gomez E, Palma JL, Gonzalez J, Monteseirin FJ, Martinez A et al: Occupational rhinoconjunctivitis and asthma caused by *Tetranychus urticae* (red spider mite). A case report. Clin Exp Allergy 24: 477-80, 1994
 - 11) Delgado J, Orta JC, Navarro AM, Conde J, Martinez A, Martinez J et al: Occupational allergy in greenhouse workers: sensitization to *Tetranychus urticae*. Clin Exp Allergy 27: 640-5, 1997
 - 12) Astarita C, Fanzese A, Scala G, Sproviero S, Raucci G: Farm workers' occupational allergy to *Tetranychus urticae*: clinical and immunologic aspect. Allergy 49: 466-71, 1994
 - 13) Astarita C, Martino PD, Scala G, Franzese A, Sproviero S: Contact allergy: Another occupational risk to *Tetranychus urticae*. J Allergy Clin Immunol 98: 732-8, 1996
 - 14) Ree HI, Jeon SH, Lee IY: Fauna and geographical distribution of house dust mites in Korea. Kor J Parasitol 35: 9-17, 1997
 - 15) Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ: Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: a prospective study. N Eng J Med 323: 502-713, 1990
 - 16) Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P et al: The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Eng J Med 336: 1356-63, 1997
 - 17) Kim HY, Park HS, Kim YK, Son JW, Nahm DH, Cho SH et al: Identification of IgE-binding components of citrus red mite in sera of CRM-induced asthmatic patients. J Allergy Clin Immunol 105: 392, 2000 ()