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Total Plasma Homocysteine Determination in Subjects with Premature Cerebral Vascular Disease

To the Editor:

In recent years, interest has been focused on the possibility that heterozygosity for cystathionine β-synthase (EC 4.2.1.22) deficiency may predispose to the development of premature cerebral vascular disease (1). These studies were mainly based on measurement of free homocysteine disulfides, either in the fasting state or after methionine loading. This methodology is critical because 75% of the total plasma homocysteine is bound to protein albumin in serum; after a few hours at room temperature or after storage for a week at -20 °C, a considerable proportion of free homocysteine becomes protein-bound (2, 3). Because of this variable affecting free homocysteine, measuring protein-bound (2) or better, total homocysteine in plasma is more nearly accurate.

Various techniques used to determine the concentration of homocysteine differ by reducing agent, deproteinization reagent, and analytical methods: amino acid analyzer (4), high-performance liquid chromatography (HPLC) with fluorometric (5) or electrochemical detection (6), radiometric assay in combination with HPLC (3) or paper chromatography (7), and gas chromatography–mass spectrometry (8). Some of these protocols are laborious, expensive, and cumbersome (5, 6, 8) so they are not suitable for clinical application; others require radioactive reagents that necessitate use of particular equipment.

We used the method of Brattstrom et al. (4) because it is simple and adaptable to an amino acid analyzer, with some modifications. We used a lithium tricitrate buffer as the reducing medium, and precipitated protein with sulfosalicylic acid (0.2 g/L final concentration at 4 °C). We quantified results with a Model LC 2000 amino acid analyzer (Biotronic, Maintal, F.R.G.). Homocysteine standard was prepared under the same conditions as the plasma samples.

We investigated 25 controls (12 men and 13 women, ages 22 to 46 years, mean 33 years) and 92 patients with premature cerebral vascular disease (ages 16 to 49 years, mean 34 years). The values for controls and patients are shown in Figure 1. Four patients had significantly high concentrations of total plasma homocysteine (17.4-25.1 μmol/L), which were still high for four to six months later. Eleven patients had concentrations slightly higher than those of the control subjects (12.1-15.9 μmol/L). The values for our controls (mean ± SEM = 8.01 ± 1.76, range 3.8-12.1 μmol/L) are lower than those reported by other authors (3-8), especially Brattstrom et al. (4), presumably attributable to differences in analytical techniques. We achieved better analytical performance with our analyzer and more nearly accurate determinations with use of the homocysteine standard, prepared under the same conditions as for the plasma. We also observed that the concentration of homocysteine in the standard decreased during storage, which may explain the high values reported by Brattstrom et al. (4) (mean 11.5, range 5.7-28.9 μmol/L).

Our study demonstrates the interest in determining homocysteine in patients with premature cerebral vascular disease. The assay of cystathionine β-synthase, now in progress, will complete this study. The human gene for this enzyme has been recently assigned to chromosome 21. In the future, the use of specific molecular probes may allow the assay of total plasma homocysteine, to establish the heterozygous state of homocystinuria attributable to cystathionine β-synthase deficiency and, thereby, to identify increased vascular risk in these subjects (1).

References

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