

Research Assessment Exercise 2020

Impact Case Study

University: The Chinese University of Hong Kong

Unit of Assessment (UoA): 02 Pre-clinical studies

Title of case study: Development of biomarker of pyrrolizidine alkaloid-induced liver injury: impact on public health with diagnosis and prevention of pyrrolizidine alkaloid poisonings

(1) **Summary of the impact** (indicative maximum 100 words)

Pyrrolizidine alkaloids (PAs) are significant phytotoxins and induce liver injury (PA-ILI) via human exposure to PA-producing natural products/PA-contaminated foodstuffs. Currently there is no definitive diagnosis of PA-ILI. Lin and co-workers developed a biomarker for diagnosing PA-ILI. This methodology provides a paradigm shift in diagnosis, and has been recognized internationally (i.e. USA, Africa, Asia) and recommended in China for testing PA-ILI patients. To prevent PA poisonings in public, her team also developed a novel methodology for rapid analyzing of PAs in natural products. Her works have significant impact on clinical diagnosis, public awareness to the risk and prevention of PA-ILI.

(2) **Underpinning research** (indicative maximum 500 words)

Needs for diagnosis of PA-ILI

PAs are phytotoxins present in numerous plants. Approximately 660 PAs have been identified in ~6,000 plants. PA poisoning is the consequence of inadvertent consumption of PA-producing herb/plant-derived natural products, like medicinal herbs, or PA-contaminated foodstuffs, like wheat flours. International Programme on Chemical Safety has announced that PAs are a significant threat to human health and safety. USA Food & Drug Administration (FDA) has issued Guidance for Industry-Botanical Drug Products and recommended biological assays and/or chemical assays to be included for any investigational new drug application for botanical products known to contain PAs.

Since the first report in 1920, PA poisoning has been continued to rise yearly. Based on retrospective identification of PA exposure and liver injury symptoms, which are although unspecific to PA-ILI, to date, over 17,000 acute PA-ILI cases were reported worldwide. However, an absence of a definitive diagnosis for PA-ILI might underestimate the true incidence, and the currently known PA-ILI cases may only be the tip of the iceberg. Therefore, it is timely need for development of a specific diagnostic method for PA-ILI.

Development of biomarker for diagnosis of PA-ILI

Toxic PAs are esters of two types of necine base: retronecine-type and otonecine-type. Lin's research group at CUHK delineated that toxic mechanism of all PAs is identical. PAs require cytochrome P450-mediated metabolic activation in the liver to produce reactive dehydro-PAs, which covalently bind to proteins to form pyrrole-protein adducts (PPA) causing hepatotoxicity. Lin identified that all dehydro-PAs contain an identical core structure regardless of different structures of PAs (**section-3, ref i**). This led the foundation for developing a novel mechanism-based biomarker for diagnosis. In 2011, Lin developed a specific LC-MS method to determine PPA, and for the first time identified PPA in the blood of PA-ILI patient in China (**section-3, ref ii**).

Using this novel biomarker, Lin's group tested ~150 DILI (drug-induced liver injury) patients with unknown aetiology in China and determined that ~39% of patients were exposed to PAs, indicating a high risk of PA-ILI (**section-3, ref iii**). Using this biomarker for testing 600 blood samples, Lin collaborated with US CDC (Centers for Disease Control and Prevention) to

identified that an outbreak of unidentified liver disease affecting ~1,200 individuals in Ethiopia since 2007 was PA-ILI due to consumption of PA-contaminated foods (**section-3, ref iv-v**). This novel diagnostic method has been recognized internationally (i.e. USA and Ethiopia) and recommended in China for definitive diagnosis of PA-ILI patients.

Needs for public aware of PA-ILI

Using this biomarker, Lin's group found that PA-ILI is a significant contributor (~39%) to HILI (herb-induced liver injury) in China (**section-3, ref iii**). Because annual incidence of DILI has been reported as 7-14 (global) or 23.8 (China) per 100,000 inhabitants, and HILI accounts for 16.1% (USA) or 26.8% (China) of DILI (**section-3, ref vi**), based on the currently available data, annually ~129,484 PA-ILI patients are estimated in China. Therefore, it is timely and significant to alert public the high risk of PA-ILI.

