Usefulness of $\beta_2$-transferrin assay in the detection of cerebrospinal fluid leaks following head injury

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The clinical value of analyzing various fluids and exudates for $\beta_2$-transferrin ($\beta_2$-Tfn) to detect cerebrospinal fluid (CSF) leakage following head trauma was reviewed in a series of 11 cases. Qualitative detection of $\beta_2$-Tfn was performed by agarose gel electrophoresis of tears, ear and nose exudates, cerebral cyst fluid, and wound discharge fluid in different cases. In each instance, the presence of $\beta_2$-Tfn in the analyzed fluid supported the diagnosis of a CSF leak. Equally, the demonstration of the absence of $\beta_2$-Tfn in the fluid excluded the diagnosis of such a leak. Neither false-positive nor false-negative results were found, as indicated by separate radiological investigations and/or subsequent clinical assessment of patients. The detection of $\beta_2$-Tfn in suspect fluids thus provides a highly sensitive and selective, rapid, and noninvasive test for the detection of CSF leakage in cases of head trauma.

KEY WORDS • head injury • cranionasal fistula • cerebrospinal fluid leak • $\beta_2$-transferrin

Fractures of the anterior or middle cranial fossa may result in tearing of the dura mater and the opening of a cerebrospinal fluid (CSF) fistula into the air sinuses, nasopharynx, or middle ear. Such cranionasal fistulae are an obvious route of access for infections, and clinical management demands their closure. The most obvious sign of such a fistula is a leakage of CSF from the nose or ear. Where this leakage is profuse and clear, the diagnosis is unmistakable; however, a small and possibly intermittent leak may be overlooked or misinterpreted, especially if it is mixed with blood or nasal secretions. In such cases, intrathecal injections of radionuclides or radiopaque contrast media and radiological scanning, are routinely used both to confirm and to localize CSF leaks. These procedures are invasive, however, and involve some discomfort; the results may also be negative due to the temporary closure of the leak.

The discovery that a high proportion of transferrin (Tfn) in CSF exists as a carbohydrate-free isoform opened the way for the development of the test to detect CSF contamination in ear and nose exudates of the type produced following damage to the anterior cranial fossa. Initial electrophoresis techniques showed the absence of this Tfn isoform, which became known as $\beta_2$-transferrin ($\beta_2$-Tfn) in uncontaminated tears and ear and nose exudates. The same techniques could demonstrate $\beta_2$-Tfn in concentrated nasal or ear exudates or other fluids mixed with CSF. A rapid, noninvasive test for the detection of $\beta_2$-Tfn in such fluids, and by implication for CSF leakage, was born.

The circumstances under which this test is requested will assist in the interpretation of the results. More refined electrophoresis techniques have in fact shown that $\beta_2$-Tfn is not unique to CSF, and Tfn's with reduced sialylation can be found in the blood serum of newborns and of patients with alcoholic and non-alcoholic liver disease. The relationship between the presence of $\beta_2$-Tfn in CSF and blood serum in these subjects is unknown at present. It was initially suggested that desialated Tfn was synthesized de novo in nervous tissue. The current theory is that $\beta_2$-Tfn is produced by the desialation of normal (6) Tfn in CSF through the action of cerebral neuraminidase, in a reaction which seems to be specific to Tfn. A neural origin for $\beta_2$-Tfn in CSF is also supported by its striking association with at least one congenital demyelination disorder. This being so, testing for $\beta_2$-Tfn in tears and ear and nose exudates following craniofacial trauma should remain a useful procedure.

