The Accuracy of Indonesian New Local Skin Prick Test (SPT) Allergen Extracts as Diagnostic Tool of IgE-mediated Atopic Dermatitis

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Abstract

Background: Skin Prick Test (SPT) is good diagnostic tool of allergic disease such as Atopic Dermatitis (AD). Imported allergen extracts are expensive and not always available in Indonesia. New local SPT allergen extracts are potential as substitute.

Objective: To compare SPT results of new local and imported allergen extracts (house dust mite, chicken, egg white, cow's milk, and shrimp), and specific IgE in AD patients.

Methods: This was clinical trial of 45 non lesional AD patients. The SPT of imported allergen extracts was performed in the first week, followed by the new local and specific IgE examination in the next one week.

Result: Sensitivity (Sn) of new local compared with imported allergen extracts in this study was between 15.38%-84.61%, the highest point was house dust mite extract. Specificity (Sp) was ranged from 81.48%-93.75%, the highest rank was shrimp extract. Compared with IgE results, the Sn of new local and imported house dust mite extracts was good (85.7%; 92.9%). The Sp was good for egg white and cow's milk new local extracts (86.4%; 84.4%).

Conclusion: New local allergen extracts should be considered as a substitute of the imported diagnostic tool.

Keyword: *atopic dermatitis, allergen extracts, skin prick test, specific IgE, accuracy.*

Introduction

Atopic dermatitis (AD) is a chronic skin disease characterized by recurrent intense pruritic inflammation. This condition sometimes occurs in patients suffering from allergic rhinitis and asthma, or patient with family history of similar disease.[1] The prevalence of adult AD is varied (US 4.9%, Canada 3.5%, the EU 4.4% and Japan 2.1%). Recently, this condition was observed to increase two to three times. In general, the prevalence of AD does not increase in countries with high prevalence such as the United Kingdom. It was found that the risk of suffering from AD was associated with areas of developing countries such as Indonesia.[2]

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Avoiding precipitating factors is the most important treatment of allergic disease. House dust mite, chicken, egg white, cow's milk, and shrimp are common precipitating factors of AD. Allergic reaction will occur immediately after those proteins bind with specific IgE and Mast cell then trigger the production of histamine and other inflammatory cytokines. Skin Prick Test (SPT) is a diagnostic tool to identify the causes of allergic disease. This test is important modality for showing Ig-E mediated mechanism that causing clinical symptoms.[3] SPT is simple, accurate, and economic diagnostic tool. The incidence of adverse event is low and rarely appear as severe reaction.[4]

Quality of allergen extracts is one of important factors of valid SPT results. Allergen extracts must meet standards, in an effort to provide good evaluation and management of AD patients.[3] Imported allergen extracts are expensive and not always available in Indonesia. There is also possibility of different species used in imported house dust mite extracts that not suitable in Indonesia and influence the accuracy of the test.[4] Dr. Soetomo Academic General Hospital Surabaya Indonesia has produced allergen extracts since around 1990, but there were not much publication about the accuracy of the extracts.

A study is needed to find out the accuracy of new local and imported SPT allergen extracts as diagnostic tool of IgE related AD. The objective of this study was to compare SPT results of new local and imported allergen extracts (house dust mite, chicken, egg white, cow's milk, and shrimp), and specific IgE in AD patients. Results of the study will give consideration in SPT allergen extracts selection in daily practice.

Methods

Subjects

Subjects of the study were 45 AD patients who came to the Allergy and Immunology of Dermatology and Venereology Outpatient Clinic of Dr. Soetomo Academic General Hospital in Surabaya, Indonesia. Inclusion criteria were AD patients aged 18-64 years, willing to sign informed consent, and were in good health at the time of examination. Exclusion criteria were pregnant women, suffering from acute exacerbation of atopic dermatitis or other skin disease, having another chronic disease (stroke, kidney failure, malignancy, or diabetes mellitus), history of anaphylactic shock or other serious allergic eruption, or taking certain drugs (antihistamines, corticosteroids, antidepressants, beta blockers, or ACE inhibitors) in last 2 weeks before study. This research has been approved by Ethical Committee of Dr Soetomo Academic General Hospital Surabaya Indonesia (1493/KEPK/IX/2019).

