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Use of protein electrophoresis in the diagnosis of cerebrospinal fluid rhinorrhoea

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Abstract
The diagnosis of CSF rhinorrhoea on clinical grounds alone can be difficult. We describe how the use of non-invasive electrophoretic analysis of nasal secretions for tau protein (asialotransferrin) helped in the management of cases where the existence of a CSF leak was in doubt. Patients were thus saved unnecessary invasive investigations or surgery. A modification of the method of analysis, which improves diagnostic accuracy, is described.

Introduction

Cerebrospinal fluid (CSF) may leak into the nose either directly from the floor of the anterior cranial fossa or indirectly from the middle or posterior fossae via the eustachian tube. The causes are described as traumatic (both accidental and iatrogenic), or non-traumatic, and may be associated with pathologically raised or normal CSF pressure. The latter group includes the spontaneous CSF leaks (Bleach *et al.*, 1988).

When faced with the clinical problem of a suspected leak there are two questions to be answered: (1) Is there a CSF fistula? (2) If so, where?

Once a CSF fistula has been identified there are several methods employed to localize it; all of which are invasive. They involve the injection of a tracer in the CSF, with subsequent detection by X-ray, pledgelets or endoscopy (Oberascher, 1988a). Computerized tomography of the sinuses and skull base is essential but may only show the site of leakage in 85 to 90 per cent (Tolley and Brookes, in press).

The diagnosis of CSF rhinorrhoea is suggested by a clear watery discharge which is made worse on bending, straining or lying down (Brockbank *et al.*, 1989). Students are taught that glucose stick testing distinguishes CSF from nasal secretions (Walton, 1985). It is increasingly being recognized, however, that this test can be very misleading and has a high rate of false positive findings (Calcaterra, 1985).

Other tests suggested to distinguish CSF from normal secretions include total protein, which is higher in nasal secretions; specific gravity, which is lower in CSF; or chloride concentration which is higher in CSF. None of these tests, however are certain. To diagnose a CSF leak reliably a specific marker for CSF is required.

One promising approach involves the detection of CSF-specific marker proteins. Of these some, such as gamma-trace protein, also known as cystatin C, (Lofberg and Grubb, 1979), beta-trace protein (Felgenhauer *et al.*, 1987) and prealbumin (Herbert *et al.*, 1986) are relatively enriched in CSF. However, as they are also present in serum and nasal secretion, their usefulness in demonstrating the presence of CSF is limited. By contrast asialotransferrin, also known as tau protein, comprises 15–20 per cent of the total CSF transferrin, but is completely absent from serum. Although asialotransferrin is also formed outside the nervous system any tau protein in the plasma is rapidly removed by asialoglycoprotein receptors present on hepatocytes and reticuloendothelial cells, preventing any build up in the vascular compartment. The detection of asialotransferrin is therefore a reliable method for the identification of CSF.

The following cases illustrate the use of electrophoretic methods in confirming or refuting the existence of a CSF fistula (by the detection of tau protein) and show how the findings have aided patient management.

Case reports

Case 1

A 28-year-old male was assaulted and sustained a severe blow to the head. He immediately developed a right-sided watery nasal discharge, which was diagnosed as CSF. Despite four weeks of bed rest the leak persisted, and a CT cisternogram demonstrated the flow of metrizamide into the nose where it was detected in nasal packing, although the exact site of leakage could not be demonstrated. He subsequently underwent a transethmoid and trans-septal repair, packing of the sinuses with muscle, fascia and *Tisseel* fibrin glue, and rotation of a flap of septal mucosa.

Two weeks post-operatively, after removal of the nasal packs, he complained of a persistent clear nasal discharge which was positive to glucose stick analysis. A repeat cisternogram failed to show a leak. Fluid was collected and

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analysed for asialotransferrin, which was found to be absent. He was managed conservatively with eventual resolution of his symptoms.

Case 2

A 29-year-old female was referred by our neurosurgical colleagues with a one-year history of right-sided nasal discharge. It was of sudden onset and on examination a clear fluid appeared from the right nostril when the patient bent forwards. Glucose testing on the fluid was negative but a CT scan showed a fluid level in one maxillary sinus, with an apparent bony dehiscence in the cribriform plate on that side. A clinical diagnosis of spontaneous CSF fistula was therefore made. Analysis of the fluid, however, was negative for asialotransferrin. She was started on an ipratropium (*Rinatec*®) nasal spray and her symptoms have since resolved.

Case 3

A 43-year-old female developed a CSF leak three weeks after a trans-sphenoidal hypophysectomy for a growth hormone secreting pituitary ademona. She underwent a trans-septal repair, at which procedure the site of leakage was identified and the sphenoid sinus was packed with muscle and fascia. After the nasal packs had been removed on the tenth post-operative day, she again complained of rhinorrhoea for several weeks. The nasal secretion was tested on several occasions with glucose sticks which gave a positive reaction. When, however, the secretions were tested by electrophoresis no tau protein was detected. The patient was managed conservatively,with gradual resolution of her symptoms.

Methods

The use of protein electrophoresis to detect proteins unique to CSF is a simple, non-invasive and reliable approach to distinguish true CSF rhinorrhoea from that due to nasal or lachrymal secretion. The method used here relies upon nitrocellulose-based immunoblotting and has distinct advantages over that based upon simpler immunofixation approaches (Injala *et al.*, 1979; Oberascher, 1988b). Immobilization of the CSF proteins on nitrocellulose and the use of enzyme-labelled antibody both reduce the risk of false negative results. Our method is described below.

