

Low Potency of Indian Dust Mite Allergen Skin Prick Test Extracts Compared to FDA-Approved Extracts: A Double-Blinded Randomized Control Trial

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Abstract

Background: Skin prick testing is the most important diagnostic tool to detect immunoglobulin E-mediated allergic diseases. With increase in the number of allergy tests performed in India, it is imperative to know the potency of indigenous extracts in comparison with U.S. Food and Drug Administration (USFDA)-approved extracts.

Methods: A randomized comparison trial of Indian manufactured and USFDA-approved extracts of *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF) was done at Christian Medical College & Hospital, Vellore, India from April 2014 to June 2015, to compare the skin test reactivity of indigenous allergen extracts of dust mites against validated allergen. Study enrollment included 197 patients with allergic disorders that showed sensitivity to dust mite during routine allergy skin testing. Study participants were tested with varying dilutions of DP and DF indigenous extracts along with USFDA-approved allergens in a blinded fashion. Results were recorded, and statistical significance was calculated using the Friedman rank sum test.

Results: Using the Friedman rank sum test with a Tukey adjustment for multiple comparisons, we found that the extracts in each dilution were significantly different ($P < .0001$). The full strength indigenous extracts, B-DF (DF allergen standard extract from Bioproducts and Diagnostics, India) and C-DF (DF allergen extract from Creative Diagnostics, India) extracts, had mean wheal sizes of 7.69 (standard deviation [SD] 9.91) and 31.01 (SD 51.04), respectively. The full strength S-DF (DF allergen extract from Jubilant Hollister Stier, Spokane, WA, USA) had a mean wheal size of 109.97 (SD 162.73), which was significantly higher ($P < .0001$) than both the indigenous extracts. For each of the dilutions, the S-DF mean wheal size was significantly greater than that of the corresponding B-DF and C-DF wheal sizes. The full strength indigenous C-DP (DP allergen extract from Creative Diagnostics, India) had mean wheal size of 39.37 (SD 51.74). The full strength standard S-DP (DP allergen extract from Jubilant Hollister Stier, Spokane, WA, USA) extract had a mean wheal size of 167.66 (SD 270.80), which was significantly higher ($P < .0001$) than the indigenous C-DP extract. Similar differences were seen across all dilutions.

Conclusion: The indigenous extracts have significantly lower potency compared to USFDA-approved extracts; hence, there is an urgent need for policy makers to institute stringent criteria for standardization of antigens in India.

Keywords

immunoglobulin E, allergy testing, skin prick test, allergens, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, allergen extract standardization

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Background

Over 20% of the world population suffers from immunoglobulin E (IgE)-mediated allergic diseases such as asthma, rhino-conjunctivitis, eczema, and anaphylaxis. In India, approximately 20% of the population suffers from allergic rhinitis and 15% from bronchial asthma.¹ Even though there has been enormous development in the field of in vitro diagnostic testing, the skin prick test is the most important diagnostic tool to detect IgE-mediated disease, reliability of the results of which heavily depends on the technique and the materials used.² Knowledge of the potency of allergen extracts used for diagnostic and therapeutic products is mandatory to gain a better evaluation and treatment of allergic individuals.³ Very few studies have been published on allergy skin testing in Indian subjects.

The quality of some allergen extracts is strictly monitored within the United States by the U.S. Food and Drug Administration (USFDA) through its Center for Biologics Evaluation and Research which dictates reference tests and substances to be used for extract quality control before batch release.² In India, since the first registration for commercial manufacture and supply of allergen extracts in 1972, the allergen preparations do not have any special status and they need to comply with general requirements for medicinal products. Over the past 10 years, there have been attempts to standardize the extracts using standard immune-biochemical methods.⁴ However, the manufacturers are not constrained to adhere to any standardized protocol for preparation of allergen extracts. In view of the above, there could be a great deal of variability in the quality of the marketed allergen extracts in India.

