

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

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6 **List of Abbreviations**

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8 Sk: Salsola kali

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10 SAR: Seasonal Allergic Rhinitis

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12 SPT: Skin Prick Test  
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14 SkSPT: Salsola kali Skin Prick Test  
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16 NPT: Nasal Provocation Test  
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18 SkNPT: Salsola kali Nasal Provocation Test  
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20 AIT: Allergen Specific Immunotherapy  
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22 SkAIT: Salsola kali Allergen Specific Immunotherapy  
23  
24 PNIF: Peak Nasal Inspiratory Flow  
25  
26 PEF: Peak Expiratory Flow  
27  
28 SsIgE: Serum Specific Immunoglobulin E  
29  
30 ROC: Receiver Operating characteristics  
31  
32 CI: Confidence Interval  
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34 CRP: C-reactive protein  
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36 TNSS: Total nasal symptom score  
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38 TCSS: Total clinical symptom score

39 **Abstract**

40 **Background:** Sensitization to Salsola kali (Sk) weed pollen allergen is the most common cause  
41 of seasonal allergic rhinitis (SAR) in Middle East countries.

42 **Aim:** To identify Salsola kali skin prick test (SkSPT) wheal size cut-off, able to determine true  
43 allergy among adult patients with moderate to severe SAR, who are in need of Salsola kali  
44 allergen specific immunotherapy (SkAIT).

45 **Methods:** In 151 adults with moderate to severe SAR, mean age  $32.79 \pm 10.79$  years, of both  
46 gender (females: 43.05%), with a positive SkSPT, (i.e. cut off wheal longest diameter of 3 mm)  
47 and one or more other local weed pollens, Salsola kali nasal provocation test (SkNPT) was  
48 carried out. Response was assessed both subjectively, with scores, and objectively, by  
49 measuring peak nasal inspiratory flow (PNIF). Safety profile of SkNPT was assessed using peak  
50 expiratory flow rate (PEF) measurements.

51 **Results:** SkNPT positive response was found in 125 patients (82.78%). Mean skin prick test  
52 (SPT) wheal size to Sk was bigger in the nasal provocation test (NPT) positive group (9 mm)  
53 compared to the NPT negative patients (5 mm),  $p < 0.0001$ . ROC analysis showed that a SPT  
54 wheal size to Sk at the threshold of  $> 7.5$  mm enabled identification of SkNPT positivity with a  
55 sensitivity of 73.6% and specificity of 100.0% (area under the curve 0.9498, standard error  
56 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853;  $p < 0.0001$ ).

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

62 **Key words:** Salsola kali, skin prick test, nasal provocation test, allergy, sensitivity, specificity.

63

64 **Introduction**

65 Despite the scarce vegetation in Kuwait's desert environment, SAR is one of the most common  
66 respiratory allergies (1). Previous studies demonstrated that the prevalence of allergic rhinitis  
67 symptoms; ever, current symptoms and physician-diagnosed allergic rhinitis were 43.9%,

68 30.7%, and 17.1%; respectively (2). Chenopods weed family, including Salsola kali (Sk) are a  
69 dominant sensitizing allergen, showing almost always a markedly greater response than all  
70 other allergens (3). They are highly allergenic, very invasive and fast-growing in arid/salty  
71 areas. Maximal level of weed pollen is during March-April and September-October (2).  
72 Although more than a hundred genera comprise Chenopods family, it seems that Sk have been  
73 mostly associated with clinical symptoms of allergy in Kuwait (3), with oscillation of pollen  
74 grain in the atmosphere between 30 and 80 grains/m<sup>3</sup> (4) during peak of the season.

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

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

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

95

## 96 **Materials and methods**

97 In the 151 adult SAR patients referred to Al Rasheed Allergy Centre in Kuwait from September  
98 2017 to February 2018, with a positive SPT to Sk (i.e. cut off wheal longest diameter of 3 mm)

