REC - Its Usefulness in the Diagnosis of Wilson's Disease

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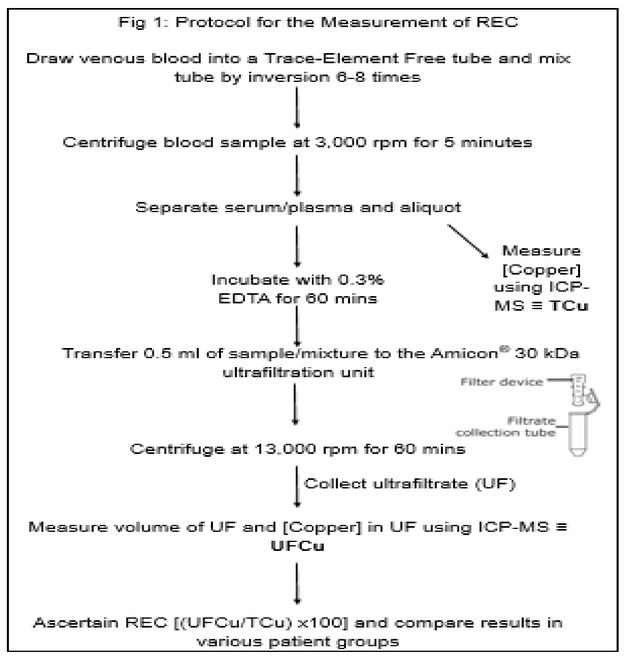
Introduction

Wilson's Disease (WD) is an autosomal recessive disorder caused by mutations in the ATP7B gene. The disorder is associated with the accumulation of copper and can be fatal if left untreated. Diagnosis of WD is challenging, as laboratory results (and symptoms) often mimic other conditions. There is currently no single test that can be used in its diagnosis. It is widely accepted that the amount of non-caeruloplasmin-bound copper (NCC) is increased in, and attributable for the toxicity, seen in WD. Mathematical calculation of NCC (from the measured copper and caeruloplasmin concentrations) often yields negative values, as the caeruloplasmin concentration is overestimated (as both the apo- and holo-forms are captured) by immunoassay techniques (1). There has therefore, been a lot of interest in the direct measurement of NCC as a diagnostic tool for WD (2).

Aim

A method for ascertaining the Relative Exchangeable Copper (REC), based on studies by El Balkhi, et al., (3, 4), was established and applied to control and patient samples.

Method



Surplus serum/plasma samples in the laboratory were utilised in setting up the method. The method involved the chelation of labile/free copper with EDTA, followed by the separation of the complexed copper by ultrafiltration (Fig 1). Copper concentration, measured by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) in the ultrafiltrate (UFCu) and expressed as a percentage of the total copper (TCu) concentration in the serum/plasma, provides the REC. The method was applied to samples from controls (n=11), patients with non-WD liver conditions (n=14) and WD patients (n=10). Statistical analyses was carried out using unpaired t-test.

Results

Lack of detectable copper in the ultrafiltrate obtained from passing saline through the ultrafiltration units, indicated that the units utilised did not contribute to any copper contamination. Using surplus serum/plasma samples, an optimal EDTA concentration and incubation time were established, along with the period required for the ultracentrifugation. Albumin and caeruloplasmin, the primary copper-transporting proteins in blood, were not detectable in the ultrafiltrates. Though transcuprein was not measured in the ultrafiltrate, it can be safely assumed that it would be retained by the filter, as it has a mass (270kDa) much larger than albumin and caeruloplasmin. The method was reproducible, as reflected by the intra-assay (n=3; 2.16 - 6.62%) and inter-assay (n=3; 1.26 - 7.29%) variabilities for two separate samples. The study also demonstrated that it was possible to prepare and analyse ultrafiltrates up to 72hrs post-separation of serum/plasma. Limited studies also indicated that storage temperature of serum/plasma (for up to 1 week), did not significantly influence the REC. Non-WD patients with liver conditions exhibited similar RECs to control patients without any liver problems (see Fig 2 - individual values shown and Table 1). All WD patients however, exhibited a raised REC compared to both other groups ($p<0.05$, Table 1).

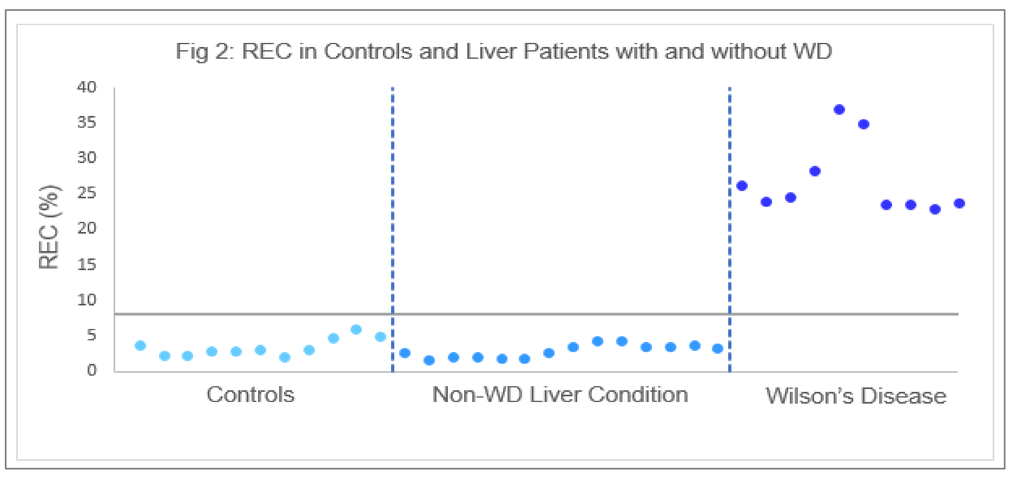


Table 1: Statistical Analyses of REC in Various Patient Groups						
	Controls	*Non-WD Liver Conditions*	*Controls*	*WD*	*Non-WD Liver Conditions*	*WD*
Mean REC (%)	3.485	2.987	3.485	26.903	2.987	26.903
Variance	1.547	0.907	1.547	25.602	0.907	25.602
p-value	0.268		6.200E-12		2.369E-14	

Conclusion

The REC method developed, has the ability to identify and differentiate patients with WD, reducing the possibility of false negatives. Non-WD patients with liver conditions (of various aetiology) were not associated with an in increase in REC. Based on the small cohort to date, a cut-off value for REC of 8% (or 12% if corrected for ultrafiltrate volumes) is postulated for the diagnosis of patients with WD.

Acknowledgements

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References

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