Despite increasing widespread use of allergen extracts in clinical practice, there has been no study done so far to compare the potency of the allergenic extracts in the Indian market with internationally approved extracts. With this background, we decided to undertake a double-blinded randomized control trial to compare the biological activity (by prick skin testing) of nonstandardized allergen extracts of *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF) from the Indian market against USFDA-approved comparator. The study was conducted at Christian Medical College (CMC), Vellore, India from April 2014 to June 2015.

Methods

Aim

To compare the skin test response to Indian manufactured allergen extracts of DP and DF against USFDA-approved comparator by skin prick testing.

Design of the Study

Double-blind randomized control trial.

Setting and Characteristics of Studies Included in the Trial

Adults of age 17 years and above who were evaluated in the outpatient department for various allergic disorders and referred for skin prick testing to the allergy testing lab of the Department of Pulmonary medicine, CMC, Vellore were approached to participate in the study.

Patients referred for allergy testing were tested with an allergy panel which included an allergen extract of USFDA-approved dust mite and the tests were carried out as per internationally accepted protocol.⁵ A positive test is a wheal diameter of 3 mm or greater relative to the negative saline control. Individuals who tested positive for the dust mite mixture, namely, DP and DF, and were willing to give informed consent, were then included in the trial.

Study participants received prick skin testing with the USFDA-approved allergens and test allergen extracts from Indian market at full concentration as well as in various dilutions on the same day.

The Skin Prick Allergen Extracts

DP and DF allergen extracts were obtained from 2 Indian pharmaceutical sources. Details of standardization of these products were not specified. The allergens were coded as follows:

- A. C-DP and C-DF (nonglycerinated allergen extracts of DP and DF from Creative Diagnostic Medicare Private Limited D-296, Vashi Plaza, Sector 17, Navi Mumbai).
- B. B-DF (nonglycerinated allergen extracts of DF from Trivandrum Bioproducts and Diagnostics Private Ltd., Plot No 22 and 23, Industrial Development Plot, Manvila, Kulathoor (p.o), Thiruvananthapuram)
- C. S-DP and S-DF (USFDA-approved allergen extracts of DP and DF from Jubilant Hollister Stier, Spokane, WA, USA)

The following dilutions were made of the allergen extracts: 1:1, 1:10, 1:100, and 1:1000.

Masking

The indigenous allergen extracts and their dilutions and the USFDA-approved extracts and their dilutions were stored in 5 mL identical coded bottles and capped with a dropper, for blinding purposes. The codes were kept at the Biostatistics department of CMC. Investigators and patients were blinded to the allergens and their strength.

Unblinding was done only after the statistical analysis was carried out.

Ethical Issues

The study protocol was approved by the institutional review board of CMC, Vellore.

Technique

The prick tests were performed on the skin surface over the upper back of the patient. After cleaning the test site with alcohol swab, the skin prick tests were performed by 1 of the 2 investigators. Circles were drawn with a ball point pen, 2 cm apart to ensure uniform spacing between the applications. The allergen extract was applied within the circle using the dropper on to the skin as per standard protocols.^{6,7} The time of the first prick was noted. Individual lancets (Manufacturer—MEDIPOINT Lancet, Mineola, NY) were used for each prick. A single technique skin prick of piercing the skin at 45° to 60° angle to the surface using the lancet was used as described in the accepted international protocol.⁵ Allergen response was measured at roughly 18 minutes from the initial prick. After careful blotting of the excess allergens, the wheal diameter (Figure 1) was measured in both the longitudinal and the transverse axis in millimeters, and the readings were recorded in the clinical reporting form. The positive reaction was considered as 3 mm above the negative control measured for the subject according to international protocol.⁵ After the test, the subjects were duly instructed and discharged in a satisfactory condition. The mean wheal surface area was calculated for each of the measurements. The 2 investigators who performed the test did a pilot testing on 10 subjects to ensure that their technique was similar and the wheal size comparable.



Figure 1. The photograph shows the wheal and erythema due to the prick skin testing.

