ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 53, N.5, 228-233, 2021

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Which skin prick test wheal size detects true allergy to Salsola kali?

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Key words

Salsola kali; skin prick test; nasal provocation test; allergy; sensitivity; specificity.

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Doi
10.23822/EurAnnACI.1764-1489.161

Summary

Background. *Sensitization to Salsola kali (Sk) weed pollen allergen is the most common cause of seasonal allergic rhinitis (SAR) in Middle East countries.* ***Aim.*** *To identify Salsola kali skin prick test (SkSPT) wheal size cut-off, able to determine true allergy among adult patients with moderate to severe SAR, who are in need of Salsola kali allergen specific immunotherapy (SkAIT).* ***Methods.*** *In 151 adults with moderate to severe SAR, mean age 32.79 ± 10.79 years, of both gender (females: 43.05%), with a positive SkSPT, (i.e., cut off wheal longest diameter of 3 mm) and one or more other local weed pollens, Salsola kali nasal provocation test (SkNPT) was carried out. Response was assessed both subjectively, with scores, and objectively, by measuring peak nasal inspiratory flow (PNIF). Safety profile of SkNPT was assessed using peak expiratory flow rate (PEF) measurements.* ***Results.*** *SkNPT positive response was found in 125 patients (82.78%). Mean skin prick test (SPT) wheal size to Sk was bigger in the nasal provocatin test (NPT) positive group (9 mm) compared to the NPT negative patients (5 mm), $p < 0.0001$. ROC analysis showed that a SPT wheal size to Sk at the threshold of > 7.5 mm enabled identification of SkNPT positivity with a sensitivity of 73.6% and specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; $p < 0.0001$).* ***Conclusions.*** *SPT wheal size of 3 mm might overestimate the presence of real allergy to Sk in a desert environment. A SPT wheal size > 7.5 mm for Sk appears to distinguish individuals who develop disease from those who does not. Physicians should select the proper SPT wheal size value as an appropriate criterion according to the allergen than using a uniform cut off value in patients eligible for SkAIT.*

Impact statement

A SPT wheal size > 7.5 mm for Salsola kali appears to distinguish individuals who develop seasonal allergic rhinitis from those who does not, with a sensitivity of 73.6% and specificity of 100.0%.

Abbreviations

Sk: *Salsola kali*
SAR: *Seasonal Allergic Rhinitis*
SPT: *Skin Prick Test*
SkSPT: *Salsola kali Skin Prick Test*
NPT: *Nasal Provocation Test*
SkNPT: *Salsola kali Nasal Provocation Test*
AIT: *Allergen Specific Immunotherapy*
SkAIT: *Salsola kali Allergen Specific Immunotherapy*
PNIF: *Peak Nasal Inspiratory Flow*
PEF: *Peak Expiratory Flow*
SsIgE: *Serum Specific Immunoglobulin E*
ROC: *Receiver Operating characteristics*
CI: *Confidence Interval*
CRP: *C-reactive protein*
TNSS: *Total nasal symptom score*
TCSS: *Total clinical symptom score*

Introduction

Despite the scarce vegetation in Kuwait's desert environment, SAR is one of the most common respiratory allergies (1). Previous studies demonstrated that the prevalence of allergic rhinitis symptoms; ever, current symptoms and physician-diagnosed allergic rhinitis were 43.9%, 30.7%, and 17.1%, respectively (2). Chenopods weed family, including Salsola kali (Sk) are a dominant sensitizing allergen, showing almost always a markedly greater response than all other allergens (3). They are highly allergenic, very invasive and fast-growing in arid salty areas. Maximal level of weed pollen is during March-April and September-October (2). Although more than a hundred genera comprise Chenopods family, it seems that Sk have been mostly associated with clinical symptoms of allergy in Kuwait (3), with oscillation of pollen grain in the atmosphere between 30 and 80 grains/m (4) during peak of the season.

Identification of clinically relevant allergen is the key step for the diagnosis of allergy. The most common diagnostic tools in identifying allergen sensitization is skin prick test (SPT) and an *in vitro* test to detect serum specific immunoglobulin E (SsIgE). SPT, is a safe and simple procedure (5, 6) and remains a fundamental diagnostic tool in the practice of clinical allergy. Although the cut off for a positive immediate skin reaction of a 3 mm wheal size diameter is a widely accepted criterion (7), there is no consensus among researchers on the diagnostic accuracy of SPT (8, 9) and a 3 mm criterion is not always sufficient for accurate diagnosis of true allergy (10, 11). However, few scientific data are available to evaluate the validity of SPT wheal size criterion (11-13).

