Oral Abstracts

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PROTEOMIC CHARACTERIZATION OF UNIQUE VENOM COMPONENTS AND DEVELOPMENT OF SANDWICH ELISA FOR DIAGNOSING CLINICALLY SIGNIFICANT SNAKE ENVENOMATION IN TAIWAN

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Objectives: Taiwan is an island located in the south Pacific, a subtropical region that has 61 species of snakes. In clinical envenomation, more than 90% of victims were bitten by four snakes, *Trimeresurusstejnegeri*(TS), *Protobothropsmucrosquamatus*(PM), *Bungarusmulticinctus*(BM) and *Najaatra*(NA). Owing to the antivenoms are available, the mortality of snakebite is less than 1% in Taiwan. Currently, there are two types of bivalent antivenom including hemorrhagic antivenom against the venom of TS and PM, and neurotoxic antivenom treating envenomation by BM and NA. However, there are no suitable detection kits guiding the physicians to use the antivenom precisely. Therefore, the present study aimed at identifying the species-specific proteins as snakebite biomarkers and developing a diagnosis assay for improving clinical management of snakebite in Taiwan.

Methods: A two-step of affinity purification was set up for generating neurotoxic species-specific antibodies (NSS-Ab) and hemorrhagic species-specific antibodies (HSS-Ab). Then, these two SS-Abs were used to develop a sandwich ELISA which could distinguish the venom from neurotoxic snakes (BM & NA) or hemorrhagic snakes (TS & PM) in human plasma. Snakebite animal models of 4 venomous snakes were used to confirm the feasibility of this ELISA for detecting venom in biological samples. Additionally, the four snake venom proteomes were characterized by liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS), and the protein components recognized by HSS-Ab and NSS-Ab were respectively identified.

Results: Our approach could purify SS-Ab and successfully develop ELISA to discriminate between neurotoxic and hemorrhagic snakebite. The limit of quantification (LOQ) of the ELISA for neurotoxic venom and hemorrhagic venom was determined as 0.39 and 0.78 ng/ml, respectively, and the venom concentration in envenomation model's plasma ranged from 7 to 600 ng/ml, depending on the species of snake. Using Western blot analysis in conjunction with LC-MS/MS analysis, we have identified several venom proteins as the antigens specifically recognized by the NSS-Ab and HSS-Ab, respectively.

Conclusion: We herein presented a feasible strategy to develop sensitive sandwich ELISAs for detecting and differentiating venom proteins between TS/PM and BM/NA. Moreover, we also identified several species-specific proteins as the major antigens recognized by the purified SS-Ab. Knowing the identities of these SS-Ab-recognizable antigens can facilitate the development of new antivenom for snakebite treatment and management in the near future.