Statistical Analysis

Relative skin test reactivity was compared using the mean wheal surface areas produced by reactivity of the test allergens and the standard allergens at full strength and at individual dilutions. To determine the diagnostic test accuracy, the Friedman rank sum test with a Tukey adjustment for multiple comparisons was used to study the difference in the distribution of wheal surface areas. Determination of statistical significance was kept at a test level alpha of 0.05. The null hypothesis is that there is no difference between the means of the wheal surface areas produced by skin reactivity to the USFDA comparator and Indian allergens. All statistical calculation was performed with R Version 3.3.2.

Results

Demographic Data of Study Patients

The 197 patients enrolled in the study were between 17 and 77 years of age with an average age of 37.2 years. Around 62% of study participants were males. Among the study population, 66% were diagnosed with asthma, 24% with food allergies, 7% with drug allergies, and 25% with skin allergy or eczema. Around 9% of patients were current or exsmokers. Among the study population, 54% lived in urban settings, 31% in rural, and 15% partially lived in urban and rural settings (Table 1).

Table 1. Demographic Data of the Study Population.

Participants	n = 197
Age (mean with SD)	37.2 years, SD = 13.63
Sex	Female = 37.6% Male = 62.4%
Baseline wheal size measured in mm ² (mean with SD)	Histamine: \bar{x} = 82.25, s = 44.89 Saline: \bar{x} = 1.29, s = 8.60 House dust Mite: \bar{x} = 36.46, s = 27.62
On antiallergic treatments	No = 64% Yes = 36%
On asthma medications	No = 60% Yes = 40%
Been treated for skin allergies/eczema	No = 93% Yes = 7%
Smoking status	Nonsmoker = 91% Smoker = 9%
Home setting	Rural = 31% Urban = 54% Partially urban and rural settings = 15%

Abbreviation: SD, standard deviation.

Adverse Events

Only 1 patient had a local reaction of intense erythema and itching on the testing site which faded with a single dose of oral antihistamine.

Potency Comparison Between Test Allergens and Comparator

Prick testing results of mean wheal sizes were compared between indigenous extracts and standard extracts. They showed significant differences across all dilution with significant P values of $<.0001$.

Comparison of DF Extracts

The S-DF extract had a mean of 109.07 (standard deviation [SD] 162.73) at full strength. However, B-DF extract had a mean wheal size of only 7.69 (SD 9.91) and C-DF had a mean wheal size of 31.01 (SD 51.04). This difference was significant between both the S-DF and C-DF ($P < .0001$) as well as between the S-DF and B-DF ($P < .0001$). The mean wheal size of S-DF was

significantly higher than the mean wheal sizes of C-DF and B-DF across all dilutions (1:10, 1:100, and 1:1000), which was also statistically significant with P value of less than .0001 (Table 2, Figure 2)

Comparison of DP Extracts

The S-DP extract had a mean wheal size of 167.66 (SD 270.80) at full strength. In comparison, the indigenous C-DP had a mean wheal size of 39.37 (SD 51.74) in full strength. The mean wheal size of S-DP was significantly higher than C-DP across all dilutions, which was highly significant with P value of $<.0001$ (Table 2, Figure 3).

The full strength indigenous extracts produced wheal area considerably smaller than even the 1:1000 dilutions of the Food and Drug Administration (FDA) standardized extracts, indicating that their potency in terms of allergen content was in each case well below one of thousands of the FDA standardized extract.

Even between the 2 indigenous extracts, there were statistically significant differences between the allergen

Table 2. Mean Wheal Sizes of the Allergen Extracts Across All Dilutions Along With the P Values.