New local and imported allergen extracts

There were two groups of allergen extracts in this study: the new local and imported allergen extracts. The new local allergen extracts were produced by Dr. Soetomo Academic General Hospital Surabaya, Indonesia. The imported allergen extracts was produced by Astromed^{R} and commonly used as standard allergen extracts. There were 5 types of allergens in this study: house dust mites, chicken, egg white, cow's milk, and shrimp. Normal saline was used as negative control, and histamine dihydrochloride 0.1% was used as positive control.

Skin prick test

SPT was examined in 2 different times, at one week interval. Subjects screening including history taking and physical examination was performed in the first visit. Subjects who met inclusion criteria and willing to join the research were given explanation related to the study and asked to sign the informed consent. First SPT using imported allergen extracts was performed in the right volar side of forearm after the subjects recruitment process was complete. The testing area was marked with minimum distance of 3 cm between each allergens. The skin of subjects dripped with imported allergen extracts in the planned area then pricked by microlancette. One microlancette was used for only one allergen. After skin pricking, allergen extract was immediately removed using sterile gauze. One gauze was used carefully for each allergen so that they were not mixed. Induration was calculated 15-20 minutes after examination. The edge of induration was marked and attached to the translucent tape, then affixed in millimeter (mm) paper and measured by caliper. SPT results are considered positive if they have induration more than 3 mm, with

positive controls more than 3 mm and negative controls less than 3 mm. Subjects were asked to stay in the outpatient clinic 30 minutes after the examination to avoid the possibility of adverse event. Oral antihistamine and topical corticosteroid were always available. Second SPT was performed one week after first SPT with the same procedure, but using new local allergen extracts in the left volar side of forearm. Adverse effects possibility of previous SPT and physical examination was checked before second SPT was performed.

Specific IgE

Spesific IgE was examined in the second visit, after performing SPT using new local allergen extracts. Specific IgE classified to be positive if the consentration is more than 0.35 kU/l. House dust mite allergen extracts consist of *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Dermatophagoides microceras*, *Thyrophagus putrescentiae*, *Glyciphagus domesticus*, and *Blomia tropicalis*.

Statistic analysis

Data was analized by SPSS version 25, and presented as number and percentage. Kappa agreement was used to evaluate the agreement between new local and imported allergen extracts. Kappa is classified to be no agreement (<0), none to slight (0,01-0.20), fair (0,21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) and almost perfect (0.81-1.00).[5] Sensitivity (Sn), Spesificity (Sp), negative predictive value (NPV), positive predictive value (PPV), likelihood ratio (LR +/-) were used to evaluate the validity of SPT results.

Results

Table 1. Validity of new local compared with imported allergen extracts

	SPT of new local allergen extracts						Disease Prevalence
	Sn	Sp	PPV	NPV	LR+	LR-	
House dust mite	84.61%	83.33%	97.1%	45.5%	5.08	0.18	86.67
Chicken	33.33%	81.48%	54.5%	64.7%	1.08	0.82	40.00
Egg white	15.37%	89.47%	66.7%	43.6%	1.46	0.95	57.78
Cow's milk	27.27%	88.23%	42.9%	78.9%	2.32	0.82	24.44
Shrimp	34.48%	93.75%	90.9%	44.1%	5.52	0.70	64.44

The validity of new local compared with imported allergen extracts is shown in Table 1. The Sn of new local compared with imported allergen extracts in this study was between 15.38%-84.61%, the highest point was house dust mite extract. The Sp was ranged from 81.48%-93.75%, the highest rank was shrimp extract. PPV of house dust mite and shrimp allergen extracts were very good (97.1%; 90.9%), while NPV only good at cow's milk extract (78.9%). Disease prevalence was good in house dust mite extracts (86.87).

Table 2. Validity of new local and imported allergen extracts compared with specific IgE

	SPT	Sn	Sp	PPV	NPV	LR+	LR-	Disease prevalence
House dust mite	New local	85.7%	41.2%	70.6%	63.6%	1.46	0.35	62.22
	Imported	92.9%	23.5%	66.7%	66.7%	1.21	0.30	62.22
Chicken	New local	0%	75%	0%	97.1%	0	1.33	2.22
	Imported	100%	61.4%	5.6%	100%	2.60	0	2.22
Egg white	New local	0%	86.4%	0%	97.4%	0	1.15	2.22
	Imported	0%	40.9%	0%	94.7%	0	2.44	2.22
Cow's milk	New local	-	84.4%	-	100%	-	-	0
	Imported	-	75.6%	-	100%	-	-	0
Shrimp	New local	0%	73.2%	0%	88.2%	0	1.36	8.89
	Imported	100%	39%	13.8%	100%	1.64	0	8.89

