Easy method of detection of chyle in urine

Sir,

Chyluria, which is passage of chyle in urine that gives its milky appearance, has many causes, which are parasitic and nonparasitic. The lymph laden with fat that has been absorbed from intestine is responsible for the milky appearance. Large amount of chyle in urine or other fluid like, ascitic or pleural fluid can be easily identified on naked eye examination. However, detection of smaller amouts requires special tests.

Milky or hazy appearance of urine also may be due to presence of high phosphate or huge pus cells. Phosphaturia, which settles on standing the urine at room temperature, can be excluded by adding few drops of 5% acetic acid. Pyuria can be confirmed by centrifuging the sample that gives a clear upper and a hazy lower zone of the fluid and then by microscopy.

There are several methods of detection of chyle in urine. Use of fat solvent (ether) almost completely clear the opacity. Chylomicrons can be directly visualized under microscope with dark ground illumination or stained with Sudan III.[1]

Recently triglyceride has been demonstrated to be universally present in chyluria, even in clear urine. The amount of triglyceride has been found to be directly proportional to the haziness of the chylous urine.[2]

When present in concentrations of 100 mg/dl or less, triglyceride does not give a hazy appearance to the naked eye. Measurement of can be done by biochemical analyzer or a photoelectric colorimeter using the standard methods of measurement of triglyceride as in serum.

Method: After adding a few drops of 5% acetic acid to 1 ml of chylous urine, urine was centrifuged for 3 minutes at 3000 rpm. If it clears, it indicates phosphate or pus, which can be confirmed by examining the deposit under microscopy. If the supernatant is still opaque (chyle), then urine from upper part of the test tube is used for the measurement of triglyceride.

Principle of the test: The assay is initiated with the enzymatic hydrolysis of the triglycerides by lipase to produce glycerol and free fatty acids. The glycerol released is subsequently measured by a coupled enzymatic reaction system with a colorimetric readout at 540 nm.

Glycerol is phosphorylated by adenosine-5′-triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5′-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P is then oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Peroxidase (POD) catalyzes the coupling of H_2O_2 with 4-aminoantipyrine (4-AAP) and sodium *N*-ethyl-*N*-(3-sulfopropyl) *m*-anisidine (ESPA) to produce a quinoneimine dye that shows an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to the free glycerol concentration of the sample.[3,4]

Procedure: After centrifugation, we used 10 μl of supernatant urine and mixed with 1 ml of reagent (Sigma: Catalog Number FG0100) [Composition of the reagent-Sigma: Catalog Number FG0100: Free Glycerol Reagent = ATP+ Magnesium salt+4-Aminoantipyrine+N-Ethyl-N-(3-sulfopropyl)m-anisidine, sodium salt+ Glycerol kinase (microbial) +Glycerol phosphate oxidase+ Peroxidase horseradish)] in a cuvette at 37°C. After 5 minutes, absorbance was measured at 540 nm against blank,[3,4] and the concentration was extrapolated against absorbance of the standard (Tg 200 mg/dl).

Calculation: Tg (urine) = (200 × absorbance of urine)/absorbance of standard

In 2010, we measured triglyceride from 31 clinically suspected cases of chyluria due to filariasis; nine of whom showed clear urine. Triglyceride is measured at 700-800 mg/dl in highly chylous urine, and between 30 and 95 mg/dl in clear urine. Controls do not show any triglyceride.

Advantages: It is a quantative method unlike others including ether as lipid solvent, darkground illumination or staining with Sudan III It is also easily available and cheap.

In an endemic zone, clinically diagnosed cases of filariasis, without any apparent chyluria, chyluria can be excluded by this procedure.

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