99 and one or more other local weed pollens, in need for SkAIT, nasal provocation test with Sk  
100 was carried out. All patients were poly-sensitized to local weeds including Sk. Mild form of  
101 SAR, pregnant women, patients with upper respiratory tract infection (confirmed by a normal  
102 C-reactive protein, CRP), patient with dermographism and those with significant comorbidities  
103 were not included. Furthermore, patients with peak nasal inspiratory flow (PNIF)  $\leq 60$  L/min,  
104 peak expiratory flow (PEF)  $< 350$  L/min, choanal atresia, nasal polyp, septal perforation,  
105 atrophic rhinitis, adenoids obstructing nasal ventilation were also not included. All patients  
106 were informed about the risk and outcomes of the procedure and provided informed consent.  
107 Ethical clearance was granted by Ministry of Health Research Ethics Committee (number  
108 2017/669)

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### 110 **Skin prick test**

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

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### 119 **Nasal Provocation Test**

120 Bilateral nasal provocation test was done at least 4 weeks after weed pollen season, and 3-4  
121 weeks after upper respiratory tract infection (confirmed by normal CRP value), 1 week after  
122 discontinuation of oral antihistamine, nasal corticosteroid, and nasal decongestant, and 2  
123 weeks after antidepressant, or oral corticosteroids ( $> 10$  mg/day). Allergen extract was  
124 provided from the same manufacturer as it was for SPT (Diater, Spain). Due to less abundance  
125 of other weeds from Amaranthaceae and Chenopodiaceae family in our environment, and our  
126 local AIT practice using Sk extract only, nasal provocation with other allergen was not done.  
127 Fifteen minutes after accommodation to room temperature and saline nasal provocation, to  
128 exclude nasal hyperreactivity, progressively increasing concentrations (0.5 and 5 HEP/mL) of  
129 freshly reconstituted, commercial freeze-dried allergen solution (10 IRHEP/ml) were  
130 administered in the inferior nasal turbinate intranasal at 20-min intervals in the form of a nasal

131 spray (100  $\mu$ L/puff). Nasal reaction was assessed following the manufacturer's  
132 recommendations 20 min (pinched nose for 10 min and 10 min un-pinched) after each dose  
133 (concentration) of allergen, as follows: sneezing: 0 (0–2 sneezes), 1 (3–4 sneezes), 2 ( $\geq$ 5  
134 sneezes); nasal itching, rhinorrhea, and nasal obstruction: 1 (mild), 2 (moderate), 3 (severe);  
135 palate, eyes, and/or ears itching: 0 (absent), 1 (present). In the case of a positive response to  
136 any concentration, further provocation was interrupted. The provocation outcome was  
137 assessed subjectively and objectively in all patients. A subjective method was based on  
138 patient's assessment, expressed as a sum of symptoms; total nasal symptom score (TNSS),  
139 and a positive score was if the sum  $\geq$ 5 of the maximal 15. Peak nasal inspiratory flow (PNIF)  
140 measurement served as objective assessment of SkNPT outcome, while peak expiratory flow  
141 (PEF) was used as a safety control. Three PNIF and PEF measurements were taken; before  
142 challenge (basal value), 20 min after placebo (saline), after each given allergen concentration  
143 and 8 hours after the challenge. The best of the three PNIF and PEF measurements at each  
144 time point was recorded. Reduction in PNIF  $\geq$ 20%, compared to a baseline value, was an  
145 objective measure of nasal patency. A reduction in PEF  $\leq$ 20% excluded the involvement of the  
146 lower airways during the procedure. A positive NPT was considered when we had both a  
147 positive TNSS and a reduction of PNIF  $>$ 20% compared to a baseline value. A (Clement-Clark  
148 Int. Ltd., Harlow, UK) device was used for both PNIF and PEF measurements.

149

## 150 **Statistic**

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

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## 165 **Results**

166 Total of 151 patients, sensitized to Sk as well as to other local weed pollens from the same  
167 less abundant weed family in Kuwait, were included. The mean age was  $31.79 \pm 10.79$  years;  
168 females: 65 (43.05%), with median total clinical symptom score (TCSS) of 12 (minimum 9 and  
169 maximum 15). SkNPT was positive in 125 (82.78%) patients, while 17.22% did not react. The  
170 mean wheal size was significantly bigger in the challenge positive group when compared with  
171 challenge negative patients (median; minimum, maximum: 9; 3, 19 vs. 5; 3, 7; challenge  
172 positive, negative patients; respectively,  $p < 0.0001$ ) (Table I). In addition to the subjective  
173 assessment of SkNPT using TNSS, positivity was proven by a reduction of PNIF value during  
174 procedure. A significant reduction in PNIF, in patients with a positive challenge response, was  
175 detected ( $96.23 \pm 22.23$ ;  $71.75 \pm 19.39$  (basal PNIF; PNIF during procedure; respectively).  
176 Furthermore, its recovering to the baseline value 5 hours after the challenge was observed  
177 ( $96.69 \pm 22.01$ ;  $96.23 \pm 22.23$ ). A measurement of PEF remained stable ( $454.33 \pm 60.86$  vs.  
178  $461.62 \pm 65.95$ ) (Table 1).

