Are Carbohydrate-Deficient Transferrin Assays Useful for the Detection of Recurrent ‘Binge Drinking’ in Children with an Alcohol Intoxication in the Emergency Department?

Margot A.L. Stokbroekx1, Saskia Houterman2, Stefan A.J. Coolen3, Nico van der Lely4 and Rolf A.A. Pelleboer1,*

1Department of Paediatrics, Catharina Hospital, Eindhoven, the Netherlands, 2Department of Education and Research, Catharina Hospital, Eindhoven, the Netherlands, 3Medical Laboratories, Department of Clinical Chemistry, Reinier de Graaf Hospital, Delft, the Netherlands and 4Department of Paediatrics, Reinier de Graaf Hospital, Delft, the Netherlands

*Corresponding author: Catharina Hospital, PO Box 1350, 5602 ZA, Eindhoven, the Netherlands. Tel.: +31-40-239-92-00; E-mail: r.pelleboer@onsnet.nu

(Received 5 February 2014; first review notified 18 March 2014; in revised form 13 May 2014; accepted 7 July 2014)

Abstract — Aims: The aim of this study was to evaluate different carbohydrate-deficient transferrin (CDT) assays for the detection of recurrent excessive alcohol abuse in adolescents prior to acute alcohol intoxication. Methods: Data on drinking behaviour and CDT levels of adolescents (13–18 years) registered at the outpatient clinic for youth and alcohol at three major district general hospitals in the Netherlands were retrospectively collected. CDT and disialotransferrin (DST) levels of binge-drinking teenagers were compared with non-binge-drinking teenagers. Results: In total 198 samples were collected for the N Latex CDT method (N = 83), no differences were found in mean CDT levels for binge versus non-binge drinkers (P = 0.8). The Helder HPLC (N = 78) showed significantly higher values for binge drinkers than for non-binge drinkers (mean 1.20%DST, SD 0.28 and mean 1.01%DST, SD 0.31, respectively (P = 0.01)). The Recipe ClinRep method (N = 37) also showed significantly higher values for binge drinkers (mean 1.17%DST, SD 0.36 and mean 0.89%DST, SD 0.34, respectively (P = 0.03)). Conclusion: With the Helder HPLC method and the Recipe ClinRep assay higher levels are measured in binge drinkers than in non-binge drinkers.

INTRODUCTION

Alcohol intoxication amongst adolescents is a growing problem in the Netherlands. The prevalence of hospital admissions for observation of acute alcohol intoxication is rising (Van Hoof et al., 2011). Most of the adolescents are brought to the emergency department by ambulance and were found unconscious on the street or in a bar (Van Zanten et al., 2013). These patients often have consumed large amounts of alcohol in a short period of time, called binge drinking. Binge drinking is defined as drinking four or more standard glasses containing 12 ml of alcohol for women and five or more standard drinks for men in a couple of hours or at one occasion (Fillmore and Jude, 2011). Excessive use of alcohol at a young age can cause serious acute effects, such as hypothermia, reduced consciousness, metabolic acidosis, hypoglycaemia and even death. But it can also have long-term consequences such as learning problems (Miller et al., 2007; Bouthoorn et al., 2011). A study by Mallet et al. showed that adolescents who have experienced negative consequences of drinking alcohol are not likely to drink less in the future and are at risk to experience these consequences again (Mallett et al., 2006). Recently, so-called ‘alcohol-clinics’ have been set up in the Netherlands (Sheldon, 2006). Adolescents who were admitted at the hospital with alcohol intoxication are invited with their parents for a follow-up at the outpatient clinic to educate the patient and to prevent recurrence.

In adults, the carbohydrate-deficient transferrin (CDT) analysis is a widely accepted method to detect excessive alcohol use. The question remains if this test could also be useful to detect binge drinking in teenagers. Patients identified as repetitive binge drinkers can have a more intensive follow-up to prevent alcohol-related problems later on in life.

