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Syed M. Hasnain, PhD, FACAAI, FAAAAI1, Halima Al Sini2, Alanoud Al-Qassim1, Abdurrahman Al Frayh, prof., allergy/pulmonology2, Mohammad Othman Gad-El-Rab2 and Ayodele Ala'Iya4,
(1)Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia, (2)King saud University &King Khalid Hospital Riadh KS ARiyadh, Riyadh, (3)College of Medicine, King Saud University

Protein Expression Profile of Indigenous and Commercial Extracts of Amaranthus Pollen in Allergy Patients

Syed M. Hasnain, Halima Al Sini, Alanoud Al Qassim, Abdurrahman Al Frayh*, Mohammad O. Gad-El-Rab*, Ayodele A. Ala'Iya
King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
*College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Background: Amaranthus viridis and Amaranthus lividus, pollen are the most prevalent in various parts of Saudi Arabia. Amaranthus species are allergenic and potential cause of respiratory allergy. However, neither are commercially available for diagnostic or therapeutic purposes.

Methods: SPT was applied in this study. Five allergy patients were skin tested with locally prepared (indigenous and commercial pollen) as well as commercial extracts. Amaranthus pollen was collected from various indigenous sources. A. palmeri, A. retroflexus, A. hybridus, A. tuberculatus were acquired from Greer and A. retroflexus, A. tamariscinus were acquired from Allergon. The raw pollen from these species was extracted in buffered saline PH 6.1. Protein patterns of eight different types of Amaranthus samples as well as serum samples from patients were analyzed using two-dimensional polyacrylamide gel electrophoresis (2-DE)/SDS PAGE and computer-assisted image analysis (PDQUEST).

Results: We have generated and characterized the expression of multiple proteins in human serum samples of patients exposed to 7 different types of Amaranthus allergens. Two patients demonstrated similar high expression changes to 2 types of Amaranthus allergens and were classified as group 1, while three samples showed low expression to Amaranthus and were referred to as group 2 of the Amaranthus allergens. Changes in the expression of 12 proteins were observed between groups 1 and group 2 samples.

Conclusion: There appear to be proteins diversity in six major Amaranthus species and similarities in the two indigenous species. While the reactive and cross-reactive proteins between the indigenous and commercial species are being investigated, the available commercial extracts appear to have different protein profile and may not be fully relevant to this region for the diagnosis of inhalant pollen allergy and subsequent specific immunotherapy. Further validation of observed protein spots is warranted in order to support their usefulness as potential Amaranthus biomarkers for the diagnosis and therapy monitoring of allergy patients.

See more of: Allergens and risk factors
See more of: Abstract Submission

<< Previous Abstract | Next Abstract >>
ABSTRACTS

December 2009 to July 2010. Demographic data and history taking on the basis of standardized questionnaires were collected from all participants. The skin prick test was performed with 12 common allergens.

**Results**
There were 218 subjects, 111 (50.9%) boys and 107 (49.1%) girls, age 8.4 to 13.4 years old, mean of age 10.4 ± 1.05 years old, enrolled in the study. Positive SPT were found in 175 subjects (80.3%), negative SPT in 31 subjects (13.8%) and severe dermatographism in 13 subjects (6%). The positive rates of inhaled and food allergens were 33.1% and 6.3% respectively whereas positive rate to both allergens was 60.6%. The most common inhaled allergens were house dust mites i.e. *Blomia tropicalis* (58%), *Dermatophagoides farinae* (55%), and *Dermatophagoides pteronyssinus* (49.5%), followed by cockroach (32.6%), cat dander (26.6%), *Alternaria alternata* (24.3%), and *Aspergillus* mix (18.3%). Yolk egg was the most common food allergen (31.7%), followed by chocolate (30.7%), shrimp (22.5%), and soya (11.5%).

**Conclusion**
The percentage of positive SPT is high. The most common allergen is house dust mite (*Blomia tropicalis*).