Validity of new local and imported allergen extracts compared with specific IgE is shown in Table 2. The range of the Sn for new local allergen extracts was 0%-85.7%, while the imported one was 0%-100%. The Sp of new local allergen extracts was ranged from 41.2%-86.4%, while the imported one was 23.5%-75.6%. The Sn of new local and imported house dust mite extracts was good (85.7%; 92.9%). The Sp was good for egg white and cow's milk new local extracts (86.4%; 84.4%). Sp of new local was better than the imported allergen extracts in all allergen types. PPV was not good in all allergen extracts. NPV was good in all allergen extracts (88.2-100%), except new local and imported house dust mite extracts (63.6%; 66.7%). Disease prevalence was not really good (0-62.225).

Table 3. Correlation of SPT results between new local and imported allergen extracts

	Patients with positive SPT		Kappa
	Imported (n)	New local (n)	
House Dust Mite	39	34	0.502
Chicken	18	11	0.158
Egg white	26	6	0.043
Cow's milk	11	7	0.177
Shrimp	29	11	0.225

The agreement results between new local and imported allergen extracts shown in Table 3. The agreement was moderate (K=0.502) for house dust mite and fair (K= 0.225) for shrimp extracts.

Table 4. Correlation of SPT results between new local and imported allergen extracts and specific IgE

	Specific IgE (+)	Kappa	
		Imported	New local
House Dust Mite	28	0.188	0.289
Chicken	1	0.066	-0.042
Egg white	1	-0.045	-0.040
Cow's milk	0	-	-
Shrimp	4	0.102	-0.150

The agreement results between new local and imported allergen extracts compared with IgE shown in Table 4. The agreement of SPT results between new local and imported allergen extracts compared to IgE was fair (K=0.289) for new local house dust mite extracts, and slight agreement (K=0.188) for imported extracts of the same allergen.

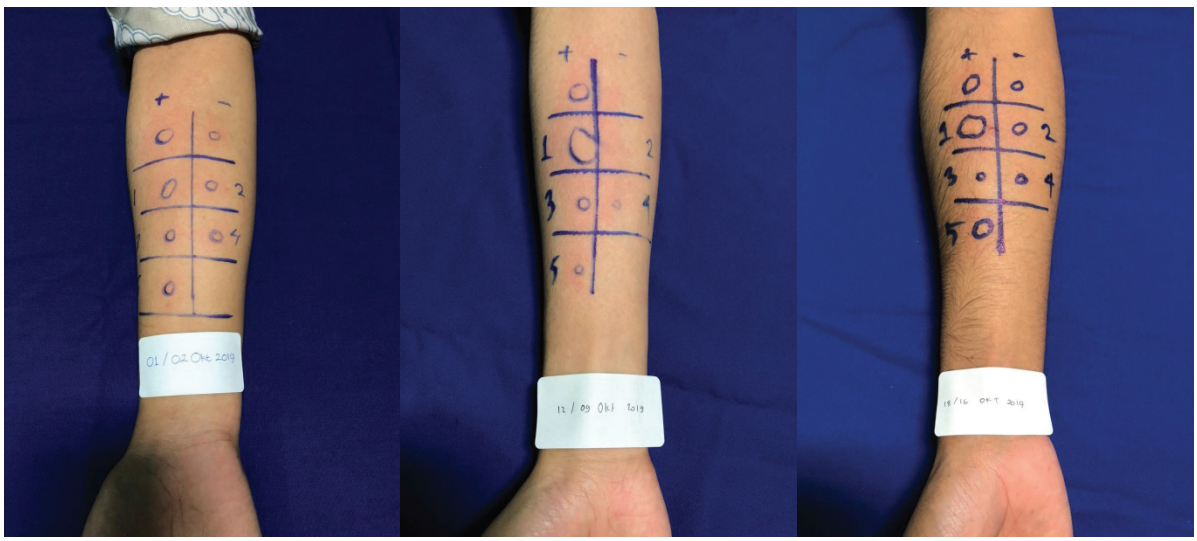

Fig. 1: SPT (Skin Prick Test) of imported allergen extracts in atopic dermatitis (AD) patients.