CDT is a protein which is present in elevated concentrations in blood under the influence of ethanol. Transferrin is a protein with two carbohydrate chains that each contains 0–4 sialic acid groups. When someone is exposed to alcohol and/or acetaldehyde, more transferrin protein that misses one or both carbohydrate chains is synthesized in the liver (Bortolotti et al., 2006). In adults the half-life of CDT is ~10 days. Adults need to drink 50–80 g ethanol/day, which is the equivalent of 5–8 standard alcohol containing glasses, for a minimum of a week to increase the serum CDT concentration (Schellenberg et al., 2005; Paling and Mostert, 2013).

In the Netherlands there are several methods used to determine CDT. Two frequently used methods are high performance liquid chromatography (HPLC) and the immunonephelometric assay, N Latex CDT (NVKC, 2012).

The sensitivity and specificity of CDT in detecting alcohol abuse in adults has been extensively investigated and discussed in literature (Bortolotti et al., 2006; Delanghe et al., 2007; Bergstrom and Helder, 2008). In most research only patients aged 18 years and older are included. There is hardly any literature describing values of the CDT in patients aged 11–18 years. One study describes HPLC evaluation of CDT in children (2 months–14 years) but they did not investigate the effect of alcohol consumption on CDT in children (Bianchi et al., 2012). Opposed to adult alcohol abusers, who often drink a lot every day, adolescents drink large amounts of alcohol once or twice a week. It is unknown whether binge drinking can raise CDT levels and whether these levels are as high as is the case with continuous drinking.

The objective of this study is to determine the usefulness of the CDT assay for detection of binge drinking teens. In this retrospective study CDT levels for teenagers who have been binge-drinking and teens that have not been binge-drinking in the month before they were admitted with alcohol intoxication were evaluated.

METHODS

Data collection

Data were collected retrospectively in three major district general hospitals in the Netherlands: Catharina Hospital (Eindhoven), Máxima Medical Centre (Veldhoven) and Reinier de Graaf Hospital (Delft). All patients aged 13–18 who were registered at the alcohol clinic between 2008 and 2013 and in
whom a CDT was measured during alcohol intoxication were included. Since the start of the ‘Clinic for youth and alcohol’ in 2008, CDT measurement is part of the paediatric protocol for alcohol intoxications and is measured in blood collected for the purpose of measuring blood alcohol levels at the emergency department. Due to different laboratories and changing methods within the laboratories, three different laboratory methods were used for CDT assay: Helander HPLC (Helander et al., 2003) was used in Reinier de Graaf Hospital, Recipe ClinRep (a HPLC method) was used in Catharina Hospital and N Latex CDT method of Siemens was used in all three hospitals. All laboratories participated in an EQA programme for CDT and performed equally well.

The HPLC method is a liquid chromatography and only measures the disialotransferrin (DST) as a percentage of total transferrin (%DST). The N Latex CDT method detects asialo-, monosialo- and disialotransferrin. This is expressed as %CDT (Delanghe et al., 2007). The cut-off value of N Latex CDT is higher than with HPLC because a greater fraction is measured (Delanghe et al., 2007; Paling and Mostert, 2013).

Data on alcohol behaviour were obtained from the Dutch Paediatric Surveillance Unit questionnaires. These questionnaires are used in all Dutch hospitals for all children (<18 years) with positive blood alcohol levels. They collect data on alcohol intoxications of adolescents in the Netherlands and consist of four parts: general and demographic information, alcohol use and other substance use patterns, characteristics of the intoxication and hospital treatment and hospital information (Van Hout et al., 2011). If information in the questionnaires was missing or unclear, medical records were searched for the missing data. The amounts of alcohol were converted to grams of alcohol when the amounts were written down in other quantities. All data were collected and edited by one researcher (M.A.L.S). Ethical approval as applicable in standard hospital care. Results were processed anonymously as valid under Dutch law.

Variables

Demographic data of the subjects included gender and age at the time of intoxication. Laboratory values that were collected consisted of blood alcohol level and CDT. Data on alcohol consumption comprehended the amount of alcohol consumed on the day of intoxication and the amount of alcohol consumed in the month before intoxication. Furthermore, whether the teenagers have been binge-drinking in the month prior to their intoxication was noted. Binge drinking was defined as drinking four (girls) or five (boys) standard drinks of alcohol during one occasion.

