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# 1803 Protein expression profile of indigenous and commercial extracts of amaranthus pollen in allergy patients

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**Protein Expression Profile of Indigenous and Commercial Extracts**

**of *Amaranthus* Pollen in Allergy Patients**

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**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. 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* 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.

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# 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%). Yolc 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 mites, IgE-mediated allergy, skin prick test

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

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**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.

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## NATURAL RUBBER LATEX-RELATED OCCUPATIONAL ASTHMA: SUCCESSFUL SUBLINGUAL DESENSITIZATION

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**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 diisocyanates). 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.

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

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

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## INTRODUCTION:

• Allergy and asthma in both children and adult 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 is *A. viridis* [1].

Despite of 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 immunochemical aspects of the *A. viridis* in relation to allergy and asthma in Saudi Arabia.

## MATERIALS & METHODS:

• ***Preparation of extracts:***

Extracts were prepared using buffered saline.[2]
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 Millipor filters (0.45µm) then (0.22µm).

For the biochemical studies in 2D SDS-PAGE, Phenol extraction [3] protocol was used, with some modification, followed by methanolic ammonium acetate precipitation.
Protein content of each extract was determined by Bradford[4] 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 gel 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.

seven (7) different types of Amaranthus allergens as listed in table I, blood sera from positive reacting patients was analyzed using two-dimensional polyacrylamide gel electrophoresis (2-DE).

Table 1

| No. | Name | |
|---|---|---|
| 1 | *Amaranthus viridis* | *(indigenous)* |
| 2 | *Amaranthus lividus* | *(indigenous)* |
| 3 | *Amaranthus retroflexus* | *(Allergon)* |
| 4 | *Amaranthus retroflexus* | *(Greer)* |
| 5 | *Amaranthus tuberculatus* | *(Greer)* |
| 6 | *Amaranthus hybridus* | *(Greer)* |
| 7 | *Amaranthus palmeri* | *(Greer)* |

- ***Abbreviations:***

2-DE- Two-dimensional gel electrophoresis, MALDI –TOF-MS – Matrix Assisted Laser Desorption Ionization Time of Light Mass Spectrometry

***Electrophoresis, scanning and image analysis***

Crude serum samples were diluted to a total volume of 350 µl, in a solution containing 8 M urea, 2M thio urea, 0.2% Pharmalyte, 0.3% DTT, 2 M CHAPS and a trace of bromphenol blue. A total amount of 75 µg of protein was loaded on each strip via rehydration using linear pH 4-7 Ready IPG, strips (Bio-Rad, Hercules, and Ca, USA). First-dimension isoelectric focusing was carried out for a total of 45,500 Vh in a PROTEAN IEF cell (Bio-Rad).

Following Isoelectric focusing, the strips were first equilibrated in a 15 ml solution containing (8 M urea, 75 mM Tris (pH 8.8), 30% (w/v) glycerol, 2% (w/v) SDS, and 0.002% bromophenol blue) and reduced with 65 mM DTT for 15 min, followed by 15 min second equilibration in a solution containing 135 mM iodoacetamide. The IPG-strips were then loaded and run on a 12.5% SDS-PAGE criterion mini gel and run over 2 hours at 200V constant until the bromophenol blue dye front had reached the bottom of the gel. The gels were stained with silver nitrate and scanned using a calibrated densitometer, GS 800.

***Data preprocessing / Data analysis:***

2-DE gels were analyzed using Progenesis SameSpots software (Nonlinear Dynamics).

Variables were selected using ANOVA analysis. Quantitative datasets from the Progenesis gel analysis package was exported in the form of data table, with rows representing gels and columns representing spots.

The pre-processed data were analyzed by hierarchical clustering and PCA analysis using the J Express software (java.sun.com).[5,6]

Fig 1. Representative 2 D gel images from patient (A) and Control (B) serum samples (Below are gel segments showing two differentially expressed spots between patient and control samples, Crude serum sample was subjected to 2-DE using IPG strip pH 4-7 in the first dimension and 12.5% mini criterion pre cast SDS polyacrylamide gel in the 2nd dimension)

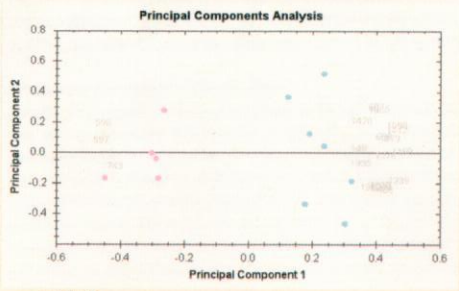


Fig 2A. Principal Component Analysis (PCA) plot using the expression patterns of 19 protein datasets that are differentially expressed between the control and patient samples.

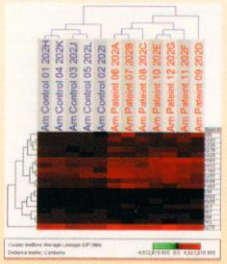


Fig 2B. Hierarchical cluster analysis using the expression patterns of 19 protein datasets that are differentially expressed between the control and patient samples

Fig 3. Representative 2 D gel image from a patient serum sample (Marked are gel distribution and location of differentially expressed spots between patient and control samples)

## CONCLUSION:

In summary, we have used 2-DE to separate proteins from serum samples of allergy patients exposed to different Amaranthus allergens. We have identified some protein spots on 2-DE gels that should be further validated in order to support their usefulness as potential Amaranthus biomarkers for the diagnosis and therapy monitoring of allergy patients.


## ACKNOWLEDGEMENT:

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