	Full strength (mean \pm SD)	P	1:10 dilution (mean \pm SD)	P	1:100 dilution (mean \pm SD)	P	1:1000 dilution (mean \pm SD)	P
B-DF	7.69 \pm 9.91	$<.0001$	7.42 \pm 10.89	$<.0001$	6.97 \pm 9.66	$<.0001$	6.57 \pm 9.42	$<.0001$
C-DF	31.01 \pm 51.04	$<.0001$	29.75 \pm 69.23	$<.0001$	22.84 \pm 38.67	$<.0001$	16.78 \pm 24.99	$<.0001$
S-DF	109.97 \pm 162.73		106.32 \pm 221.47		77.56 \pm 103.35		79.13 \pm 114.52	
C-DP	39.37 \pm 51.74	$<.0001$	36.72 \pm 62.22	$<.0001$	29.39 \pm 41.08	$<.0001$	21.91 \pm 29.13	$<.0001$
S-DP	167.66 \pm 270.80		143.67 \pm 232.78		131.15 \pm 193.66		110.40 \pm 191.62	

Abbreviations: B-DF, *Dermatophagoides farina* (DF) allergen extract from Bioproducts and Diagnostics, India; C-DF, DF allergen extract from Creative Diagnostics, India; C-DP, DP allergen extract from Creative Diagnostics, India; S-DF, DF allergen extract from Jubilant Hollister Stier, Spokane, WA, USA; S-DP, DP allergen extract from Jubilant Hollister Stier, Spokane, WA, USA.

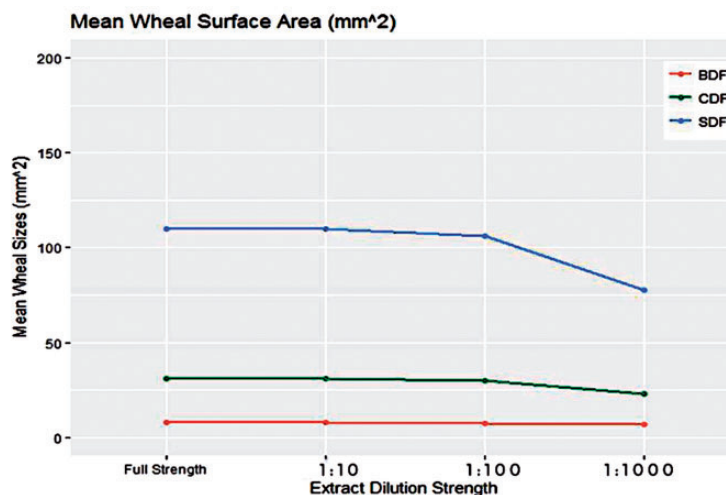


Figure 2. Comparison of mean wheal surface area of *Dermatophagoides farina* (DF) extracts. B-DF, DF allergen extract from Bioproducts and Diagnostics, India; C-DF, DF allergen extract from Creative Diagnostics, India; S-DF, DF allergen extract from Jubilant Hollister Stier, Spokane, WA, USA.

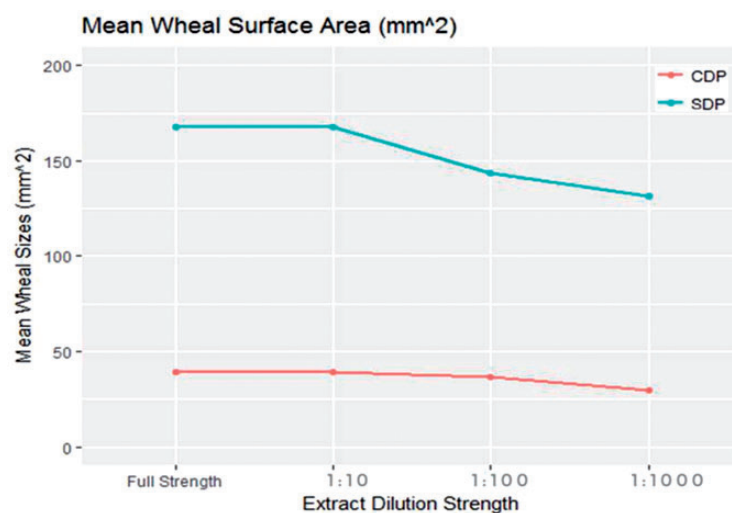


Figure 3. Comparison of mean wheal surface area of *Dermatophagoides pteronyssinus* (DP) extracts. C-DP, DP allergen extract from Creative Diagnostics, India; S-DP, DP allergen extract from Jubilant Hollister Stier, Spokane, WA, USA.

extracts with C-DF showing larger mean wheal sizes at all dilutions than B-DF (Figure 2).