179 Comparing SPT wheal size in NPT positive and negative patients (median SPT wheal size: 9 mm  
180 vs. 5mm; challenge positive vs. negative patients), the optimal skin prick wheal size cut off for  
181 Sk was determined using ROC curves, constructed by plotting sensitivity vs specificity at  
182 various skin prick wheal size diameters for Sk provocation positive and negative patients  
183 (Graph I). SkSPT at threshold of  $>7.5$  mm enabled the identification of Sk provocation positivity  
184 with sensitivity of 79.6% and specificity of 100.0% (area under the curve 0.9498, standard  
185 error 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853;  $p < 0.0001$ ) (Table II, Graph 1)

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## 187 **Discussion**

188 Although advanced diagnostic tools in allergy might improve the selection of patients for AIT  
189 (17). SPT is highly specific (79-86%) and sensitive (9) (85-87%), and remains the technique of  
190 choice in allergy practice for identification of causative allergens in patients with allergic  
191 rhinitis. The reliability of SPT depends on the skill of the tester, the test instrument (14),  
192 potency and stability of test reagents, skin colour and patient's age, as well (6). Those factors,  
193 besides the lack of SsIgE cut offs based on the SPT, might influence the interpretation of SPT

194 (18). A positive immediate skin reaction at the threshold of a 3 mm wheal of the longest (19)  
195 or mean diameter (6) is a widely accepted criterion. However, 3 mm wheal threshold might  
196 lead to the overestimation of allergic disease and increase the risk of inadequate AIT (20). To  
197 improve clinical interpretation of SPT results in terms of its clinical relevance, Hahtela et al  
198 (21) calculated quantitative decision points for 18 inhalant allergens with the wheal size in  
199 mm and found that the risk of allergic symptoms to particular allergen increased significantly  
200 with larger wheal sizes for 17 of the 18 allergens tested (the 80% PPV varied from 3 to 10 mm  
201 depending on the allergen). Similar observation was documented in our study. The mean  $\pm$  SD  
202 of Sk wheal longest diameter size was  $8.24 \text{ mm} \pm 2.79 \text{ mm}$  (Table 1). However, 15% of our SPT  
203 positive patients on threshold of 3 mm wheal did not react to nasal provocation. Furthermore,  
204 we observed that all Sk provocation negative patients had a significantly lower wheal diameter  
205 in comparison with those who reacted positively ( $4.88 \pm 1.21$  vs  $8.94 \pm 2.5$ ;  $p < 0.0001$ ) (Table  
206 2). This observation support results obtained by others (22), that larger skin reactions predict  
207 higher likelihood of positive nasal response and better correlate with clinical allergen  
208 reactivity with inhalant allergens, as well. Zarei group (12), using cat NPT, documented that a  
209 3 mm skin prick wheal will overestimate the presence of cat allergy. They found that a 6 mm  
210 wheal size appears to distinguish those individuals who are cat allergic from those who are  
211 not. The authors concluded that instead of taking skin prick wheal cut offs of 3 mm as standard  
212 criterion, the prick wheal size cut off for each allergen should be determined. Similar results  
213 are documented by others (23). These results are in concordance with ours from the present  
214 study, as well as our other study (24) done with a cat allergen. We found, similar to Zarei group  
215 (12), that positive cat NPT detected true cat allergy in an environment with a low cat  
216 ownership, that was predicted by a cat SPT wheal size  $>6.5 \text{ mm}$  with a sensitivity of 71.11%  
217 and a specificity of 100%.

