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Review

Copper toxicity induced hepatocerebral and neurodegenerative diseases: An urgent need for prognostic biomarkers

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ABSTRACT

Copper (Cu) has been the subject of intensive research over several decades as numerous evidence robustly support the involvement of excess Cu induced neurotoxicity in hepatocerebral (Wilson's disease) and neurodegenerative disorders (especially Alzheimer's disease and Parkinson's disease); notwithstanding, the ideal Cu neurotoxicity biomarker/s for early prognosis remains elusive. Non-ceruloplasmin bound Cu is a biological marker of Wilson's disease and recent studies have shown that its levels are also increased in Alzheimer's disease. Copper chaperone for superoxide dismutase seems to be the other most promising biomarker of Cu toxicity (subject to its validation). Serum/plasma Cu, urine Cu and ceruloplasmin concentrations, most widely used laboratory indicators to diagnose Wilson's disease, are not specific for Cu excess milieu as these are also influenced by age, sex, inflammation and hormonal status. High inter-individual variability, nonexistence of standardized assays and non-specificity limit the use of other cuproenzymes as biomarkers of Cu neurotoxicity. The majority of Cu neurotoxicity biomarker research has focused in plasma/serum where other factors including inflammation, oxidative stress, dietary and environmental factors influence the Cu condition being studied. Proteomics study of cerebrospinal fluid, due to its high specificity and sensitivity represents an alternative approach to study early peripheral Cu neurotoxicity biomarker/s in experimental animals. In addition, network biology, transcriptomics in conjunction with novel *in vivo* Cu imaging techniques allow us to explore other potential candidates and propose new targets to be studied for chronic Cu neurotoxicity biomarker/s, and for possible therapeutic interventions.

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1. Introduction

Copper (Cu), an essential trace element, is vital for sustaining life and growth of the organism (Madsen and Gitlin, 2007). Due to lack of a sensitive and specific biomarker/s for body Cu status (primarily due to complex interplay of Cu with iron (Fe) and zinc (Zn), and other nutrient factors affecting Cu absorption/bioavailability) there remains on-going struggle to set a precise recommended dietary allowance (RDA) and recommending safe Cu levels in water and mineral/vitamin supplements (Brewer, 2011); however, a range of estimated safe and adequate daily dietary intakes (ESADDIs) have been established for it. RDA for Cu is in the range of 1.5–3.0 mg/day (Recommended Dietary Allowances, 1989); however, the actual requirement may be <1.5 mg/day in diet (Turnlund et al., 1989). Owing to its redox active property, Cu is an essential component of many enzymes/proteins [(92 reviewed Cu binding proteins in *Homo sapiens*) (source: http://www.uniprot.org/) (Electronic supplementary material 1]. Deficiencies and excess of Cu have been associated with

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genetically inherited fatal Menkes and Wilson's diseases (WD), respectively (Strausak et al., 2001). Indian childhood cirrhosis (ICC) (Tanner, 1998) and idiopathic Cu toxicosis (ICT) (Muller et al., 1998) have also been reported due to Cu toxicity. In addition, Cu toxicity has also been implicated in the pathoetiology of hepatocerebral (WD, acquired non-Wilsonian hepatocerebral degeneration, post-shunt myelopathy and hepatic encephalopathy) (Butterworth, 2010) and neurodegenerative diseases [particularly Alzheimer's disease (AD) and Parkinson's disease (PD)] (Desai and Kaler, 2008); yet till date no fully validated Cu neurotoxicity biomarker/s have been defined (Harvey and McArdle, 2008; Olivares et al., 2008). Free Cu excess, rather than Cu deficiency, is more widespread in human population (Brewer and Althaus, 2008) primarily due to high intake of inorganic Cu in the form of mineral/vitamin supplements and Cu plumbing (for details, refer to Brewer, 2009).

It is intriguing to note that very recent studies have demonstrated involvement of ATP7B gene (encodes Cu transporting P-type ATPase) in AD, further supporting the role of Cu dyshomeostasis as an initiating/contributing factor in the development of neurodegeneration and cognitive dysfunctioning (Squitti et al., 2013a,b; Bucossi et al., 2012; Liu et al., 2013). These thought provoking studies have shown that (1) single-nucleotide polymorphisms (SNPs) in transmembrane domain of ATP7B may have a strong association with AD risk (Squitti et al., 2013a); (2) AD patients who were carriers of the ATP7B gene variants exhibited increased free Cu levels (Squitti et al., 2013b; Squitti and Polimanti, 2012); (3) loci of susceptibility for AD is present in the ATP7B gene (Bucossi et al., 2012; Squitti, 2012); and (4) genetic variations in ATP7B gene might contribute to AD pathogenesis (Liu et al., 2013).