Data analysis

Values were expressed as means and standard deviation (SD) for normally distributed data, or as median and minimum-maximum for non-normally distributed data. Unpaired t-test and one-way analysis of variance (ANOVA) were used to analyse normally distributed data. Multiple comparison testing (Bonferroni) was used to indicate differences between the different methods of CDT measurement. Non-normally distributed data were analysed using the Mann–Whitney and the Kruskal–Wallis test. A chi-square test was used to compare categorical data. A two sided P-value of <0.05 was considered to be statistically significant. A receiver operating characteristic (ROC) curve was plotted to determine the optimal cut-off point with optimal sensitivity and specificity. Data were analysed using SPSS for Windows, version 20.

RESULTS

Between 2008 and 2013, a total of 198 CDT assays were conducted. In the Catharina Hospital 104 patients were included, 7 patients were eligible in the Máxima Medical Centre and 87 patients were included in the Reinier de Graaf Hospital. Due to alternating laboratory methods within the different hospitals, results for the three CDT assays were presented separately. No differences were found in gender, mean age, blood alcohol concentration and percentage binge drinking between the three CDT methods (Table 1). All analyses were also performed for male and female patients separately. A significant difference was only found in the N Latex group. Boys drank more alcohol at the time of the intoxication than girls (median 120 versus 80 g, P < 0.01). No difference was found in CDT levels between males and females; all results are given for boys and girls together.

Table 1. Characteristics of study population for three measurement methods of carbohydrate-deficient transferrin

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Helander HPLC</th>
<th>N Latex CDT</th>
<th>Recipe ClinRep</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
<td>Mean (SD)</td>
<td>n</td>
</tr>
<tr>
<td>78</td>
<td>16.2 (1.0)</td>
<td>83</td>
<td>16.3 (1.0)</td>
<td>37</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>44</td>
<td>16.5 (1.0)</td>
<td>20</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>16.1 (1.2)</td>
<td>39</td>
<td>16.2 (1.1)</td>
</tr>
<tr>
<td>BAC</td>
<td>78</td>
<td>1.91 (0.54)</td>
<td>83</td>
<td>1.97 (0.56)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol intake (grams) at intoxication</th>
<th>Helander HPLC</th>
<th>N Latex CDT</th>
<th>Recipe ClinRep</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Median</td>
<td>Min–Max</td>
<td>n</td>
<td>Median</td>
</tr>
<tr>
<td>65</td>
<td>80</td>
<td>40–230</td>
<td>77</td>
<td>100</td>
</tr>
<tr>
<td>Alcohol intake (grams) in month before intoxication</td>
<td>Helander HPLC</td>
<td>N Latex CDT</td>
<td>Recipe ClinRep</td>
<td>P-values</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------</td>
<td>-------------</td>
<td>---------------</td>
<td>----------</td>
</tr>
<tr>
<td>n</td>
<td>Yes % (n)</td>
<td>n</td>
<td>Yes % (n)</td>
<td>n</td>
</tr>
<tr>
<td>65</td>
<td>43% (28)</td>
<td>77</td>
<td>42% (32)</td>
<td>32</td>
</tr>
</tbody>
</table>

BAC, blood alcohol concentration in grams of alcohol/litre blood; CDT, carbohydrate-deficient transferrin; SD, standard deviation.

*Recipe ClinRep is significantly different from N Latex CDT and Helander HPLC.
**Recipe ClinRep is significantly different from N Latex CDT.
TABLE 2. Mean CDT values for binge-drinking and non-binge-drinking adolescents for Helander HPLC, N Latex CDT and Recipe ClinRep assay

<table>
<thead>
<tr>
<th>Method</th>
<th>Binge drinking</th>
<th>No binge drinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helander HPLC</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>N Latex CDT</td>
<td>32</td>
<td>45</td>
</tr>
<tr>
<td>Recipe ClinRep</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Values measured with Helander HPLC and Recipe ClinRep are expressed as percentage disialotransferrin of total transferrin (%DST); N Latex CDT values are expressed as percentage carbohydrate-deficient transferrin of total transferrin (%CDT).