Keywords: Allergen, house dust mite, IgE-mediated allergy, skin prick test

1118

PROTEIN EXPRESSION PROFILE OF INDIGENOUS AND COMMERCIAL EXTRACTS OF AMARANTHUS POLLEN IN ALLERGY PATIENTS

Hasnain, S. M., 1 Al Sini, H., 1 Al-Qassim, A., 1 Al Frayh, A., 2 Gad-El-Rab, M. O., 3 and Alaiya, A. 1

1Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. 2King Saud University &King Khalid Hospital Riyadh K SA Riyadh, Riyadh. 3College of Medicine, King Saud University.

**Background:** *Amaranthus viridis* and *Amaranthus lividus* pollen are the most prevalent in various parts of Saudi Arabia. *Amaranthus* species are allergenic and potential cause of respiratory allergy. However, neither are commercially available for diagnostic or therapeutic purposes.

**Method:** SPT was applied in this study. Five allergy patients were skin tested with locally prepared (indigenous and commercial pollen) as well as commercial extracts. *Amaranthus* pollen was collected from various indigenous sources. *A. palmeri*, *A. retroflexus*, *A. hybrius*, *A. tuberculatus* were acquired from Greer and *A. retroflexus*, *A. tamariscinus* were acquired from Allergon. The raw pollen from these species was extracted in buffered saline pH 8.1. Protein patterns of eight different types of *Amaranthus* samples as well as serum samples from patients were analyzed using two-dimensional polyacrylamide gel electrophoresis (2-DE)/SDS PAGE and computer-assisted image analysis (PDQUEST).

**Results:** We have generated and characterized the expression of multiple proteins in human serum samples of patients exposed to 7 different types of *Amaranthus* allergens. Two patients demonstrated similar high expression changes to 2 types of *Amaranthus* allergens and were classified as group 1 while three samples showed low expression to *Amaranthus* and were referred to as group 2 of the *Amaranthus* allergens. Changes in the expression of 12 proteins were observed between groups 1 and group 2 samples.

**Conclusion:** There appear to be proteins diversity in six major *Amaranthus* species and similarities in the two indigenous species. While the reactive and cross-reactive proteins between the indigenous and commercial species are being investigated, the available commercial extracts appear to have different protein profile and may not be fully relevant to this region for the diagnosis of inhalant pollen allergy and subsequent specific immunotherapy. Further validation of observed protein spots is warranted in order to support their usefulness as potential *Amaranthus* biomarkers for the diagnosis and therapy monitoring of allergy patients.

1119

NATURAL RUBBER LATEX-RELATED OCCUPATIONAL ASTHMA: SUCCESSFUL SUBLINGUAL DESSENSITIZATION


Allergy Department, Catholic University, Rome, Italy.

**Background:** Occupational Asthma (OA) has become the most common work-related lung disease in industrialized countries. The most common triggers are wood dust, grain dust, latex (especially among health care workers associated with use of gloves) or other chemicals (especially disocyanates). Specific desensitization represents an important therapeutic tool in the management of patients with latex allergy. The aim of the study is to evaluate the safety and effectiveness of sublingual desensitization in patients with latex-induced asthma and its impact on patient capability to reintegrate at the previous work.

**Method:** We selected 13 patients affected by occupational latex-induced asthma. The diagnosis of NRL allergy was based on a positive allergological work-up, included execution of allergological tests (skin prick test and *in vitro* laboratory tests) and provocation challenges (glove-wearing, conjunctival, bronchial and sublingual provocation test) to confirm clinical latex allergy. Based on clinical history and positive allergological work-up, we decided to carry on a rush sublingual desensitization with latex, performed in 4 days with increasing doses of latex extract under patient's tongue until the highest dose of 500 µg of undiluted latex solution. A maintenance therapy (10 drops of undiluted solution three times a week) was recommended. After 1-year treatment challenges were repeated and those with negative bronchial test underwent a 8-hour work place challenge in an operating room.

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86

FINAL PROGRAM
Protein Expression Profile of Indigenous and Commercial Extracts of Amaranthus Pollen in Allergy Patients

Syed M. Hasnain, Halima Al Sini, Alanoud Al-Qassim, Abdulrahman Al Frayh*, Mohammad O. Gad-El-Rab*, Ayodele A. Alaiya
King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
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INTRODUCTION:

- Allergy and asthma in both children and adults can be caused by many allergenic pollen grains from weeds, trees, and grasses. World allergenic pollen flora varies in their nature and quantity from place to place and fluctuates with geography and climate.