Given the high rate of sensitization to Sk in our atopic population (76.7%) (3), we validated the scientific basis of the 3 mm threshold of SPT wheal size for Sk as the key diagnostic tool for allergen specific immunotherapy (AIT) and compared that with Salsola kali nasal provocation test (SkNPT), as a more accurate and specific diagnostic tool (13, 14). Nasal provocation test (NPT) is recommended whenever discrepancies arise or difficulties exist in the assessment of patient's medical history and results of SPT (15, 16). This is important in avoiding overestimation of true allergy and miscalculation of SkAIT.

The aim of this study was to assess the reliability of SkSPT wheal size in detecting positive SkNPT to determine true allergy in adult patients with SAR, polysensitized, positive to Salsola and one or more allergens from *Chenopodiacae* and *Amaranthaceae* families, eligible for SkAIT.

Patients and methods

In the 151 adult SAR patients referred to Al Rasheed Allergy Centre in Kuwait from September 2017 to February 2018, with a positive SPT to Sk (*i.e.,* cut off wheal longest diameter of 3 mm) and one or more other local weed pollens, in need for SkAIT, nasal provocation test with Sk was carried out. All patients were poly-sensitized to local weeds including Sk. Mild form of SAR, pregnant women, patients with upper respiratory tract infection (confirmed by a normal C-reactive protein, CRP), patient with dermographism and those with significant comorbidities were not included. Furthermore, patients with peak nasal inspiratory flow (PNIF) < 60 L/min, peak expiratory flow (PEF) < 350 L/min, choanal atresia, nasal polyp, septal perforation, atrophic rhinitis, adenoids obstructing nasal ventilation were also not included. All patients were informed about the risk and outcomes of the procedure and provided informed consent. Ethical clearance was granted by Ministry of Health Research Ethics Committee (number 2017/669).

Skin prick test

SPT was used as the gold standard to describe atopic status. SPT was performed by single head prick lancets on the volar aspect of the forearm, 2 to 3 cm from the wrist and the antecubital fossae as recommended (6). We used a battery of indoor and outdoor inhalant allergens (Diater, Spain) which included Sk and other local pollens, from the same family. All patients refrained 7 days from treatment with antihistamines. Histamine (10 mg/mL) and saline were used as positive and negative controls, respectively. Results were read 15–20 minutes following allergen extract application.

Nasal Provocation Test

Bilateral nasal provocation test was done at least 4 weeks after weed pollen season, and 3-4 weeks after upper respiratory tract infection (confirmed by normal CRP value), 1 week after discontinuation of oral antihistamine, nasal corticosteroid, and nasal decongestant, and 2 weeks after antidepressant, or oral corticosteroids (> 10 mg/day). Allergen extract was provided from the same manufacturer as it was for SPT (Diater, Spain). Due to less abundance of other weeds from *Amaranthacae* and *Chenopodiacae* family in our environment, and our local AIT practice using Sk extract only, nasal provocation with other allergen was not done. Fifteen minutes after accommodation to room temperature and saline nasal provocation, to exclude nasal hyperreactivity, progressively increasing concentrations (0.5 and 5 HEP/mL) of freshly reconstituted, commercial freeze-dried allergen solution (10 IRHEP/ml) were administered in the inferior nasal turbinate intranasal at 20-min intervals in the form of a nasal spray (100 µL/puff). Nasal reaction was assessed following the manufacturer's recommendations 20 min (pinched nose for 10 min and 10 min un- pinched) after each dose (concentration) of allergen, as follows: sneezing: 0 (0–2 sneezes), 1 (3–4 sneezes), 3 (≥ 5 sneezes); nasal itching, rhinorrhea, and nasal obstruction: 1 (mild), 2 (moderate), 3 (severe); palate, eyes, and/or ears itching: 0 (absent), 1 (present). In the case of a positive response to any concentration, further provocation was interrupted. The provocation outcome was assessed subjectively and objectively in all

patients. A subjective method was based on patient's assessment, expressed as a sum of symptoms; total nasal symptom score (TNSS), and a positive score was if the sum ≥ 5 of the maximal 15. Peak nasal inspiratory flow (PNIF) measurement served as objective assessment of SkNPT outcome, while peak expiratory flow (PEF) was used as a safety control. Three PNIF and PEF measurements were taken; before challenge (basal value), 20 min after placebo (saline), after each given allergen concentration and 8 hours after the challenge. The best of the three PNIF and PEF measurements at each time point was recorded. Reduction in PNIF ≥ 20%, compared to a baseline value, was an objective measure of nasal patency. A reduction in PEF ≤ 20% excluded the involvement of the lower airways during the procedure. A positive NPT was considered when we had both a positive TNSS and a reduction of PNIF ≥ 20% compared to a baseline value. A device (Clement-Clark Int. Ltd., Harlow, UK) was used for both PNIF and PEF measurements.

