Analysis of serum peptidome patterns of patients stung by honey bees

Sir,

Recently, the number of the people who died from bitten by honeybees is gradually increasing. Hymenoptera venom of component is extremely complicated, and can impact function of multi-organ *in vivo*, such as intravascular hemolysis, rhabdomyolysis, acute renal failure, liver loss, heart loss, nervous system loss, disseminated intravascular coagulation, etc., Especially, the renal damage is common.[1] Stings of Hymenoptera may lead to anaphylaxis. Anaphylaxis to insect stings has occurred in 3% of adults and can be fatal even on the first reaction. The body systems of patients stung by honey bees exert differentially expression proteins, which can threat human lives.[2] 2-D gel electrophoresis (2-DE) combined with protein identification by mass spectrometry (MS) is the most classic technology used for proteomics and is now recognized as an accurate method to determine and quantify human proteins, can reduce inter-gel variability and simplifying gel analysis. It is also a rapid and sensitive method.[3]

We used the technique of 2-DE to separate differentially expression proteins in the serum of patients [Figure 1] stung by honey bees, and compared with healthy control individuals [Figure 2]. Then, we identified the bee venom-associated key protein molecules by MS analysis. The function and classification of these proteins were determined by searching the public database.

Protein samples were separated on 13 cm linear pH 4-9 IPG strips, followed by sodium salt-Polyacrylamide gel electrophoresis (SDS-PAGE), and stained with Coomassie Blue R-250. The images were analyzed using the image master 2D platinum 5.0 software. An effective exclusion, in advance, of the given high-abundance proteins is the key to a successful 2-DE. In this study, the trials consequently contributed to the methods in the exclusion of serum albumin and Immunoglobulin G (IgG) through affinity and absorption by ProteoSeek™ Albumin/IgG Removal Kit, the purification of the protein samples by 2D clean-up kit and also an improved protocol of silver staining.[4] This technique is very useful for applications requiring accurate quantization and direct differential proteomic analysis of some tissues or serum.[5]

Another finding was that the abundances of some cytoskeletal proteins including Keratin1 type II (KRT1- II), alpha-2-macroglobulin and Spectrin beta chain, brain 1. They were decreased in the patients group. There has been reported alpha-2-macroglobulin which related to patients with bees venom. However, there is still one protein, protein-coding gene SDA1, domain containing (Sdad1), whose function is totally undetermined. These novel candidates would be more worthy for further investigation.

The goal of this preliminary work focuses on primary delineating comparative protein profiles of patients with sting by boney bees and wasps and healthy controls with two dimensional electrophoresis and matrix assisted laser desorption/ionization time of flight MS. The study shows the potential application of MALDI-TOF-TOF spectrometry proteomics in identifying protein changes and detecting interested biomarker candidates in some diseases.

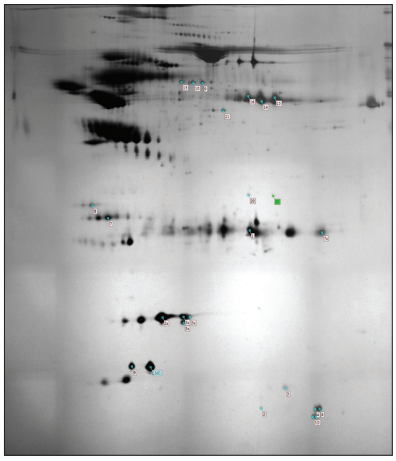

Figure 1: Patient group gel. The pH direction of the IEF is indicated on the top of the figure. Isoelectric Focusing pH 4~9

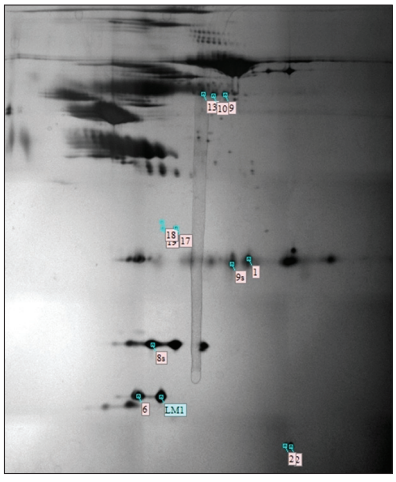

Figure 2: Control group gel. The pH direction of the IEF is indicated on the top of the figure. Isoelectric Focusing pH 4~9

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