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Current status of Wilson disease in Japan 2017: challenges toward precise diagnosois by synchrotron-generated X-ray

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1. Introduction

Copper and iron are essential nutrients for all organisms. Both metals in excess are toxic with strong redox-activity. Humans as well as higher vertebrates developed the ingenious strategy to manage these double–edged swords; the effective absorption and excretion system for copper, and the strictly regulated absorption and no specific excretion system for iron. However, medical interests of copper in the beginning had been focused on the toxic effect, acute poisoning or chronic exposure by mine pollution. Since the discovery of mammalian copper transporters, ATP7A and ATP7B in 1993, molecular mechanisms of copper homeostasis and trafficking pathways in physiological and pathological conditions have been widely explored. In this symposium, we would like to introduce about what is copper toxicosis, namely Wilson disease (WD), and several problems encountered in clinics and laboratories. We then show a trial to solve the copper detection by synchrotron-based technique. We hope that scientists in the field of X-ray imaging optics are continuing persistent hard works which brings biomedical researchers like us up to the top level, beyond “precision medicine”.

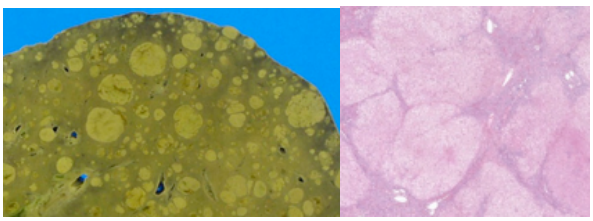

Figure 1 Classic Wilson disease with liver cirrhosis. Macroscopic (left) and Hematoxylin and eosin stained pictures (right).

2. Current status of diagnosis of Wilson disease, challenges and solutions

2.1 Brief introduction of Clinical Practice Guideline of Wilson disease in Japan 2015

There has been two guidelines for diagnosis and treatment of Wilson disease proposed by societies for liver disease in Europe and North America, EASL and AASLD, respectively [1, 2]. Because patients with Wilson disease in Japan as well as east asia are relatively similar compared to those in Europe and North America, and because the disease forms, genotypes, and national health care policy are different between nations, the Wilson disease guideline working group (WG) lead by professor Hiroko Kodama has just published the guideline in Japanese [3]. The approach to diagnosis of WD is similar in these three guidelines, but we include an unique disease form, presymptomatic form as different entity because it is difficult to diagnose and the number of patients with this form is increasing in Japan as the health care of children is relatively generous [4].

2.2 Problems in diagnostic tests

When patients were presented with unexplained liver disease or with slightly elevated liver enzymes, the attending doctors conducted copper-related laboratory tests; such as serum ceruloplasmin, serum copper, urinary copper according to the health care plan in Japan. If these tests are positive, physicians ask surgeons for invasive liver biopsy to examine liver copper content and pathological examination. First, biochemical liver copper content measurement is not easy for most laboratories in hospitals. The samples must be transferred to the special center that can

perform atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectroscopy (ICP-AES). In this step, there were several factors affected its accuracy. Secondly, although molecular genotyping of ATP7B could be decisive and precise, certain fraction of patients with clinically apparent WD showed negative results [4]. Thirdly, histochemical copper staining methods were insufficient which frequently failed to detect copper deposits [5, 6]. Taken together, we often have critical difficulty in the diagnosis.

2.3 A possible solution

To overcome these challenges and difficulties, we tried to improve the mutation analysis, standardization of biochemical tests, and applied synchrotron-radiation X-ray fluorescence to pathological examinations [7-9]. Whereas other tests were negative or unable to perform, copper could be detected and visualized in some hard-to-diagnose cases.

3. Experiments and Results

The double crystal spectrograph placed upstream of the hatch was used to monochromatize the X-ray and to change the energy range appropriately for the elements of interest. The X-ray beam was focused to spot size with about 30 square micrometers focused by the poly-capillary system. Formalin-fixed paraffin-embedded (FFPE) tissue sections from were irradiated by the 11 keV X-ray beam. The silicon drift X-ray detector (SDD, Vortex-EX, Hitachi, Japan), a type of energy dispersive X-ray detector (XEDS), connected to the current amplifier (Model 428, Keithley, U.S.A.) was placed on the mobile rack that could be readily moved to adjust the distance between the tip of probe and samples. The thermoelectrically cooled SDD does not require liquid nitrogen. The resultant fluorescence X-ray emission was analyzed with energy dispersive spectrometry (XEDS) by spot analysis and presented as a histogram. Copper Ka peak was clearly distinguished by other elemental peaks. This tissue section was completely negative for copper stained deposits. Therefore, the SXRF analysis is much higher sensitivity compared to the routinely used histochemical copper staining methods.

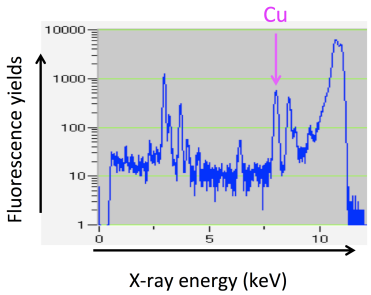

Figure 2 XEDS histograms of FFPE tissue specimens of patient with WD. X axis:energy range. Y axis: fluorescence yields

4. Conclusion

Combination of several techniques, precise diagnosis of Wilson disease become possible in the early stages even before development of clinical manifestations. Synchrotron X-ray fluorescence analysis may become a new tool to estimate copper accumulation in the liver even with fine needle biopsy specimens. The pathogenesis of Wilson disease should be clarified in a variety of aspect [10, 11].

5 References

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