Statistic

Accuracy and normality were determined using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Non-parametric and parametric methods were used to calculate statistical significance. Student's t test, the Mann-Whitney U test, Fisher's test, and the χ^2 test were used to calculate the differences between groups. ANOVA was used to calculate the relative difference distribution variance between variables. Receiver operating characteristics (ROC) analysis was used to determine the optimum value of the SPT wheal size predictive score, and the Hanley and McNeil methods were used to calculate the area under the curve. The statistical hypotheses were tested at the level of $\alpha = 0.05$, and the difference be- tween the groups in the sample was considered significant with two-sided $p < 0.05$. Statistical significance was considered to be achieved at $p < 0.05$, $p < 0.01$, and $p < 0.001$. All data was analysed using GraphPad Prism 7 (San Diego, CA, USA).

Results

Total of 151 patients, sensitized to Sk as well as to other local weed pollens from the same less abundant weed family in Kuwait, were included. The mean age was 32.79 ± 10.79 years; females: 65 (43.05%), with median total clinical symptom score (TCSS) of 12 (minimum 9 and maximum 15). SkNPT was positive in 125 (82.78%) patients, while 17.22% did not react. The mean wheal size was significantly bigger in the challenge positive group when compared with challenge negative patients (median; minimum; maximum: 9; 3; 19 *vs* 5; 3; 7; challenge positive, negative patients, respectively, $p < 0.0001$) (**table I**). In addition to the subjective assessment of SkNPT using TNSS, positivity was proven by a reduction of PNIF value during procedure. A significant reduction in PNIF, in patients with a positive challenge response,

Table I *- Patients' baseline and follow up characteristics.*

Patients; number		151		
Females; number (%)		65 (43.05%)		
Age (years) (mean ± standard deviation)		32.79 ± 10.79		
Total Clinical Symptom Score (TCSS) (median [minimum, maximum])		12 [9, 15]		
SPT mean wheal size (mm) (median [minimum, maximum])		7 [3, 14]		
SkNPT positive; number (%)		125 (82.78%)		
SPT mean wheal size (mm) (median [minimum, maximum])	SkNPT positive 9 [3, 19]	SkNPT negative 5 [3, 7]	p value < 0.0001*	
PNIF (mean ± standard deviation)	Before SkNPT 96.23 ± 22.23	After positive SkNPT 71.75 ± 19.39	8 hours after positive SkNPT 96.69 ± 22.01	p value < 0.0001*
PNIF fall after SkNPT (median [minimum, maximum])		- 20.0 [- 90.0, 30.0]		
PEF (mean ± standard deviation)	Before SkNPT 454.33 ± 60.86	After SkNPT 461.62 ± 65.95	p value 0.7437	
PEF fall after SkNPT (median [minimum, maximum])		0.0 [- 120, 80]		

was detected (96.23 ± 22.23; 71.75 ± 19.39: basal PNIF; PNIF during procedure, respectively). Furthermore, its recovering to the baseline value 8 hours after the challenge was observed (96.69 ± 22.01; 96.23 ± 22.23). A measurement of PEF remained stable (454.33 ± 60.86 *vs* 461.62 ± 65.95) (**table I**).

Comparing SPT wheal size in NPT positive and negative patients (median SPT wheal size: 9 mm *vs* 5 mm; challenge positive *vs* negative patients), the optimal skin prick wheal size cut off for Sk was determined using ROC curves, constructed by plotting sensitivity *vs* specificity at various skin prick wheal size diameters for Sk provocation positive and negative patients (**figure 1**). SkSPT at threshold of > 7.5 mm enabled the identification of Sk provocation positivity with sensitivity of 73.6% and specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; p < 0.0001) (**table II**, **figure 1**).