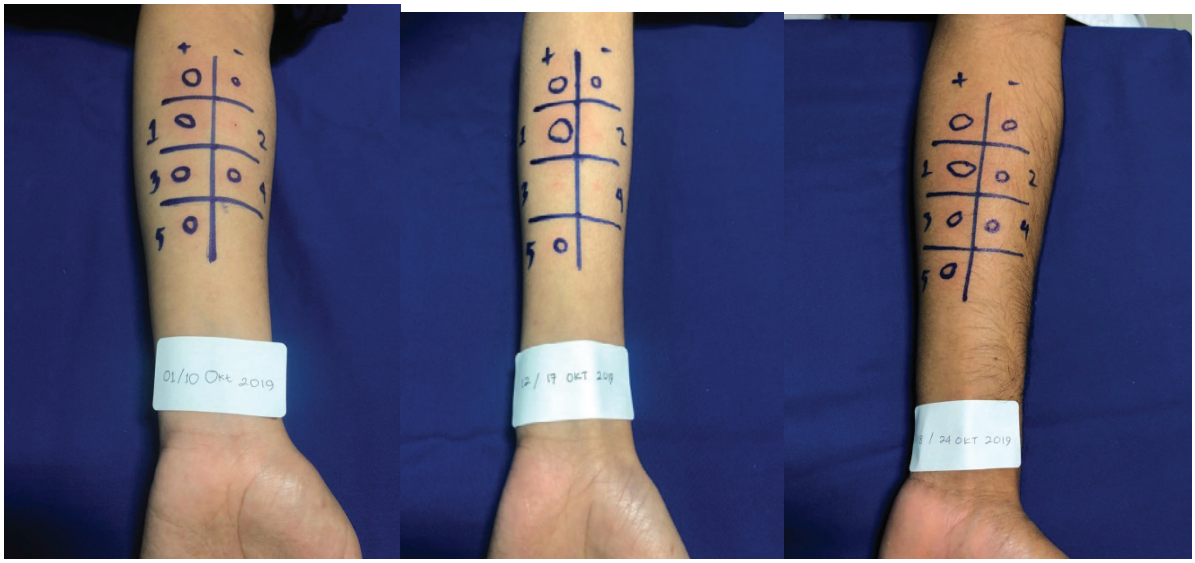

Fig. 2: SPT (Skin Prick Test) of new local allergen extracts in atopic dermatitis (AD) patients.

Discussion

SPT is one of many diagnostic tool to confirm sensitization process of an allergen by detection of specific IgE antibodies in allergic disease such as AD. This method is considered as good diagnosis tool in allergic disease because it is simple, safe, accurate, and economic.[4] SPT is expected to easily stated whether the patients have sensitization to certain allergen or not, in order to avoid the exacerbation of the disease. Similar to other diagnostic tool, the accuracy of the test is important. Accuracy is result of validity appraisal that commonly presented as Sn, Sp, NPV, PPV, LR, and disease prevalence. Negative Sn rules OUT the disease (SnNOUT), and Positif Sp rules IN the disease (SpPIN).[6] Negative NPV is probability that patient will truly not have the disease, while positive PPV is probability that patient will truly have the disease. LR is commonly used to evaluate the ability of test, higher LR + and lower LR - is indication of better test.

This study aimed to compare SPT results of new local and imported allergen extracts (house dust mite, chicken, egg white, cow's milk, and shrimp), and specific IgE. The sensitivity of new local compared with imported allergen extracts in this study was 15.38%-84.61%, the highest point was house dust mite extract. The specificity was ranged from 81.48%-93.75%, the highest rank was shrimp extract. Compared with IgE results, the sensitivity of new local and imported house dust mite extracts was good (85.7%; 92.9%). The specificity was good for egg white and cow's milk new local extracts (86.4%; 84.4%).

