

A preliminary fabrication of a protein microarray biochip to detect allergic sensitization of tropical grass pollen allergens

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Purpose: Limited research on grass allergic sensitization and cross-reactivity in the tropical zone creates a critical gap in clinically diagnosing grass pollen allergy. To address this challenge, a preliminary platform of a protein microarray chip was set up and developed to detect grass allergic sensitization in Thai atopic individuals.

Methods: A protein-array biochip was fabricated by spotting a mixture of partially purified pollen extract of a representative grass species: para grass (*Urochloa mutica*), a predominant grass species in Thailand, and a photoactive copolymer onto untreated polymethyl methacrylate substrates. UV-irradiation cross-linked the polymer and proteins, forming a biofunctionalized hydrogel immobilized on the biochip's surface. These microarrays were then incubated with serum samples containing IgE from Thai atopic donors (n=33), who had a positive skin test to para grass extract. Subsequently, biotinylated anti-human IgE antibody and streptavidin conjugated Cy5 were applied, and the fluorescence signal was quantified using a fluorescence array imaging reader, which was then converted to a signal-to-background ratio (SBR) for analysis. Spearman's rank correlation was used to assess the correlation between IgE reactivity measured by the microarray, skin prick test (SPT), and ELISA.

Results: A preliminary prototype of a protein microarray chip was successfully set up and capable of detecting IgE reactivity in Thai atopic sera against partially purified para grass pollen extract. Optimal concentrations of coating protein, serum samples, detection antibody, and detection dye were determined. The SBRs obtained from the microarray chip demonstrated the ability to differentiate between patient serum IgE levels even in cases with low wheal sizes in SPT or low ELISA optical density. Correlation analysis revealed a moderate and statistically significant positive correlation between SPT and microarray results ($r_s=0.514$, $p=0.002$), and a weaker but still significant correlation between SPT and ELISA ($r_s=0.384$, $p=0.028$).

Conclusion: This preliminary protein microarray chip holds promise as a valuable tool for research studies and could potentially serve as a more precise alternative method for clinically diagnosing tropical/subtropical grass pollen allergy in the Asia-Pacific region in the future.

Keyword: Para grass, *Urochloa mutica*, Hydrogel polymer, IgE reactivity, Grass pollen allergy

Evaluation of basophil activation test and other biomarkers before and after the exercise provocation test in children with exercise-related symptoms

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Background: Current knowledge regarding the mechanisms underlying exercise-induced anaphylaxis (EIA) or bronchospasm (EIB) remains limited. This study aimed to investigate biomarkers including basophil activation test (BAT) before and after exercise provocation test (EPT).

Method: A total of 12 patients (7 male, 5 female; median age 15, range 9–18 years) with a history of EIA or EIB were enrolled. EIA included food-dependent EIA (FDEIA) and isolated EIA. Fractional exhaled nitric oxide (FeNO), serum tryptase, complements (C3 and C4), osmolality, eosinophil cationic protein (ECP), and lactic acid were measured before and after the EPT. BATs based on stimulation of heparinized whole blood cells measuring CD63 activation of basophils by flow cytometry (FACS Canto II, BD Biosciences) were performed before and after the EPT.

Results: The median levels of FeNO, serum tryptase, C3, and C4 before and after the EPT were 16 vs. 14 ppb, 2.71 vs. 2.96 $\mu\text{g/l}$, 115.5 vs. 119.0 mg/dl, and 20.5 vs. 23.0 mg/dl, respectively. The median levels of pre-EPT and post-EPT serum osmolality, ECP, and lactic acid were 294 vs. 297 mOsm/kg, 13.5 vs. 17.1 $\mu\text{g/l}$, and 1.22 vs. 7.62 mmol/l, respectively. Among the measured biomarkers, FeNO, serum C3, C4, osmolality, and lactic acid showed significant differences after the EPT. In BAT, the median percentages of CD63+ basophils before and after the EPT were 67.9% and 75.0% respectively, and the difference was significant ($p = 0.002$).

Conclusions: The significant changes in the BAT observed during the EPT suggests that exercise induces basophil degranulation, which may be implicated in the mechanisms of EIA and/or EIB. Further research is required to understand the implications of other biomarkers.

Keyword: Anaphylaxis, Basophil activation test, Exercise-induced bronchoconstriction, Exercise provocation test, Food-dependent exercise-induced anaphylaxis