FC37 ANALYSIS OF BIOPTERIN AND NEOPTERIN IN AMNIOTIC FLUID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Atypical phenylketonuria (PKU) due to tetrahydrobiopterin (BH_4) deficiency may be caused by a defect in 6-pyruvoyl tetrahydropterin synthase (6PTPS). Urinary biopterin (B) and neopterin (N) analysis by high performance liquid chromatography (HPLC) has been developed to differentiate variant forms of PKU. For prenatal diagnosis, the determination of B & N in amniotic fluid was studied. Reduced forms of B & N in amniotic fluid were oxidized by 0.1% iodine in 2% KI solution under acidic conditions. Total B & N was then analyzed by reverse phase C18 HPLC with an isocratic elution of 3% methanol and with fluorescent detection (ex 350 nm, em 450 nm). Recoveries of B and N were both above 87% with within-run imprecision of 4%. The reference ranges (14-21 weeks of gestation, n=26) of B(pmol/mL), N(pmol/mL) and total biopterin ratio (B%=B/(B+N)%) in amniotic fluid were 9.8-24.3, 20.1-70.7 and 14.8-36.9, respectively. B & N profiles in amniotic fluid, taken from two pregnant cases (19th week) at risk of 6PTPS deficiency, were consistent with the urinary B & N results analyzed by HPLC after birth or by determination of 6PTPS activity in the fetal blood. The results indicate that analysis of B & N in amniotic fluid by HPLC can be applied to prenatal diagnosis for PKU caused by 6PTPS deficiency.

FC38 ENZYME IMMUNOASSAY OF 17-HYDROXYPROGESTERONE IN DRIED BLOOD SPOTS FOR NEONATAL SCREENING OF CONGENITAL ADRENAL HYPERPLASIA

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The commonest form of congenital adrenal hyperplasia (CAH), resulting from 21-hydroxylase deficiency, is treatable if detected early. An enzyme immunoassay (EIA) kit (Tomakomai Clin. Lab. Co., Japan), using competitive double antibody method for quantitative determination of 17-hydroxyprogesterone (17-OHP) in dried blood spots collected on filter paper has been evaluated for screening of CAH. The within-assay imprecision (n=15) for three different concentrations (6.3-62.5 ng/mL) was 5.4%-7.5%. The linearity was good (r=0.999) and the analytical recovery was 94-98.4%. Detection limit was determined to be 2.0 ng/mL of whole blood. For comparative study, the 17-OHP in plasma and in dried blood collected from the same subject was determined by a radioimmunoassay (RIA; Sorin Biomedica, Italy) and the present EIA method. The correlation was good (EIA)=1.13(RIA) + 1.07, r=0.881, ($p < 0.001$, n=23). The reference interval of 17-OHP in dried blood spot (collected 3-7 days after birth) determined by this EIA method was estimated to be 3.2-29.1 ng/mL (n=126). All 3 known CAH patients had high 17-OHP concentration (>80 ng/mL) in dried blood spots. The results indicate that this EIA method may be suitable for mass neonatal screening of CAH.

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