CARBOHYDRATE-DEFICIENT TRANSFERRIN IN VITREOUS HUMOUR: A MARKER OF POSSIBLE WITHDRAWAL-RELATED DEATH IN ALCOHOLICS

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Abstract — The possibility of performing reliable post-mortem analysis of carbohydrate-deficient transferrin (CDT) concentration in vitreous humour (VH) by using a commercial assay designed for serum analysis (CDTect™) as well as the usefulness of VH-CDT as a marker of alcohol misuse and possible withdrawal-related death were evaluated in a forensic sample. Detectable VH-CDT was found in 20 of 21 alcoholic subjects and in two of seven controls. By using the detection limit of the CDTect™ method (VH-CDT = 5 U/l) as cut-off level for a positive test, the alcoholic group was significantly separated from the control group (P = 0.0024, Fisher’s exact test). The sensitivity and specificity of the test was 95% and 71%, giving a positive and a negative predictive value of 91% and 83%, respectively. Time-dependent changes of VH-CDT in the dead body could not unequivocally be excluded, which must be considered when selecting cases suitable for VH-CDT analysis. We conclude that adding VH-CDT analysis to ordinary alcohol tests may become useful in forensic medicine for establishing the so-called ‘alcoholic state’, which may provide a tool in research dealing with the relation between alcohol withdrawal and various causes of death in alcoholics.

INTRODUCTION

Complications to acute alcohol withdrawal, rather than to acute or chronic alcohol intoxication, may be responsible for some of the excess mortality in alcohol-dependent individuals (Denison et al., 1995, 1997b). Acute alcohol withdrawal should thus be considered a medical emergency during which alcoholics seem to be liable to sudden death from, e.g., withdrawal seizures and cardiac complications (Denison et al., 1994, 1995, 1997a). Due to the problem of establishing such causes of death at autopsy and the lack of biochemical methods to support the diagnosis of death during acute alcohol withdrawal, it has clearly been difficult to study the context of withdrawal-related death in alcoholics.

In the clinical setting, elevated serum concentration of carbohydrate-deficient transferrin (CDT) is used as a specific marker of potentially harmful alcohol consumption (Stibler, 1991). An increased serum CDT concentration corresponds to continuous ingestion of large amounts of alcohol (Salmela et al., 1994). Due to the long half-life of CDT in serum it is possible to detect high serum concentrations several days after an alcohol debauch (Stibler, 1991). The CDT method thus seems to be well-suited to indicate pre-mortal alcohol misuse in an alcoholic. Thus, an elevated serum CDT concentration in combination with negative blood and/or urine ethanol concentrations would support a withdrawal-related death. However, in post-mortem analysis, clinically used cut-off levels do not seem to be applicable to serum CDT (Sadler et al., 1996). High values of CDT may thus be false due to carbohydrate-chain dissociation from the transferrin molecule by enzymatic or bacterial interactions in blood after death (Sadler et al., 1996).

Our hypothesis was that some of these methodological problems could be overcome if a fenced body fluid such as vitreous humour was used for post-mortem CDT analysis. Consequently, we aimed to investigate whether in the present study reliable post-mortem analysis of vitreous humour CDT concentration (VH-CDT) could be achieved by use of a commercially available assay designed for serum analysis (CDTect™). Moreover, we intended to evaluate the usefulness of VH-CDT as a marker of alcohol misuse and possible withdrawal-related death in forensic medicine.

MATERIALS AND METHODS

Subjects

A convenience sample of 21 alcoholic men aged 32–61 years (median 52 years) and seven non-alcoholic men aged 19–57 years (median 24 years) was recruited from a population of men who had died under circumstances necessitating forensic examination by decision of the police authority. In each case, the diagnosis of alcoholism was primarily based upon information about long-standing alcohol misuse in the police report. The diagnosis was further supported by non-specific but possibly alcohol-related autopsy findings in the liver (fatty liver, cirrhosis), in the pancreas (atrophy, fibrosis), in the brain (atrophy, contusions), and/or in the heart (fibrosis, hypertrophy). Detailed information concerning the past and recent alcohol history of each individual was not possible to obtain.

For each subject, the time-points were noted regarding when the person had been seen alive for the last time and when the body was found (see Table 1). Cause of death was established based on clinical and biochemical investigations according to the routines at the forensic department (Table 1). The study was approved by the Research Ethics Committee of Göteborg University.

Sampling procedures

After the dead subjects were found, they were transported to the forensic department and kept in a supine position at +4°C. At the start of the autopsy, vitreous humour was aspirated.

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from the eye by means of a sterile syringe and needle. The vitreous humour was then collected in sterile tubes and kept at \(-70^\circ C\) at the forensic department. When all specimens had been collected the frozen tubes were sent to Pharmacia & Upjohn Diagnostics Sverigef AB for analysis. The specimens had been assigned codes, thus making the laboratory staff blinded to whether the samples were taken from alcoholics or controls. Blood samples from the femoral vein and urine samples from the bladder were collected at autopsy for measurement of ethanol concentrations and for drug screening tests.