House dust mite extracts generally have higher Sn and Sp compared with other allergen extracts. It is in accordance with several studies that stated good value of Sp (70-95%) and Sn (80-97%) in allergic inhalation disease, compared with food allergies with Sp (30-90%) and Sn (20-60%). Type of allergen and technique also can causing variation of test results.[7] Composition of allergen components is also important, there may be differences in house dust mite species in both extracts. The most common species of house dust mite in Indonesia are *Dermatophagoides farinae* and *Dermatophagoides pteronissynus*, which are the same with content of imported house dust mite extract. PPV was very good (97.1%), LR - was lowest (0.18), and disease prevalence was higher than the other allergen extracts (86.67%), indicate that this new local extract is potential to be a good substitute of imported allergen extract. Other study in Thailand also reporting high value PPV and superiority of house dust mite extract than specific IgE.[8]

Chicken SPT in Malaysia has Sn 33.3% and Sp 57.5%.[9] This study showed the Sn and Sp values of 33.33% and 81.48%. Compared to specific IgE, the Sn and some parameters were zero due to no true positive result in this group. It was also occur in egg white SPT

group.

The Sn and Sp of egg SPT in Czech Republic were 68.7% and 93.3%, while in Thailand 82% and 25%, and Sweden were 60% and 41%.[10,11,12] Sn and Sp of egg white new local extract were 15.38% and 89.47%.[13] The agreement result in this study was K=0.043, while in other studies showed that result was K=0.625.[14] Compared to specific IgE, Sp of this extract is highest among the other allergen extracts (86.4%). It also has high NPV (97.4%) and lowest LR- (1.15).

Cow's milk SPT reported varied value of Sn and Sp. Research in Czech Republic (Sn 33.3%; Sp 97.9%), Thailand (Sn 22%; Sp 85%), and Sweden (Sn 41%; Sp 99%).[10,11,12] New local cow's milk in this study has Sn and Sp values of 27.27% and 88.23% compared to the imported extract. Compared to specific IgE, the Sp is highest than other allergen extracts (84.4%). The NPV was highest (78.9%; 100%), but some parameters could not be evaluated because there were no positive specific IgE in this group. More selective inclusion criteria about patient's history of selected food allergy is important to gain better evaluation results.

Shrimp SPT in America has high value of Sn and Sp (100% and 77%), while study in Thailand reported low value of Sn and high value of Sp (0% and 100%).[4,11] The Sn and Sp of new local shrimp extract in this study were 34.48% and 93.75%. The Sp was highest among other allergen extracts, so that the PPV (90.9%) and LR + (5.52). The agreement of new local shrimp extract was fair (K=0.225), while substantial in other studies (K=0.627). Studies population seemed play a role, subjects in this study was not specified to food allergy patients.[14] Mismatch results and differences of SPT Sn and Sp between regions can occur due to reagents, methods, and host gastrointestinal defenses. Recently there is no international standard of allergen extracts, the reagent production in each region is various. The SPT procedures and interpretations are also varied. Single international standardization is needed because each allergic reagent manufacturer can differ in the content of ingredients that result in different outputs.[7]

Specific IgE antibodies can show positive results in patients without clinical manifestations of AD, and vice versa. Retrospective history taking of allergens through history is subjective depending on individual memories, as in this study.[8] Difference population, sample, genetics, environmental and test methodology can produce various result. In this study, we found positive result of house dust mite (n=28) was higher than other allergen. It is confirmed by the agreement of SPT results compared to IgE that was fair (K=0.289) for new local and slight agreement (K=0.188) for imported house dust mite extracts. SPT is proved to be accurate diagnostic tool of AD. Compared to IgE, SPT is less expensive and the new local house dust mite extract seem to be more specific than the imported one. The Sn was good (85.7%), PPV (70.6%) and disease prevalence (62.22%) also higher than the other allergen extracts. Those data conclude that the new local allergen extracts should be considered as a substitute of the imported diagnostic tool.

Conclusion

The Sn of new local compared with imported allergen extracts in this study was between 15.38%-84.61%, the highest point was house dust mite extract. The Sp was ranged from 81.48%-93.75%, the highest rank was shrimp extract. The agreement of SPT results between new local and imported allergen extracts was moderate (K=0.502) for house dust mite and fair (K= 0.225) for shrimp extracts. Compared with IgE results, the Sn of new local and imported house dust mite extracts was good (85.7%; 92.9%). The Sp was good for egg white and cow's milk new local extracts (86.4%; 84.4%). The agreement of SPT results between new local and imported allergen extracts compared to IgE was fair (K=0.289) for new local house dust mite extracts, and slight agreement (K=0.188) for imported extracts of the same allergen.

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Ethical Clearance: Taken from Ethical Committee of Dr Soetomo Academic General Hospital Surabaya

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