Proteins in the samples are separated by electrophoresis for 45 mins in an agarose gel (Jeppsson *et al.*, 1979), followed by squash blotting on to nitrocellulose membrane for 15 mins. After blocking unoccupied protein binding sites with skimmed milk for 30 mins, transferrins are detected by sequential addition of goat anti-human transferrin followed by peroxidase-conjugated rabbit anti-goat serum. The membrance is washed well between antibody additions and after the final incubation. Colour is developed using ethyl-amino carbazole and hydrogen peroxide in 0.02M acetate buffer. In additional to nasal secretion samples of normal serum, diluted 1/100 in saline, and normal CSF are run in parallel. A more detailed description of the method is available elsewhere (Keir *et al.*, 1992).

Normal serum transferrin, which is tetrasialated, shows up as a single band to the anodic (positive) side of the sample application point, whilst asiatransferrin, which lacks the four negative charges associated with the sialic acid residues, migrates as a second band to the cathodic (negative electrode) side of the native transferrin band, and is only seen in CSF (Fig. 1).

Discussion

The most common cause of CSF rhinorrhoea is trauma. CSF rhinorrhoea has been estimated to complicate 2–3 per cent of all head injuries, rising to 27 per cent when basilar skull fractures in children are selected (Liu-Shindo and Hawkins, 1989).

Both neurosurgical and otolaryngological procedures may produce CSF rhinorrhoea. Between 8 and 16 per cent of patients undergoing posterior fossa craniotomy or translabyrinthine surgery for acoustic schwannomas have developed post-operative CSF otorhinorrhoea (Shiobara *et al.*, 1988) although this incidence has recently been reduced by improved surgical techniques (Bentivoglio *et al.*, 1988).

Both intra- and extra-cranial tumours may cause CSF otorhinorrhoea by erosion of the bone. Encephalocoeles and infections are other rare causes. In addition to these iatrogenic and secondary causes of CSF otorhinorrhoea, primary CSF rhinorrhoea can occur when the dural membrane herniates through congenital boney dehiscences, which can occur in the walls of the sphenoid and ethmoid sinuses. A sudden rise in intracranial pressure caused, for example, by coughing or straining, can lead to rupture of the membrane and spontaneous CSF leak. There is always a risk of developing a potentially fatal meningitis, and long-term prophylactic antibiotic cover is inappropriate and not always effective. If conservative measures fail then surgical intervention is required either by the extracranial or intracranial approach.

It is essential for CSF to be unambiguously identified as a component of the rhinorrhoea fluid before embarking upon surgical management. The important problem is to differentiate between cases of true CSF otorhinorrhoea asnd those having profuse rhinorrhoea arising from, for

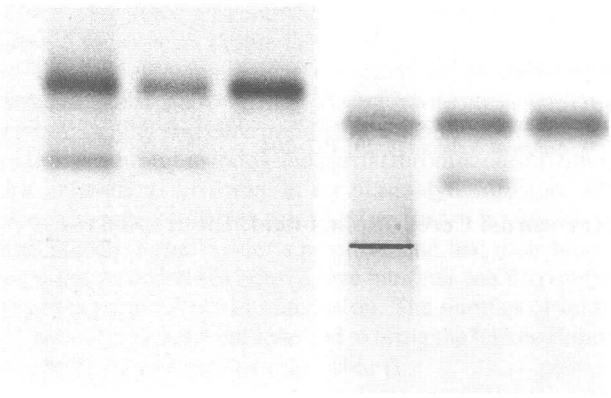

Fig. 1

Lanes 1 and 4 are nasal secretions. Lanes 2 and 5 from control CSF samples. Lanes 3 and 6 from control serum samples. All lanes contain a transferrin band at the top. Lanes 1, 2 and 5 contain Tau protein. The sample no. 1 is therefore positive for CSF, but sample 4 is negative. The dense band at the bottom of Lane 4 is an application artefact.

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example, allergic rhinitis, rhinosinusitis, or non-allergic vasomotor rhinitis (Brockbank *et al.*, 1989). Further diagnostic difficulty may arise in patients with clear nasal discharge following transnasal or transethmoidal surgical repair of CSF fistulae. Under these circumstances 'trauma' to nasal mucosa may result in reactive watery rhinorrhoea.

Asialotransferin, is found in appreciable quantities only in CSF (Verheecke, 1975), aqueous humor (Tripathi *et al.*, 1990) and perilymph (O'Connor *et al.*, 1982). The ratio of tau protein to transferrin is lower in aqueous humor and perilymph than it is in CSF (Keir, unpublished observations), and it is unlikely that there would be confusion in a clinical setting. The presence of asialotransferrin in a sample of nasal secretion, therefore, is diagnostic of a CSF rhinorrhoea. Although we have no figures from our own studies yet, Oberascher (1988b), using classical immunofixation, found only one false positive and two false negatives in a total of 88 cases studied. For reasons discussed above, we would anticipate the method we use to be at least as reliable.

We disagree with Oberascher who states that it is 'imperative' to run a sample of the patients serum in parallel, although it is desirable. Control serum and CSF should always be run alongside the nasal fluid. Although atypical forms of transferrin are sometimes found in serum in chronic alcohol intake and some forms of liver disease, these disialotransferrins have an electrophoretic mobility which is midway between that of native transferrin and tau protein and should not cause confusion. Genetic variants of transferrin exist and have a mobility different from that of the most common C allele of transferrin. Heterozygotes will display double transferrin bands, but these again should not cause confusion as neither band will have the mobility of tau protein.

The technology required for these studies is not complicated and should be available in any hospital where protein electrophoresis is undertaken. The test is not expensive and results should be available within one working day. If the test is not available locally, then requests for analysis can be referred to Dr G. Keir, Department of Clinical Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG (tel: (071) 837 3611, extn. 3814).

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