Discussion

Although advanced diagnostic tools in allergy might improve the selection of patients for AIT (17), SPT, is highly specific (79-86%) and sensitive (9) (85-87%), and remains the technique of choice in allergy practice for identification of causative allergens in patients with allergic rhinitis. The reliability of SPT depends on the skill of the tester, the test instrument (14), potency and stability of test reagents, skin colour and patient's age, as well

Figure 1 *- ROC analysis for Salsola kali positive and negative SkNPT.*

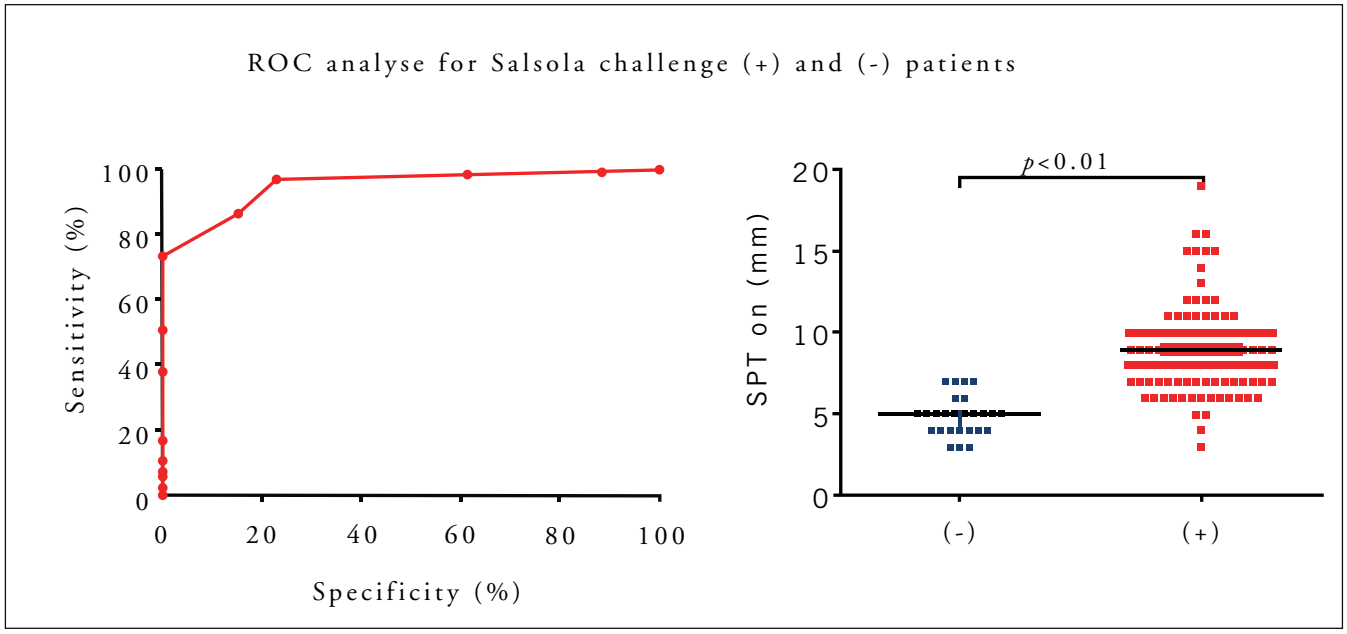

Table II *- Sensitivity and specificity at different cut off of SPT to Salsola kali in regard of SkNPT positivity.*

Cut off of Salsola SPT	Sensitivity (%)	95% confidence interval (CI)	Specificity (%)	95% confidence Interval (CI)	Likelihood Ratio
> 6.500	86.40	79.12% to 91.87%	84.62	65.13% to 95.64%	> 6.500
> 7.500	73.60	64.97% to 81.08%	100.0	86.77% to 100.0%	> 7.500
> 8.500	50.40	41.32% to 59.46%	100.0	86.77% to 100.0%	> 8.500
> 9.500	37.60	29.10% to 46.70%	100.0	86.77% to 100.0%	> 9.500
> 10.50	16.80	10.71% to 24.53%	100.0	86.77% to 100.0%	> 10.50
> 11.50	10.40	5.655% to 17.13%	100.0	86.77% to 100.0%	> 11.50

