Development of an ICP-MS method for the measurement of exchangeable copper

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Introduction

Wilson's disease (WD) is an inherited disorder of copper metabolism which results in the build-up of unbound copper within tissues, causing hepatic, neurological and other end-organ damage. It is primarily diagnosed and monitored using clinical findings and measurement of biochemical parameters. Patients generally present with low serum caeruloplasmin, low plasma total copper and high 24- hour urine copper. Recently, interest has grown in the measurement of exchangeable copper (CuEX), which includes both free and loosely protein-bound copper, as an accurate and sensitive method for the diagnosis and monitoring of WD[1]. The relative exchangeable copper (REC) can be calculated as the percentage ratio of total:exchangeable copper.

Methods

We developed an exchangeable copper method based on the routine plasma copper assay used in the Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory (STEMDRL). Standards and QCs were prepared using copper stock solution traceable to an international reference standard. Patient samples were pre-treated with 5g/L EDTA for 60 minutes and centrifuged at 4000rpm for 40 minutes using an Amicon Ultra 30 kDa filter. Samples were analysed using the Agilent 7900 inductively-coupled plasma mass spectrometer (ICP-MS).

Objective

To develop an ICP-MS assay for the measurement of exchangeable copper, suitable for routine use for the diagnosis and monitoring of Wilson's disease in the STEMDRL laboratory.

Results and Conclusions

The assay was linear at concentrations up to 17 µmol/L, with an LOQ of 0.27 µmol/L. The maximum intra-assay precision observed was 4.9% with a maximum inter-assay precision of 7.8%. The exchangeable copper reference interval for normal individuals (n=21) was 0.58-1.36 µmol/L which is in agreement with published studies. Results were reproducible when samples were stored at 2-8°C for 72 hours or at -20°C for 7 days with one freeze-thaw cycle. A preliminary study of WD patients (n=5) showed a mean REC of 32% (range 20-44%) compared to individuals with a low copper level likely due to nutritional deficiency (n=5) who showed a mean REC of 18% (range 3-18%).

Table: Total copper (µmol/L) and exchangeable copper (µmol/L) were used to calculate the % relative exchangeable copper (%REC) in WD and non-WD patients.

	Copper concentration (µmol/L)		
Sample	Total Cu (µmol/L)	Exchangeable Cu (µmol/L)	%REC
Low Cu 1	6.6	1.18	18
Low Cu 2	4.5	0.42	9
Low Cu 3	4.6	0.67	15
Low Cu 4	7.50	0.19	3
Low Cu 5	4.60	0.78	17
Wilson's 1	0.6	0.19	32
Wilson's 2	4.7	1.18	25
Wilson's 3	3	0.60	20
Wilson's 4	1.3	0.50	39
Wilson's 5	1.1	0.49	44

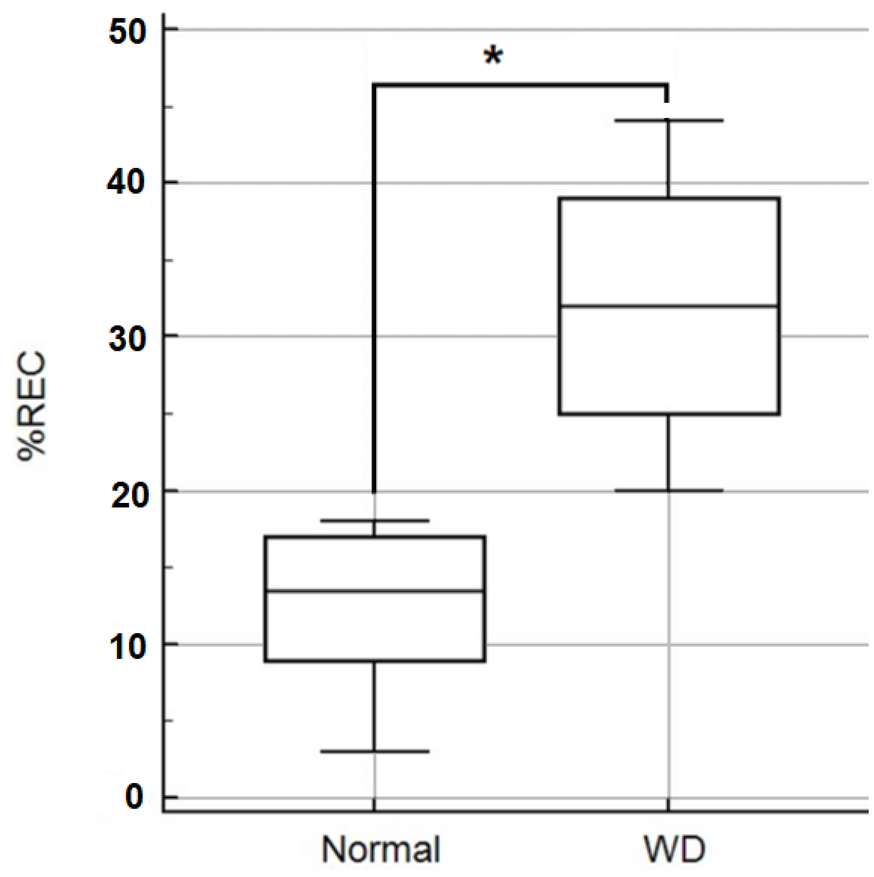

Figure: % relative exchangeable copper (%REC) calculated for WD and non-WD samples. There was a statistically significant difference (p=0.01, Mann Whitney U test) in %REC in patients with WD (n=5) and those with low total copper likely due to nutritional deficiencies (n=5).

Conclusions

- An assay for the measurement of exchangeable copper (CuEX) has been developed within STEMDRL
- The assay shows acceptable performance with regards to linearity, precision, LOQ, and other validation parameters.
- The stability of samples using this method has also been determined and this is viable for use based on the samples received by the STEMDRL service throughout Scotland.
- A preliminary sample comparison demonstrates that the assay is able to discriminate between WD and non-WD patients.

1. El Balkhi, S., Trocello, J.-M., Poupon, J., Chappuis, P., Massicot, F., Girardot-Tinant, N. and Woimant, F. (2011). Relative exchangeable copper: A new highly sensitive and highly specific biomarker for Wilson's disease diagnosis. *Clinica Chimica Acta*, 412(23-24), pp.2254–2260.

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