Discussion

This is the first double-blind randomized control study from India to report the differences in biopotency between indigenous allergens and an USFDA-approved allergen. Our study was done as a single center study to prevent the bias of the variations in the techniques.

Biological methods of comparison have been performed in both United States and Europe with 3-fold dilution series⁸ and hence, we decided to follow a similar procedure.

The study was also done with a single batch of all the products obtained from the 3 manufacturers. Batch-to-batch variability could lead to significant variations as has been found in a study done by Pagani et al.³ from Italy. Researchers in this study analyzed different batches and noticed significant differences between batches. The reasons for the differences were found to be dependent on both the type of allergens and the manufacturers.

Our study showed that the biological activity of the full strength of the indigenous allergen was statistically significantly lower than the USFDA-approved antigen extracts even at 1:1000 dilutions. This lack of potency may have been in part due to the indigenous extracts, unlike the FDA standardize extract, being in a nonglycerinated solution.⁹

A similar variation in potency of *Dermatophagoides* extracts was noted by a study done in United States which compared European and Mexican Allergens to North American *Dermatophagoides* allergens using skin prick test. This study showed European and Mexican

extracts to be less potent. It also revealed that the relative potency of the European diagnostic extracts of house dust mite is about half that of the U.S. reference extract, whereas Mexican dust mite diagnostic extracts varied between 20% and 70% of the U.S. reference extract.¹⁰ Another study comparing Bermuda grass and cat extracts of European and Mexican allergens against U.S. allergens showed similar results. The mean wheal surfaces varied from 16% to 77% for European Bermuda grass extracts and from 44% to 125% for cat extracts in comparison to U.S. extracts.² Mexican extracts also showed similar results. They concluded that diagnostic extracts for Bermuda grass from European and Mexican extracts were generally less potent than those from United States. For cat extracts, the potency of the extracts varied as well, with the U.S. extract being of intermediate potency.²

The results of our study has significant implications in the diagnosis and treatment of allergic disorders in India. House dust mites (DP and DF) are major allergens responsible for nearly 80% of the sensitization among allergic individuals.¹¹ The results of the study should discourage the clinician from using indigenous allergens.

Based on above observations, we recommend that Indian manufacturers prioritize the standardization of their allergen extracts with international standards. Protocols could involve either in vivo standardization as we have done or in vitro (Radio Allergen Sorbent Test, ImmunoCap, or ELISA inhibition assays).¹²

Results of the study also urgently call for coordinated efforts like development of “certified reference materials for allergenic products and validation of methods for their quantification (CREATE)” project in Europe which implements a synergistic in vivo and in vitro

validated methods, for standardization of the indigenous extracts by various stakeholders.¹³ There is also a need to do similar studies with other allergen extracts in India such as House dust mite, Bermuda grass and cat allergen, all of which showed varying potency when European and Mexican extracts were compared to USFDA standard allergens.^{2,10}

The study has limitations in that it has been assumed that the USFDA-approved allergen extract is the gold standard for skin prick testing. This is, however, debatable. But the study was constructed pragmatically to obtain comparative results using the best standard extracts available. Another limitation of our study was that we were not able to include all the marketed DP and DF allergens in India.

Conclusion

Our study shows that the Indian manufactured allergen extracts of DP and DF were less potent than USFDA-approved allergen extracts. Therefore, using the tested Indian allergen extracts for testing would give unreliable results, and so there is an urgent need for standardization of allergens by the concerned statutory bodies.

Availability of Data and Material

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Ethical Approval

This study was approved by our institutional review board.

Statement of Human and Animal Rights

This article does contain studies with human subjects. The consent form was obtained from all subjects which was submitted along with the article.

Statement of Informed Consent

There are human subjects in this article and informed consent is applicable. This study involves human subjects and information sheet and consent form is applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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