The value of qualitative $\beta_2$-Tfn detection in ear and nose exudates, as a rapid diagnostic test supporting the clinical approach to craniofacial injuries, is reviewed here in a series of 11 patients.
TABLE 1
Summary of 11 cases in which β2-transferrin (β2-Tfn) measurements were used to indicate leakage of cerebrospinal fluid (CSF)*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age &amp; Sex</th>
<th>Reason for Request</th>
<th>Clinical Summary</th>
<th>Specimen Characteristics</th>
<th>Subsequent Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 yrs, M</td>
<td>postop nasal discharge</td>
<td>transcranial removal of naseoencephalocele</td>
<td>bloodstained, watery, nasal discharge</td>
<td>β2-Tfn detected; dural tear confirmed by metrizamide</td>
</tr>
<tr>
<td>2</td>
<td>8 yrs, F</td>
<td>postop nasal discharge</td>
<td>craniofacial surgery (midface advance)</td>
<td>clear nasal discharge</td>
<td>β2-Tfn detected; dural tear confirmed by metrizamide; surgical repair</td>
</tr>
<tr>
<td>3</td>
<td>18 mos, M</td>
<td>postop bloodstained &quot;tears&quot; watery discharge from wound site</td>
<td>transcranial removal of naseoencephalocele</td>
<td>bloodstained watery discharge from l ty eye clear, watery wound discharge</td>
<td>β2-Tfn detected; CSF leak resolved spontaneously</td>
</tr>
<tr>
<td>4</td>
<td>1 mo, F</td>
<td>origin of cyst?</td>
<td>congenital cerebral cyst closed head injury</td>
<td>clear yellow cyst aspirate bloodstained ear discharge</td>
<td>β2-Tfn detected; cystoatral shunt</td>
</tr>
<tr>
<td>5</td>
<td>3 yrs, M</td>
<td>bloodstained discharge from rt ear</td>
<td>severe head injury</td>
<td>clear nasal discharge</td>
<td>β2-Tfn not detected; no further action taken</td>
</tr>
<tr>
<td>6</td>
<td>21 yrs, M</td>
<td>rhinorrhea</td>
<td>postcranialfacial surgery; CSF leak not proved by metrizamide; surgical exploration equivocal excision of frontal meningocerebrophalic &amp; extensive craniofacial surgery spina biffida, postop Cotrel Dubousset instrumentation</td>
<td>clear nasal discharge</td>
<td>β2-Tfn not detected; no further action taken</td>
</tr>
<tr>
<td>7</td>
<td>19 yrs, F</td>
<td>persisting rhinorrhea</td>
<td>postcranialfacial surgery; CSF leak not proved by metrizamide; surgical exploration equivocal excision of frontal meningocerebrophalic &amp; extensive craniofacial surgery spina biffida, postop Cotrel Dubousset instrumentation</td>
<td>bloodstained wound aspirate</td>
<td>β2-Tfn not detected; wound leak ceased when hemotoma aspirated</td>
</tr>
<tr>
<td>8</td>
<td>5 yrs, M</td>
<td>leak from wound</td>
<td>excision of frontal meningocerebrophalic &amp; extensive craniofacial surgery spina biffida, postop Cotrel Dubousset instrumentation</td>
<td>bloodstained wound aspirate</td>
<td>β2-Tfn not detected; wound leak ceased when hemotoma aspirated</td>
</tr>
<tr>
<td>9</td>
<td>14 yrs, M</td>
<td>watery leak from wound</td>
<td>clear, watery wound discharge</td>
<td>β2-Tfn detected; revision of shunt; CSF leak persisted; subchronchial drain inserted; CSF leak ceased</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2 yrs, F</td>
<td>discharge from lty eye</td>
<td>postop frontal meningocerebrophalic</td>
<td>clear, watery discharge</td>
<td>β2-Tfn detected; discharge resolved to a lesser serous discharge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>discharge from lty eye</td>
<td>yellow serous discharge (2 specimens)</td>
<td>β2-Tfn not detected; discharge completely resolved</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1 yr, M</td>
<td>leak from neck wound</td>
<td>revision of VP shunt to a VA shunt</td>
<td>wetary wound fluid</td>
<td>β2-Tfn detected; VA shunt reviewed; neck wound resecured; wound leak ceased</td>
</tr>
</tbody>
</table>

* Abbreviations: VP = ventriculoperitoneal; VA = ventriculoatrial.

Clinical Material and Methods

**Fluid Samples**

Samples of ear or nose exudate were obtained from patients who had been involved in accidents resulting in forehead and facial injury, or who had undergone craniofacial surgery. A minimum of 100 μl of exudate was secured. Upon receipt, samples were centrifuged to remove cell debris and were concentrated 10-fold prior to electrophoresis. Where possible, a sample of the patient’s serum was submitted with each exudate.

