Thesis Title	Development of the Micro-Assay for Quantitation of Urinary Ammonia and Citrate : their Applications in Evaluation of Renal Acidification Function
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ABSTRACT

Previous studies conducted by investigators from the Faculty of Medicine, Siriraj Hospital, have confirmed the high prevalence of endemic distal renal tubular acidosis (EdRTA) within the northeast region of Thailand. The prevalence of the condition is around 3 to 5 %. Two key biochemical tests had been used by clinicians to screen for potential cases of EdRTA, including urinary citrate and ammonia. Conventional assays for the detection of both chemical relied on classical biochemical methodologies not suitable for screening large number of samples as required in large population screening.

In this study, we developed two assays that were based on the use of microtiter plates that allowed some degree of automation, giving the possibility of handling large number of samples. The methods relied on the

enzymatic methods which give high accuracy and sensitivity, enabling the assays to be carried out using small samples of urine.

The urinary ammonia assay was based on an enzymatic method using glutamate dehydrogenase (GLDH) catalyzing a mixture of ammonia, nicotinamide adenine dinucleotide (NADH) and alpha-ketoglutarate (α-KG). The reduction of NADH as assayed by the reduction of absorbance at 340 nm, would reflect the amount of ammonia in the solution.

Citrate assay was carried out using citrate lyase as the specific enzyme catalyzing the conversion of citrate into oxaloacetate in the presence of Zn^{+2}. The resulting oxaloacetate enter another reaction mixture of NADH, catalyzed by malate dehydrogenase (MDH). The reduction of NADH would reflect the amount of citrate present in the mixture.

Both assays were accurate and reliable giving values with coefficient of variations between 1.7-10.3 % with 94-104 % of recovery. Both correlated well with respective conventional assays (modified microdiffusion method for ammonia and Nielsen's enzymatic assay for citrate) with correlation coefficient of 0.75 and 0.98 respectively

The assays had been used to quantitate urinary ammonia and citrate in 6 groups of subjects: 6 normal Bangkokians, 6 normal Northeasterners, 6 control relatives from families of adult polycystic kidney disease, 4 patients of EdRTA, 8 adult polycystic kidney disease patients with impaired urinary concentrating ability but with normal acidification functions, and 5 patients with medullary defects (inability to raise urine concentration after DDAVP challenge). Pairs of urine samples were collected prior to and after acid loading test (oral ingestion of NH_4Cl at 0.1 g/kg body weight/day).

Both assays were sensitive and accurate; showing significant difference in urinary ammonia and citrate in samples before and after acid

loading. In all groups of subjects studied , Increase in urinary ammonia and reduction in urinary citrate had been consistent findings when comparing samples prior to and after acid loading tests. EdRTA patients always excrete low amount of urinary citrate, not detected by the assay.

The "micro assay" as developed in this study have been proven to be functional and accurate. They are most suitable for screening large number of patients, for the detection of potential case of patients with tubular defects-as found prevalence, in the northeast part of Thailand.