While severe Cu toxicity is relatively easy to recognize due to the obvious clinical signs, it is virtually impossible to identify marginal Cu excess, and the biological effects arising from these settings. According to Brewer and Althaus (2008) and Danzeisen et al. (2007), there is no current way to evaluate Cu excess. Notwithstanding, serum non-ceruloplasmin bound Cu ("free" Cu) estimation is acceptable for some purposes (expanded "free" Cu pool as a biological marker for WD and excess "free" Cu in AD) (Brewer and Althaus, 2008; Squitti and Polimanti, 2012). In concurrence, there is an urgent need to establish highly sensitive biomarker/s for Cu overload induced neurodegenerative conditions for the wide-spread screening, permitting early detection of neurotoxicity in asymptomatic individuals, prognosis, precise evaluation and management of disease at different stages. Comprehensive review articles covering the search for Cu biomarkers have been published in year 2007–2008 (Harvey and McArdle, 2008; Olivares et al., 2008), and this article summarizes some of those material along with potential new approaches and sources for search of Cu excess induced neurotoxicity biomarker/s, while also considering the pictorial representation of current development and understanding in the field of molecules involved in intracellular Cu homeostasis.

2. Biomarkers of copper excess

Hepatic Cu concentration quantification by liver biopsy is considered as the gold standard for confirming the WD, an autosomal recessive disorder of Cu metabolism resulting in Cu accumulation mainly in liver and brain, and to compare the performance of various laboratory tests used to detect abnormal Cu buildup. Nonetheless, liver biopsy is only justified when there is robust evidence of liver damage due to excessive Cu accumulation. Ceruloplasmin (an acute phase protein which binds 6–7 Cu atoms), serum/plasma Cu and urine Cu levels are the most commonly used laboratory indicators for detection and monitoring of WD patients. It is worth noting that these three previously outlined Cu toxicity indicators experience fluctuations related to sex, age, hormonal status and pregnancy, and are also increased due to inflammation (particularly interleukin-6), neoplasm and estrogen therapy (European Association for Study of Liver, 2012; Milne, 1998). High levels of dietary Cu do not affect the ceruloplasmin at the level of either mRNA transcription or protein translation (Feillet-Coudray et al., 2000); conversely, it has been recently reported that ceruloplasmin protein expression was found to be increased in Wistar rats fed with diet having extra Cu (~28 parts per million Cu) (Ranganathan et al., 2011). However, no significant effect on ceruloplasmin mRNA levels was observed in the same study. The peculiar characteristic of ceruloplasmin is that though it contains the predominance of serum Cu, absence or abnormality in its functioning does not lead to alterations in Cu homeostasis; rather, it exerts preferential effects on Fe homeostasis. There are mainly two methods for estimation of ceruloplasmin levels: enzymatic [using either *p*-phenylenediamine (Ravin, 1961) or *o*-dianisidine dihydrochloride (Schosinsky et al., 1974) as a substrate] and immunologic (antibody based) method. Enzymatic method is the more ideal one for quantifying ceruloplasmin bound Cu as immunologic method measures holo-ceruloplasmin as well as apo-ceruloplasmin, thus, overestimating the bound Cu (Brewer et al., 2010). On the other hand, ratio of enzymatic ceruloplasmin activity to immunoreactive protein concentration is not influenced by age, sex, or hormonal therapy making it a better indicator of Cu status (Milne, 1998).

The serum non-ceruloplasmin bound Cu level (serum free Cu) (>1.6 μmol/L) has been proposed as a diagnostic test for detection of WD (European Association for Study of Liver, 2012). In a series of investigations, Squitti and colleagues have demonstrated that (1) mean serum free Cu (non-ceruloplasmin bound Cu) is raised in AD (Squitti et al., 2005); (2) Mini-Mental State Examination (MMSE) of AD subjects shows correlation between free Cu levels and cognitive function (the higher the serum free Cu levels, the lower the MMSE score (Squitti et al., 2006); (3) Cu and serum free Cu were higher in the apolipoprotein E ε4 (APOE4) carriers (Squitti et al., 2007); and (4) serum free Cu levels are predictive of decrease in MMSE score (Squitti et al., 2009; Brewer et al., 2010). From the outlined studies, it can be concluded undeniably that serum free Cu levels are associated with cognitive loss in AD patients. Recently, the same group led by Squitti has further reconfirmed and strengthened their earlier findings by documenting meta-analysis of serum samples from AD patients and reported that indeed AD patients have higher levels of serum Cu than healthy controls (Squitti et al., 2009; Bucossi et al., 2011). Of notice is the finding that cognitive function is inversely correlated with serum free Cu levels in normal subjects (Salustri et al., 2010). Brewer et al. (2010) have also reported significantly higher percentage of serum free Cu levels in 28 AD patients compared to healthy controls.

Nevertheless, serum non-ceruloplasmin Cu concentration may also be raised in acute liver failure due to any etiology, in chronic cholestasis, and in Cu-intoxication cases. The amount of Cu associated with ceruloplasmin is approximately 3.15 μg of Cu per milligram of ceruloplasmin. We calculated serum non-ceruloplasmin bound Cu levels by subtracting ceruloplasmin-bound Cu (3.15 multiplied by ceruloplasmin in mg/L equals the amount of ceruloplasmin bound Cu in μg/L) from the total serum Cu concentration (in micrograms per liter; serum Cu in micromoles per liter multiplied by 63.5 equals serum Cu in micrograms per liter) (European Association for Study of Liver, 2012) and found increase in its serum levels in Wistar rat model for non-Wilsonian brain Cu toxicosis (Pal et al., 2013b), which exhibited cognitive waning, astrogliosis, Alzheimer type II cells and neurodegeneration (Pal et al., 2013a). Further, Cu content of hair and intracellular Cu in blood cells have failed to provide any valuable information.