*Amaranthus* (pigweed) is an allergenic weed shedding pollen in the air throughout the year in Saudi Arabia with peaks in autumn months. There are a number of *Amaranthus* species in Saudi Arabia. However, the dominant species on the ground and frequently encountered pollen in the air belongs to *A. viridis*.

Despite this fact, either *Amaranthus* is not included in the diagnostic profile in Saudi Arabia by the clinics and hospitals or an unrelated imported/commercial extract of other *Amaranthus* is included. This may result in false negative test in those patients who are exposed to *A. viridis*. There are only up to 30% cross-reactivity between the weeds pollen allergy but no such cross-reactivity has been documented in *Amaranthus* species.

In this project, we have made initial attempt to study biochemical and immunochromatographic aspects of *A. viridis* in relation to allergy and asthma in Saudi Arabia.

MATERIALS & METHODS:

- Preparation of extracts:
Extracts were prepared using buffered saline. The collected pollens were defatted with excess of diethyl ether / n-butanol and treated with chilled acetone. Antigen was extracted from the defatted pollen with 1:10 weight per volume (w/v) concentration. The extract was prepared in Phosphate Buffered Saline (10 mM PBS pH 8, at 4°C for 72 hrs). Extract obtained was dialyzed (mol. wt cut limit 3500) exhaustively against 85% PBS, lyophilized and kept at −20°C and reconstituted when and as required. Extracts were sterilized using Millipore filters (0.45µm) then (0.22µm).

For the biochemical studies in 2D-SDS-PAGE, Phenol extraction protocol was used, with some modification, followed by methanolic ammonium acetate precipitation. Protein content of each extract was determined by the Bradford method.

RESULTS:

- Protein expression patterns between different species of *Amaranthus* samples:
Serum samples obtained from seven patients inoculated with different types of *Amaranthus* as well as sample from 5 healthy subjects as negative control samples were analyzed. Crude serum samples were prepared and analyzed by 2-DE for both qualitative and quantitative differences in the expression of multiple polypeptides. An average total number of 548 spots were resolved and more than 95% of the resolved protein spots were successfully matched between all the gels. Representative 2-D gels images from one control and one patient serum samples are shown in Figure 1.

Global Protein Expression Profiling of Serum Samples
We have generated and characterized the expression of multiple proteins in human serum samples of patients exposed to 7 different types of *Amaranthus* allergens using the technique of two-dimensional gel electrophoresis (2-DE). Sera were collected from patients diagnosed with allergic rhinitis or asthma. Samples were divided into two groups as patients and controls.

Changes in the expression of 19 proteins were observed between patient and control samples. These differential changes were considered significant (P < 0.05 with 98%CI) using combined ANOVA and more than 2-fold difference in the levels of expression of these protein spots. Two representative 2-D gel images from patient and control serum samples are shown in Figure 1 with gel segments indicating two differentially expressed spots between patient and control samples.

These 19 dataset of protein spots were used in the Principal Component Analysis (PCA) and unsupervised hierarchical cluster analysis and the samples were correctly classified into two distinct groups (Figures 2A & 2B).

The location and distribution pattern of some of these proteins are shown in Figure 3. Fifteen (15) of the 19 protein spots were at least more than 2-fold highly expressed in the patient group than in control sample. The differential expressions of two of these proteins are shown as gel segments in Figure 1.


## Acknowledgement

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## Conclusion

The results of this study indicate that the proposed method is effective in solving the optimization problem. The proposed method outperforms several existing methods in terms of both accuracy and computational efficiency. The future work will focus on extending the proposed method to more complex problems.

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**Tables**

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
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<td>2</td>
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</tr>
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<td>4</td>
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**Figures**

1. Figure 1: Description of Figure 1
2. Figure 2: Description of Figure 2
3. Figure 3: Description of Figure 3
4. Figure 4: Description of Figure 4

**Appendices**

- A
- B
- C
- D
- E
- F
- G
- H
- I
- J
- K
- L
- M
- N
- O
- P
- Q
- R
- S
- T
- U
- V
- W
- X
- Y
- Z

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