1H NMR Spectroscopy for Diagnosis of Malabsorption Syndrome

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SYNOPSIS

The use of proton NMR spectroscopy for D-xylose test in the diagnosis of malabsorption syndrome (MAS) is proposed. Urinary excretion (in 5h) of xylose after its oral ingestion (5g) was estimated and the values compared with those from conventional colorimetric method, for 35 adults with suspected MAS. The diagnosis was confirmed by fecal fat, Sudan stain and/or endoscopic duodenal biopsy. Colorimetry and NMR diagnosed 11/12 and 10/12 (p = non-significant) patients with MAS and 14/23 and 20/23 ($p < 0.05$) without MAS, respectively. NMR is rapid with better precision and specificity (7% and 86.9%) compared to colorimetry (20% and 60.7%).

INTRODUCTION

D-xylose absorption test is one of the diagnostic methods for MAS (1). When given orally, it is absorbed from proximal small intestine and is partly metabolized in the liver. 23-50% of the absorbed D-xylose is excreted in urine in healthy subjects. In patients with MAS, the excretion is less than 20%. Urinary estimation of xylose is preferred over plasma as the former is non-invasive and the values from both compare well with each other except in patients with renal failure. Colorimetric method is routinely used for xylose estimation in urine. However, this method is laborious and requires stringent conditions of temperature, reagents and reaction times and therefore it is prone to more errors (2). In addition, if the urine specimen contains other pentose or hexose sugars, they interfere giving erroneous results particularly for patients with uncontrolled diabetes mellitus. We have carried out this pilot study with a hypothesis that NMR spectroscopy may be superior to the conventional colorimetric method for D-xylose test in patients with MAS.

MATERIALS AND METHODS

Thirty-five patients with suspected MAS having no renal failure underwent tests for total serum protein, albumin, blood hemoglobin, upper gastrointestinal endoscopy, duodenal biopsy, stool test for fat (72 h excretion using Van de Kamer's technique, normal <7 g/24-h and/or Sudan III stain of spot stool specimen, normal $\leq$10 droplets/high power field) and/or endoscopic duodenal biopsy. After D-xylose ingestion (5 g), urine specimens were collected for 5 h, following the standard protocol and they were used to estimate excreted xylose using colorimetric and NMR methods, independently. For colorimetric estimation urine specimens were diluted to 10 to 80 times depending on the volume collected with saturated solution of benzoic acid. One mL of diluted urine sample was added into a 5 mL solution of 2% p-bromoaniline in 83 % glacial acetic acid saturated with thiourea and the solution was incubated at 55^{o}C for 40 min. The solution was cooled to 22^{o}C and kept in the dark for 70 min. Its optical density (OD) was measured at 520 nm against unheated blank and used to estimate xylose. Xylose in standard *in vitro* samples were also estimated using similar protocol. For estimation using NMR, 600 µL of untreated urine specimens with a reusable sealed capillary containing 30 µL of 0.375 % sodium salt of trimethyl silyl propionic acid (TSP) in deuterium oxide were used. 1D ^{1}H NMR spectra were obtained on a Bruker Avance 400 MHz spectrometer with water suppression. NMR spectra of four standard solutions of D-xylose, in duplicate, were obtained using similar conditions. Parameters used were, spectral width: 8000 Hz; data points: 32 K, flip angle: 45^{o}: number of scans: 24; recycle delay 5s and FT size: 32K. A computer program was developed which calculates quantity of xylose using integrated areas of NMR signals. Differences between categorical variables were analyzed using Chi-squared test with Yates' correction, as applicable. Continuous data were analyzed using Wilcoxon's sign rank test and Mann Whitney U test. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of colorimetric and NMR methods were calculated.

RESULTS

Demographic, clinical and laboratory parameters of patients with MAS (n=12) and without MAS (n=23) are expressed in median, range and p- value. They are, hemoglobin (g/dL): with MAS 11.5 (6-14.2) and without MAS 11.1 (6.3-15.6), p=ns; Serum albumin (g/dL): with MAS 3.6 (2.1-4.9), without MAS 3.7 (3.3-4.7), p=ns; Fecal Sudan stain (droplets/high power field): with MAS 14 (10-22), without MAS 11 (6-35), p=non significant (ns); Fecal weight(g/24h): with MAS 617 (375-1116), without MAS 325 (216-550), p=0.017; Fecal fat (g/24h): with MAS 8.6 (5.2-10.9), without MAS 6.0 (3.5 – 10.6), p=0.05; D-xylose (colorimetry, g/5h): with MAS 0.36 (0.17-1.2) without MAS 1.03 (0.32-1.89), p=0.001 and D-xylose (NMR, g/5h): with MAS 0.45 (0.17-2.87) and without MAS 1.58 (0.72-3.2)' p=0.001. Colorimetry showed a lower value for the quantity of D-xylose excreted in urine than NMR [median 0.73 (0.17 to 1.89 g) versus 1.37 (0.17 to 3.23 g), respectively; p <0.0001, Wilcoxon's signed ranks test]. Colorimetry and NMR correctly diagnosed 11/12 and 10/12 (p = ns) patients with MAS and 14/23 and 20/23 ($p < 0.05$) without MAS, respectively. Sensitivity and specificity of colorimetry and NMR were 91.6 and 60.7% versus 83.3 and 86.9%, respectively. Positive predictive value, negative predictive value and diagnostic accuracy of colorimetric and NMR were 55.0, 93.3 and 71.4 % versus 76.9, 90.9 and 85.7 %, respectively. In *in vitro* experiments xylose estimated by colorimetry showed a maximum error of 20% (median 12.5 (0 to 20)) while that in NMR showed 7% (median 4.2 (0 to 7)).

DISCUSSIONS

In solution, D-xylose molecules exist as two structural isomers; α-D-xylose and β-D-xylose. Each of the isomers gives a distinct set of signals in the ^{1}H NMR spectrum. Total concentration of D-xylose present in the urine specimen is equal to the sum of the concentrations of α and β isomers. Quantitative estimation requires the measurement of the integrated area of the peak(s) arising from at least one proton from each of the isomers. However, in order to minimize the errors, we have used all non-overlapping signals. Significantly, a comparison of NMR and colorimetric results demonstrates that the latter underestimates quantity of D-xylose as compared with the former in the same urine specimens. This could perhaps be attributed to the time, temperature and photo sensitivity of the colored complex (2). Further, colorimetry is less specific though marginally more sensitive in diagnosis of MAS, as it underestimates the quantity of D-xylose excreted in urine, particularly in those without MAS. The estimation of D-xylose carried out on standard solutions using colorimtric method gave more erratic results (deviations up to 20%) compared to NMR (deviations up to 7%). Though the study on larger number of subjects may provide more definite clues, the observations reported herein along with the studies on standard solutions point out that NMR would score over colorimetry. This is, perhaps, the first NMR study for the diagnosis of MAS.

REFERENCES

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