(3) References to the research (indicative maximum of six references)

- i. **G. Lin***, Y.Y. Cui and E. M. Hawes (2000). Characterization of rat liver microsomal metabolites of clivorine, an hepatotoxic otonecine-type pyrrolizidine alkaloid. *Drug Metabolism and Disposition*, **28(12)**, 1475-1483. (Times Cited: 61 (*web of science*) or 81 (*Google Scholar*))
- ii. **G. Lin***, J.Y. Wang, N. Li, M. Li, H. Gao, Y. Ji, F. Zhang, H. Wang, Y. Zhou, Y. Ye, H.X. Xu and J. Zheng (2011). Hepatic sinusoidal obstruction syndrome associated with consumption of *Gynura segetum*. *Journal of Hepatology*. **54(4)**, 666-673. (Times Cited: 105 (*web of science*) or 137 (*Google Scholar*))
- iii. J.Q. Ruan, H. Gao, N. Li, J.Y. Xue, J. Chen, C.Q. Ke, Y. Ye, P.P. Fu, J. Zheng, J.Y. Wang and **G. Lin*** (2015). Blood pyrrole-protein adducts – A biomarker of pyrrolizidine alkaloid-induced liver injury in humans. *Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews*. **33(4)**, 404-421. (Times Cited: 28 (*web of science*) or 27 (*Google Scholar*))
- iv. Pyrrolizidine Alkaloid-Induced Liver Disease (PAILD) Outbreak, Tigray, Ethiopia: Levels of Pyrrolizidine Alkaloids (PA) Biomarkers among Members of the Affected Community (CUHK Project Code: 7050511, **2011-2012**, Agreement for a collaborative contract/grant information could be provided if needed)
- v. Pyrrolizidine Alkaloid-Induced Liver Disease (PAILD) Outbreak, Tigray, Ethiopia: Levels of Pyrrolizidine Alkaloids (PA) Biomarkers among Members of the Affected Community (CUHK Project Code: 7050723, **2014-2015**, Agreement for a collaborative contract/grant information could be provided if needed)
- vi. T. Shen, Y. Liu, J. Shang, Q. Xie, J. Li, M. Yan, J. Xu, J. Niu, J. Liu, P.B. Watkins, G.P. Aithal, R.J. Andrade, et al (2019). Incidence and Etiology of Drug-Induced Liver Injury in Mainland China. *Gastroenterology*. **156(8)**, 2230-2241. (Times Cited: 9 (*web of science*) or 23 (*Google Scholar*))

Remarks: * = Corresponding author | Times Cited figures as at 18-Oct-2019

(4) Details of the impact (indicative maximum 750 words)

Impact of the diagnostic biomarker for PA-ILI

The developed biomarker is mechanism-based and specific with PPA only present in biological specimens after PA exposure. This piece of work has been featured in an Editorial of Journal of Hepatology (**section-5, ref a**) and stated as that, "Lin et al. have reached a new step of overcoming the difficulties encountered with herbal medicine hepatotoxicity.....They have constructed a sensitive and specific assay enabling for the detection of a reactive pyrrole–

protein adduct in serum.....providing direct evidence of the direct role of pyrrolizidine alkaloids in a clinical situation.”

This novel biomarker for definitive diagnosis of PA-ILI has been well-received nationally (**section-3, ref: ii-iii, 164 citations**). Medical doctors in China have advocated improving the existing guidelines for PA-ILI by adopting PPA as diagnostic and prognostic index (**section-5, ref: b**). In 2017, this biomarker has also been included in clinical diagnostic criteria for PA-ILI in China by an expert consensus (Nanjing Criteria) (**section-5, ref: c**). In 2018, the usage of this biomarker has been recommended in the Guidelines for the diagnosis and management of HILI in China (**section-5, ref: d**).

Furthermore, with the successful development of this novel biomarker, USA CDC had invited Lin to collaboratively investigate an outbreak of an unidentified liver disease (ULD) that has affected over 1200 persons with mortality over 30% in Tigray, Ethiopia since 2007. From 2011 to 2015, Lin’s group had analysed ~600 blood samples collected from Ethiopia subjects and confirmed that this ULD was PA-ILI due to the consumption of PA-contaminated foodstuffs, which were subsequently found to be contaminated with PA-containing plants (**section-5, ref: e**). To date, this study is the largest investigation for measuring PPA in human blood specimens and for confirming PA exposure via food contaminations. The success of this study for identifying the ULD as a PA-ILI is an excellent story of integrative epidemiology and laboratory science for translational research from the basic bench-work research to the application for clinical usage.

Impact of PA analytical methods in natural products for prevention of PA exposure

Although PA-ILI can be more quickly and accurately diagnosed in clinic by using Lin’s developed specific biomarker in the further, there is no complete cure for this deadly disease, while only supportive and/or symptomatic treatment are commonly used. Therefore, elimination of the source of PAs in foodstuffs, cessation of PA-intake, and education of public to prevent PA exposure are essential and more important.

