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Introduction: Before the availability of levothyroxine (LT4), patients were treated with desiccated thyroid extract (DTE). When switching from DTE to LT4, despite adequate dosing based on serum TSH levels, some patients still feel unwell with fatigue, mental fogginess, weight gain etc. A recent randomized, crossed over study between DTE vs. LT4 conducted in our department showed that once-daily DTE caused modest weight loss and possible improvement in mental health scores without appreciable adverse effects; also, nearly half of the study patients preferred DTE over LT4. A few studies have shown that LT4/T3 combination had beneficial effects in improving quality of life relative to LT4 alone. Furthermore, it has been reported that patients with CC genotype in the deiodinase type 2 polymorphism responded more favorably with LT4/T3 combination than T4 monotherapy. **Hypothesis**: This study investigated the efficacy and effectiveness of DTE vs. LT4/T3 combination vs. LT4 monotherapy in hypothyroid patients based on genotypic differences of deiodinase type 2.
Methodology: This was a prospective, randomized, double-blind, crossover study. 75 subjects completed the study. There were 3 arms: DTE, LT4+T3 combination, and LT4 alone. Each subject was randomly allocated to one of these 3 arms for 12 weeks randomly. The study was powered to detect the primary outcome. The primary endpoint was post-treatment score on the 36-point thyroid symptom questionnaire. Secondary endpoints were weight, general health questionnaire, the Beck depression inventory, Wechsler Memory testing, lipid panels and thyroid function tests. Analysis was performed with a linear mixed model using subject as a random factor and group as a fixed effect.
Results: There was no significant difference between the 3 arms on the thyroid symptom questionnaire (p=.32), and the secondary outcomes showed no between group differences. Auditory memory index (p=.008), and visual working memory index (p=.02) were higher in the Hashimoto's than non-Hashimoto's group. There was no significant primary or secondary outcome difference among various genotypes of deiodinase 2. There was no relationship between Hashimoto's vs. non-Hashimoto's based on genotypes or likelihood of carrying Thr92AlaD2 polymorphism. Though there was no statistically significant preference for any treatment, numerically more patients with Hashimoto's preferred DTE and LT4/T3 combination than LT4-monotherapy.
Conclusions: There was no significant difference between hypothyroid patients taking DTE vs. LT4/T3 combination vs. LT4 monotherapy. Numerically, Hashimoto's patients tended to prefer DTE and LT4/T3 combination. Also, there was no observed relationship between Hashimoto's and polymorphism. Further studies with more patients may be needed.

Thyroid
FROM HYPO- TO HYPERTHYROIDISM

Development of High-Throughput Measurement of Free Thyroxine in Serum Using Equilibrium Dialysis in Couple With Liquid Chromatography-Tandem Mass Spectrometry

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Free thyroxine (FT4) measurements are critical in the diagnosis, classification, and treatment of thyroid diseases. It is estimated that about 18 million FT4 tests are requested in the USA per year annually. In clinical laboratories, most of FT4 assays are performed by using immunoassays (I As). However, the significant bias of IAs and large variation between laboratories have been reported. The reference measurement procedures (RMPs) of FT4based on equilibrium dialysis (ED) - liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been established and recognized by the clinical chemistry community. However, the FT4 RMP is relatively low throughput and labor-intensive. Also, an aliquot of 1 mL sample is required for an RMP. A routine FT4 assay high-throughput procedure that is based on ED LC-MS/MS and utilized less sample volume will allow to conduct large biomonitoring studies and establish FT4 levels in US population. In the described method, FT4 in 150 uL of serum was separated from protein-bound T4 at 37.0 ºC in 96-well Micro-ED Teflon devices from HTDialysis. The ED conditions suggested by CLSI C45-A guideline were followed. A volume of 150 uL dialysate samples with FT4 was obtained after the ED step. FT4 in the dialysate was purified by extractions before LC-MS/MS analysis. Chromatographic separation of T4 from the sample matrix is achieved on a C18 UPLC column with a gradient of methanol and water with 0.1% formic acid. Quantification of FT4 was performed by using selective reaction monitoring in positive electrospray ionization mode. The IRMM-468 certified primary reference material (JRC, Belgium) of T4 is used for the calibration curves. FT4 concentrations reached equilibrium after 4 hours under current dialysis conditions, which was also observed in the RMP setting. The developed routine FT4 assay 96-well ED system is within 5% bias from the FT4 RMP based on preliminary method comparison study using human serum. The studies to further characterize the FT4 routine method performance are ongoing. In summary, we are developing an analytical method based on a 96-well ED-LC-MS/MS system for the measurements of FT4 in serum. By comparison with RMP, the described method significantly improve throughput and reduce sample volume, which will fulfill the requirement of FT4 routine assay in clinical laboratories and allow for its use in the large biomonitoring studies and other activities in the research and public health settings.

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