**Electrophoresis**

Thin-layer agarose gel electrophoresis was performed using commercial gels* running in barbital buffer (75 mmol/liter, pH 8.6) at 230 V for 40 minutes. In total, 2 μl of sample was applied. After electrophoresis, anti-human Tfn† was applied directly to the gel to fix the Tfn’s at 37°C for 45 minutes. The gel was then washed and stained with Coomassie blue dye.

* HREP agarose gel kit manufactured by Ciba Corning Diagnostic Corp., Medfield, Massachusetts.
† Anti-human transferrin obtained from the Binding Site, Ltd., University of Birmingham Research Institute, Birmingham, England.

Results

The results of analysis of ear and nose exudates from 11 patients presenting between July, 1989, and August, 1991, are shown in Table 1, together with additional clinical information. Among the four patients with rhinorrhea, the existence of a CSF leak indicated by the presence of β2-Tfn in the nasal fluid was subsequently demonstrated by intrathecal injection of metrizamide and computerized tomography scanning in two (Cases 1 and 2). In Case 5, a child with posttraumatic otorrhea, the immunochemical diagnosis was verified when the leakage (initially bloody) became so profuse that operative repair was necessary. In a patient with cyst aspiration (Case 4), the clinical diagnosis of an arachnoid cyst was supported by the finding of β2-Tfn in the cyst fluid, and treatment proceeded on that assumption. Verification of a CSF leak from the surgical wound site in a patient after removal of a naseoencephalocele (Case 3) provided important information at the time for the management of the case, even though subsequent treatment of the leak was not necessary. Of the three cases where β2-Tfn could not be demonstrated in the exudate (Cases 6, 7, and 8), the subsequent clinical courses supported this diagnosis.
Detection of CSF leaks with $\beta_2$-transferrin

Discussion

Following head trauma, immunochemical detection of $\beta_2$-Tfn can be reliably performed on a small volume of ear or nose exudate obtained at the bedside or in an emergency room. Technically, there is nothing beyond the resources of a modern clinical laboratory in the method used to detect $\beta_2$-Tfn in the exudates presented, and a result is routinely produced within 3 hours of sample receipt. The entire procedure is noninvasive. The only problem encountered to date is the occasional difficulty in concentrating nasal discharge fluids which have a high mucous content.

Clinically, the presence of $\beta_2$-Tfn in exudates supports the rapid diagnosis of a cranionasal fistula due to injury to the anterior cranial fossa. Of equal importance is demonstration of the absence of $\beta_2$-Tfn in the exudate, which tends to exclude the diagnosis of a tear in the dura mater and obviates the need for further invasive investigations and surgery. The investigation is also useful in circumstances where the nature of a cyst fluid or wound discharge is in doubt. In this limited series, the immunochemical determination of $\beta_2$-Tfn in ear and nose exudates produced neither false-positive nor false-negative results, as assessed by radiological techniques and/or subsequent clinical assessment.

For practical purposes, however, the value of the result depends heavily on the context of the request for analysis. After craniofacial surgery a serous or serosanguinous nasal discharge is not uncommon. Similarly, posttraumatic rhinorrhea is common following facial impact. Under such circumstances, a demonstrated absence of $\beta_2$-Tfn in the nasal discharge is reassuring, and may avoid investigative surgery to close a presumed cranionasal leak. In this report, the likely diagnosis in those cases where $\beta_2$-Tfn could not be demonstrated in the nasal exudates was allergic rhinorrhea. The demonstrated presence of $\beta_2$-Tfn in a persistent, and possibly intermittent, rhinorrhea long after injury unquestionably requires follow-up investigation. Thus, both positive and negative results from this determination have a high degree of clinical significance.

References

4. Oberascher G, Arrer E: Erste klinische Erfahrungen mit $\beta_2$-Transferrin bei Ototo- und Rhinoliquorrhoe. HNO 34: 151–155, 1986

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