(6). Those factors, besides the lack of SsIgE cut offs based on the SPT, might influence the interpretation of SPT (18). A positive immediate skin reaction at the threshold of a 3 mm wheal of the longest (19) or mean diameter (6) is a widely accepted criterion. However, 3 mm wheal threshold might lead to the overestimation of allergic disease and increase the risk of inadequate AIT (20). To improve clinical interpretation of SPT results in terms of its clinical relevance, Haahtela *et al.* (21) calculated quantitative decision points for 18 inhalant allergens with the wheal size in mm and found that the risk of allergic symptoms to particular allergen increased significantly with larger wheal sizes for 17 of the 18 allergens tested (the 80% PPV varied from 3 to 10 mm depending on the allergen). Similar observation was documented in our study. The mean ± SD of Sk wheal longest diameter size was 8.24 mm ± 2.79 mm (**table I**). However, 15% of our SPT positive patients on threshold of 3 mm wheal did not react to nasal provocation. Furthermore, we observed that all Sk provocation negative patients had a significantly lower wheal diameter in comparison with those who reacted positively (4.88 ± 1.21 *vs* 8.94 ± 2.5; $p < 0.0001$) (**table II**). This observation support results obtained by others (22), that larger skin reactions predict higher likelihood of positive nasal response and better correlate with clinical allergen reactivity with inhalant allergens, as well. Zarei group (12), using cat NPT, documented that a 3 mm skin prick wheal will overestimate the presence of cat allergy. They found that a 6 mm wheal size appears to distinguish those individuals who are cat allergic from those who are not. The authors concluded that instead of taking skin prick wheal cut offs of 3 mm as standard criterion, the prick wheal size cut off for each allergen should be determined. Similar results are documented by others (23). These results are in concordance with ours from the present study, as well as our other study (24) done with a cat allergen. We found, similar to Zarei group (12), that positive cat NPT detected true cat allergy in an environment with a low cat ownership, that was predicted by a cat SPT wheal size > 6.5 mm with a sensitivity of 71.11% and a specificity of 100%.

Nasal provocation is more specific, accurate (13) and safe (25) test that is considered as the best diagnostic "gold standard" (16), if culprit allergen is elusive. Accuracy of NPT in this study was supported with results obtained by objective measurements of nasal patency using PNIF, and by safety profile, showing no significant changes in PEF rate during procedure for all patients. NPT is a valuable method in determining cut off level of SPT wheal, whenever discordance between clinical history and SPT and/or SsIgE is present. In the absence of a positive NPT, positive SPT results might be related to the presence of cross-reactivity between weed pollen species (10, 20, 26). Furthermore, the amount of pollen each subject is exposed to, in real life, depends on several uncontrollable factors like climate, lifestyle and the actual pollen load in the air (27).

We used receiver operating characteristic (ROC) curves to determine optimal cut off values by plotting sensitivity *vs* specificity at various skin prick wheal diameters for Sk, challenge positive and negative patients. Results are shown on **table II** and **figure 1**. We observed that SkSPT at the threshold of > 7.5 mm enabled the identification of Sk provocation positivity with a sensitivity of 73.6% and a specificity of 100.0% (area under the curve 0.9498, standard error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853; $p < 0.0001$) (**table II**).

Therefore, patients eligible for SkAIT whose SPT wheal is less than 7.5 mm should be taken into consideration to carry out nasal provocation to verify a clinically relevant allergen. Similar suggestions are given by others (28).

As a limitation of the current study, a relatively small number of patients were included. Due to missing data in majority of included patients, the correlation of SsIgE with SPT wheal cut off was not evaluated. In addition, it has been previously shown that patient's age might influence on SPT cut offs for different inhalant allergens (11): since our group of patients was relatively homogenous in regard to age, we have not focused on this issue.

In conclusion, a SPT wheal size ≥ 7.5 mm for Sk might be considered as an appropriate wheal size in confirming Sk allergy in adult patients with moderate to severe SAR. SkNPT might be recommended if SkSPT wheal size is < 7.5 mm. Selection of the proper SPT wheal cut off value rather than using a uniform value might be important in the accurate treatment with AIT. More studies with higher number of patients with moderate to severe SAR sensitized to other allergens typical for desert climate, are necessary. Furthermore, similar evaluation of cases with a mild SAR, in comparison to more severe form of allergic rhinitis, would be interesting for potential AIT, which is still the only treatment modality capable of preventing further progression of allergic disease.

Conclusions

SPT wheal size of 3 mm might overestimate the presence of real allergy to Sk in a desert environment. A SPT wheal size > 7.5 mm for Sk appears to distinguish those individuals who develop disease from those who does not. Physicians should select the proper SPT wheal size value as an appropriate criterion according to the allergen rather than using a uniform cut off value in patients eligible for AIT.

Ethics

All patients were informed about the risk and outcomes of the procedure and provided informed consent.

Conflict of interests

The authors declare that they have no conflict of interests.

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