DST, disialotransferrin; CDT, carbohydrate-deficient transferrin; CI, confidence interval.

DISCUSSION

In this retrospective study, teenagers who have been binge-drinking had higher CDT levels (measured with Helander HPLC or Recipe ClinRep CDT) than adolescents who did not binge-drink in the month prior to their intoxication. In the group of children measured with the N Latex CDT method no differences were found between binge drinkers and non-binge drinkers. An explanation could be that N Latex CDT method is less sensitive for the detection of less alcohol intake, possibly due to the fact that this assay measures not only the disialotransferrin but also asialo- and monosialotransferrin.

All teenagers had CDT levels below the cut-off points for adults in the Netherlands: <2.6 for N Latex and <2.0 for Helander HPLC and Recipe ClinRep (NVKC, 2012). It should be pointed out that these cut-off values are set to be sure that an adult is not unfairly accused of excessive alcohol use. For this, a critical difference is added to the 95% upper limit of a non-drinking reference group (NVKC, 2012). The results of this study obtained with the N Latex CDT method differ from a study that reported mean %CDT values similar to levels in adults in a group of 141 non-drinking adolescents (aged 11–18 years) (Delanghe et al., 2007). They reported a median of 1.91%CDT and 2.5th and 97.5th percentiles of 1.45 and 2.40% while the drinking subjects in our study had a mean of 1.61%CDT. It remains unclear why our results are different from those values. To our knowledge there is no literature for values in adolescents for the Helander and Recipe ClinRep method.

The best cut-off point found with the ROC curve for the Helander HPLC would be 1.08%DST, with a sensitivity of 75% and specificity of 65% in our study. This is much lower than the cut-off point for adults, which is 2.0%DST. It can be assumed that this is due to the lesser amount of alcohol the adolescents have consumed in total. The sensitivity and specificity for this cut-off point are also lower than the reported sensitivity (51–92%) and specificity (90–96%) for adults (Bortolotti et al., 2006; NVKC, 2012). The sensitivity and specificity of the optimal cut-off point found in our study are too low to be used clinically.

To our knowledge this is the first study conducted to evaluate the CDT assay in alcohol consuming adolescents younger than 18 years. The sample size of this study was comparable with the number of patients in similar studies conducted with adults (Bortolotti et al., 2006). Since the aim of this study is to determine whether the CDT assay can be used to detect recurrent alcohol abuse (binge drinking) in patients at the outpatient clinic for youth and alcohol, who have experienced intoxication, we choose to take patients at this outpatient clinic who did not binge-drink as controls instead of a group of children who have not been drinking alcohol at all.

The results should be evaluated in light of limitations of the data and of the design. Since this is a retrospective study, details of alcohol consumption were not written down consistently in the same way and were sometimes missing. Furthermore, the alcohol consumption was self-reported by the teenagers. They reported the numbers of drinks they remembered consuming before their intoxication. Due to the intoxication their memories of the event can be heavily impaired (White, 2003). Often the numbers of drinks were given by bystanders who came to the emergency department, usually friends who have also been consuming large amounts at the occasion. Finally, as a consequence of different, not comparable methods in different hospitals,
we could not create one study group but had to investigate for three different smaller groups.

A prospective study should be done to generate sufficient data for the development of reliable cut-off points. It might be useful to work with validated questionnaires to obtain information on alcohol consumption habits more objectively. The inclusion of a group of non-drinking children should be considered to determine cut-off points more accurately. Choosing a specific laboratory method deserves attention in order to ensure that all data can be compared. The investigation of other biomarkers of alcohol consumption, such as ethyl glucuronide (EtG), might be a useful addition in future studies.

The results of our study indicate that the Helander HPLC and the Recipe ClinRep assays might be a useful assay to detect recurrent binge drinking in adolescents admitted with alcohol intoxication.

Conflict of interest statement. None declared.

REFERENCES


Fillmore MT, Jude R. (2011) Defining ‘binge’ drinking as five drinks per occasion or drinking to a 0.08% BAC: which is more sensitive to risk? Am J Addict 20:468–75.