218 Nasal provocation is more specific, accurate (13) and safe (25) test that is considered as the  
219 best diagnostic “gold standard” (16), if culprit allergen is elusive. Accuracy of NPT in this study  
220 was supported with results obtained by objective measurements of nasal patency using PNIF,  
221 and by safety profile; showing no significant changes in PEF rate during procedure for all  
222 patients. NPT is a valuable method in determining cut off level of SPT wheal, whenever  
223 discordance between clinical history and SPT and/or SsIgE is present. In the absence of a  
224 positive NPT, positive SPT results might be related to the presence of cross-reactivity between  
225 weed pollen species (10, 20, 26). Furthermore, the amount of pollen each subject is exposed

226 to, in real life, depends on several uncontrollable factors like climate, lifestyle and the actual  
227 pollen load in the air (27).

228 We used receiver operating characteristic (ROC) curves to determine optimal cut off values by  
229 plotting sensitivity vs specificity at various skin prick wheal diameters for Sk, challenge positive  
230 and negative patients. Results are shown on Table II and Graph I. We observed that SkSPT at  
231 the threshold of >7.5 mm enabled the identification of Sk provocation positivity with a  
232 sensitivity of 73.6% and a specificity of 100.0% (area under the curve 0.749), standard error  
233 0.01808; 95% confidence interval (CI): 0.9144 to 0.9853;  $p < 0.0001$ ) (Table III).

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

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

242 In conclusion, a SPT wheal size  $\geq 7.5$  mm for Sk might be considered as an appropriate wheal  
243 size in confirming Sk allergy in adult patients with moderate to severe SAR. SkNPT might be  
244 recommended if SkSPT wheal size is  $< 7.5$  mm. Selection of the proper SPT wheal cut off value  
245 rather than using a uniform value might be important in the accurate treatment with AIT.

246 More studies with higher number of patients with moderate to severe SAR sensitized to other  
247 allergens typical for desert climate, are necessary. Furthermore, similar evaluation of cases  
248 with a mild SAR, in comparison to more severe form of allergic rhinitis, would be interesting  
249 for potential AIT, which is still the only treatment modality capable of preventing further  
250 progression of allergic disease.

251

## 252 **Conclusion.**

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

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260 **Declaration of interest statement:**

261 None of the authors declare any conflict of interest.

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351 **Tables and graphs**

352 **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]	<i>p</i> 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
PNIF fall after SkNPT (median [minimum, maximum])	-20.0 [-90.0, 30.0]		
PEF (mean ± standard deviation)	Before SkNPT 474.33 ± 60.86	After SkNPT 461.62 ± 65.95	<i>p</i> value 0.7437
PEF fall after SkNPT (median [minimum, maximum])	0.0 [-120, 80]		

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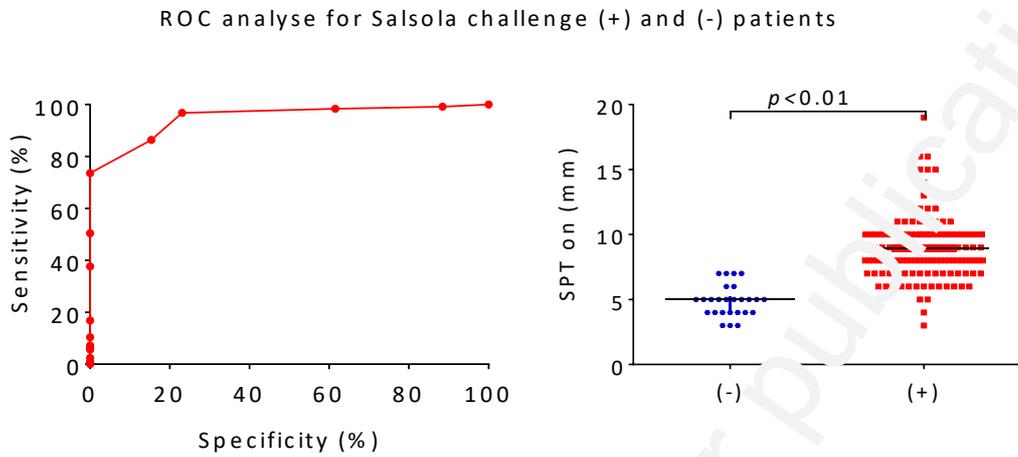
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366 **Graph I.** ROC analysis for Salsola kali positive and negative SkNPT



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369 **Table II.** Sensitivity and specificity at different cut off or SPT to Salsola kali in regard of SkNPT  
370 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 97.87%	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

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