Given there are ~660 different PAs, standards for these PAs are not all available, complicating quality control of PAs in natural products, including herbs, health supplements, teas, honey and foodstuffs used in daily life. With her seminal discovery of characteristic mass fragmentation ions specific to different PAs, Lin developed a novel, rapid and specific LC-MS method to determine toxic PAs in natural products without requiring all corresponding standards. The developed analytical method provided a revolutionary step in identifying contaminated PAs and screening for the safe use of natural products, and resulted in international adoption of the approach enabling rapid assessment of PAs in natural products and the determination for whether further evaluation of individual PAs in the products is needed. (**section-5, ref f**). The developed analytical method for quality control of PAs has been transformed to botanical/food products companies. This is evidenced in that Nin Jiom Medicine Mfy. (H.K.) Ltd requested Lin’s group for the analysis of potential presence of PAs in Nin Jiom cough syrup, a highly popular Chinese herbal remedy with annual sales of ~100 millions US\$ worldwide, to ensure no acute/chronic adverse effect/toxicity to human health as a consequence of consumption of their products (**section-5, ref: g**).

Impact of public awareness

The discovery of a novel biomarker for PA-ILI diagnosis and the development of a rapid and specific method for analysis of PAs in PA-producing natural products and PA-contaminated foodstuffs have uncovered a high incidence of PA-ILI in human and a potential high risk of daily PA exposure in public, which had increased public awareness of the risk of PA exposure and PA-ILI. For instances, Centre for Food Safety, Hong Kong Government, and some public mediums have warned potential dietary PA exposure and PA-producing herbs-induced HILI to

public (section-5, ref: h-i).

Overall, 1) the established biomarker certainly has a significant impact on definitive diagnosis of PA-ILI for immediate treatment and necessary cessation of PA-intake; and 2) the developed PA analytical method and making public awareness have a significant impact on stopping/reducing PA exposure in the public, and thus preventing PA-ILI in human.

(5) **Sources to corroborate the impact** (indicative maximum of 10 references)

- a. D. Larrey, S. Faure (2011). Herbal medicine hepatotoxicity: A new step with development of specific biomarkers. *Journal of Hepatology*. **54(4)**, 599-601. (Times Cited: 36 (*web of science*) or 51 (*Google Scholar*) on 18 Oct., 2019)
- b. H. Gao, J.Q. Ruan, J. Chen, N. Li, C.Q. Ke, Y. Ye, **G. Lin*** and J.Y. Wang* (2015). Blood pyrrole-protein adducts as diagnostic and prognostic Index in pyrrolizidine alkaloids-hepatic sinusoidal obstruction syndrome. *Drug Design, Development and Therapy*. **9**, 4861-4868. (Times Cited: 19 (*web of science*) or 25 (*Google Scholar*) on 18 Oct., 2019)
- c. Y. Zhuge, Y. Liu, W. Xie, X. Zou, J. Xu, J. Wang and Chinese Society of Gastroenterology Committee of Hepatobiliary Disease (2019). Expert consensus on the clinical management of pyrrolizidine alkaloid-induced hepatic sinusoidal obstruction syndrome. *Journal of Gastroenterology and Hepatology*. **34(4)**, 634-642. (Times Cited: 2 (*web of science*) or 2 (*Google Scholar*) on 18 Oct., 2019)
- d. J.B. Wang, Y. Zhu, Z.F. Bai, F.S. Wang, X.H. Li, X.H. Xiao (on Behalf of the Branch Committee of Hepatobiliary Diseases and Branch Committee of Chinese Patent Medicines, China Association of Chinese Medicine 2018). Guidelines for the Diagnosis and Management of Herb-Induced Liver Injury (2018). *Chinese Journal of Integrative Medicine*. **24(9)**, 696-706. (Times Cited: 9 (*web of science*) or 18 (*Google Scholar*) on 18 Oct., 2019)
- e. Investigating Liver Disease in Ethiopia. Success Stories: Disease Detectives in Ethiopia.
- f. L. Zhu, J.Q. Ruan, N. Li, P.P. Fu, Y. Ye and **G. Lin*** (2016). A novel ultra-performance liquid chromatography hyphenated with quadrupole time of flight mass spectrometry method for rapid estimation of total toxic retronecine-type of pyrrolizidine alkaloids in herbs without requiring corresponding standards. *Food Chemistry*. **194**, 1320-1328. (Times Cited: 14 (*web of science*) or 19 (*Google Scholar*) on 19 Oct., 2019)
- g. CUHK- Nin Jiom Medicine Mfy. (H.K.) Ltd Agreement for Service (No. TN1712309, 2018).
- h. Centre for Food Safety, The Government of the Hong Kong Special Administrative Region *Pyrrolizidine Alkaloids in Food (Report)* 2017.
The link provided report, presentation, and press, regarding to “Dietary Exposure to Pyrrolizidine Alkaloids of Hong Kong Adult Population”. In the report, some of our works were cited, such as references 3, 4, and 16 of the report.
- i. 多维分析 HILI 影响因素。药肝研究联盟 8-10-2018.