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Sandwich-Type Enzyme Immunoassay for Amyloid A4 Protein in Cerebrospinal Fluid From Patients with Head Trauma

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Abstract: The aim of this study was to design a simple method for detecting the amyloid A4 protein in the cerebrospinal fluid (CSF) of patients who had severe brain trauma caused by traffic or other accidents and to evaluate its diagnostic and prognostic value. For this purpose, a sandwich type ELISA assay was used. A4, a 4kDa protein of 39-43 amino acids, is a metabolic product of a membrane-spanning, 695-770 aa, precursor molecule, the b-amyloid precursor protein (b-APP). The polyclonal rabbit antibody against synthetic A4 protein (1-40 residues) was used as an immobilized antibody. A mouse monoclonal antibody against synthetic A4 protein (1-28 residues) was used as a different immunoglobulin to attach the A4. Enzyme-labelled anti-immunoglobulin (peroxidase rabbit anti-mouse antibody, PRAM) was used for reaction with mouse immunoglobulin. The assay was highly specific for A4, demonstrating no cross reactivity between polyclonal anti A4 and monoclonal A4 antibodies (<0.7% cross reactivity). The lowest detectable value in the assay was 100ng/ml (1ng/well). Our results showed that, in CSF samples of 30 patients with head trauma caused by traffic or other accidents and 14 controls drawn from children and adults who presented with acute headache or meningeal irritation and were tested by ELISA for A4 protein, only the CSF samples of patients with head trauma displayed elevated A4 reactivity. This assay thus permits the detection of abnormal fragments of b-APP (A4) within the CSF. It means that severe head trauma may cause abnormal production of A4 which leads to amyloid deposition and, in turn, neuronal degeneration.

Key Words: Beta-Amyloid protein (A4), Head Trauma, ELISA

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