**Biochemical assay**

**Carbohydrate-deficient transferrin concentration in vitreous humour (VH-CDT).** One tube of vitreous humour from the eye from each subject was thawed, well mixed and tested in double duplicate in the same assay run using CDTect\textsuperscript{TM} radioimmunoassay (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden, nowadays Axis-Shield PLC, Oslo, Norway) according to the manufacturer’s directions as detailed by Stibler et al. (1991). Briefly, after iron saturation of the samples, separation of the transferrin isoforms with pI >5.7 was accomplished by anion-exchange chromatography on microcolumns. Thereafter, these carbohydrate-deficient fractions of transferrin were quantified by a double-antibody radioimmunoassay with a standard curve comprising values from 5 to 300 U/l. The mean value was reported and used for all calculations. The intra-assay and inter-assay coefficients of variation for CDT analyses were <5 and 16.8\%, respectively.

**Cut-off level for VH-CDT.** Since the transferrin content in vitreous humour is <10% of that in serum (Devgun and Dunbar, 1986), it was unknown at the planning of the study whether CDT could be detected in vitreous humour. It thus seemed appropriate to use the detection limit of the commercially available test, which is 5 U/l, as the highest acceptable cut-off level of VH-CDT for recent alcohol abuse.

**Ethanol concentrations.** Ethanol concentrations were measured by headspace gas chromatography (Jones and Schuberth, 1989) at the Department of Toxicology, National Institute of Forensic Medicine (Linköping, Sweden).

**Statistics**

Due to the small sample size, Fisher’s exact test was used to compare categorical data between alcoholics and controls. \(P < 0.05\) was considered as statistically significant.

## RESULTS

Twenty of the 21 alcoholic subjects and two of the seven controls had detectable VH-CDT (Fig. 1). By using a VH-CDT of 5 U/l as a cut-off level for a positive test, the alcoholic group was significantly separated from the control group (\(P = 0.0013\), Fisher’s exact test). The sensitivity of the test was 95\% and the specificity 71\%, giving a positive and a negative predictive value of 91 and 83\%, respectively.
DISCUSSION

The rationale for the use of vitreous humour for post-mortem analysis of CDT is that this body fluid seems to be more protected from bacterial action during putrefaction than blood (Harper, 1989). It thus seems possible that determination of CDT in vitreous humour may reflect more adequately the biochemical situation at the time of death, than analysis of CDT in serum. In a post-mortem analysis of CDT in serum, Sadler et al. (1996) found that not only alcoholics but also controls had CDT levels well above the clinically used in vivo reference level, probably due to post-mortem changes. Such processes may be time-dependent, which causes problems in setting reference levels and thus limits the usefulness of testing CDT in serum post mortem.

The choice of CDTECT for analysis of VH-CDT in our study turned out well because, by chance, it seemed to be possible to use the detection limit of this test in a semi-quantitative way to discriminate between alcoholics and non-alcoholics. The highly significant result in the Fisher’s exact test was somewhat surprising, because it could hardly be expected that all of the alcoholics would have been in a drinking phase. However, the outcome supports that most of the alcoholics had been drinking heavily prior to death, because a raised VH-CDT level is related to alcohol consumption and not to alcoholism per se.

Two of the controls had detectable levels of VH-CDT. This may be due to selection failure; that is, they may in fact have been high-consumers of alcohol. However, since these two controls had a much longer post-mortem interval before the vitreous humour specimens were collected, time-dependent changes in CDT concentrations may have occurred also in vitreous humour, giving a false positive test. Theoretically, this hypothesis could easily be investigated by taking repeated samples of vitreous humour from some subjects at various time points for CDT analyses. However, this procedure was not considered appropriate, because it must be assumed that the barrier surrounding the vitreous humour in this way is destroyed and thus the VH-CDT will be influenced in the same manner as is noted in post-mortem serum specimens. Another cause of a false positive result could be certain eye disorders in which the protein content may increase and the protein composition may change as a consequence of the breakdown of the blood–retinal barrier (Bresgen et al., 1991).

Until more data are available on this subject, it seems reasonable to restrict the post-mortem interval for the use of VH-CDT analysis. Nevertheless, when an arbitrary, but in forensic medicine practical, post-mortem interval of 72 h for VH-CDT analysis was applied, the outcome was not considerably changed.
Table 3. Clinical application of combining the results of analyses of vitreous humour carbohydrate-deficient transferrin (VH-CDT) and urine/blood alcohol concentrations

<table>
<thead>
<tr>
<th>VH-CDT</th>
<th>Alcohol test</th>
<th>‘Alcoholic state’</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>Present long-term alcohol misuse</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>Alcohol withdrawal</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>Alcohol intoxication or recent relapse</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>Alcohol abstinence</td>
</tr>
</tbody>
</table>

Proposed interpretations of various combinations to describe during which phase of misuse an alcoholic has died (here called ‘alcoholic state’).

The conclusions from the present preliminary study, with a rather small sample, must be drawn with some caution. There is a need for more studies to compare the various CDT methods (Keating et al., 1998; Viitala et al., 1998). If the findings of this study can be reproduced and established, analysis of CDT in vitreous humour can be useful in forensic medicine with at least two applications. First, it may make it possible to detect heavy alcohol consumption before death in forensic cases in general. Such information may explain certain confusing autopsy findings, and may also be helpful in the process of establishing cause of death in obscure cases. Secondly, in persons with known alcohol-dependence, analysis of CDT in vitreous humour, in combination with blood and urine ethanol tests, may offer an opportunity to define an ‘alcoholic state’ during which an individual has died (Table 3). This approach would make it possible to link various causes of death to certain phases of alcohol misuse and thus provide a tool to identify withdrawal-related death in alcoholics.

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