DOSE-EFFECT RELATION BETWEEN DAILY ETHANOL INTAKE IN THE RANGE 0–70 GRAMS AND %CDT VALUE: VALIDATION OF A CUT-OFF VALUE

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Abstract — Aim: To evaluate the ability to infer alcohol consumption using the %CDT (carbohydrate deficient transferrin) immunoassay (TIA) kit. Methods: One hundred and eighty-three healthy subjects (143 men, 40 women) undergoing a routine medical check-up at their workplace declared frequency and quantity of alcohol consumption covering the last 4 weeks. Seven sub-groups were made up from this population, according to daily ethanol intake and by increments of 10 g from 0 to 70 g/day. A reference group that consisted of 133 healthy teetotallers (74 men, 59 women) was recruited by occupational medicine in the same conditions as the 183 subjects of the study. Percentage CDT and gamma glutamyl transferase (GGT) were assayed on a fasting blood sample. Results: There was a proportional dose-response effect of daily ethanol intake on %CDT values in the range of 0–70 g per day. A threshold effect on %CDT values for patients having an alcohol intake of over 40 g per day was found, an effect which was not observed for GGT activity. Conclusion: The kit has clinical usefulness, and the value of 2.6% proposed by the manufacturer for the cut-off for hazardous drinking in both sexes has been validated.

INTRODUCTION

Twenty years ago, CDT (carbohydrate deficient transferrin) was proposed as a promising clinical bio-marker for heavy drinking. However, up to now, the clinical usefulness of the CDT has remained controversial. This is essentially the result of the use of a large variety of different measurement techniques, and a non-homogeneous definition of the populations studied (Koch et al., 2004). Furthermore, some studies used different reference values despite the use of an identical technique (Koch et al., 2004). Therefore, the definition of normal reference values is a key point in order to establish population survey. Cut-off based on CDT values obtained with abstinent subjects leads to a low cut-off value that might not be relevant for general populations.

Recently, Axis Shield [Oslo, Norway, distributed by BioRad (Hercules, USA)] proposed a second generation of the %CDT immunoassay (TIA) kit. Using this kit, a CDT increase has been reported in patients with daily ethanol intake of over 60 g (Anttila et al., 2003). Of note, alcohol intake in the range of 20–60 g has not been investigated. In the kit, the cut-off has been set by the manufacturer at 2.6%. This cut-off corresponds to the 90th percentile of CDT values obtained in a population of 199 healthy subjects with a daily ethanol intake <20 g. However, for this assay, other cut-off values have been proposed (Helander et al., 2001; Fleming and Mundt, 2004).

The two objectives of our study were to assess the manufacturer’s cut-off value using the diagnostic value of %CDT at various levels of alcohol intake, and to check for a correlation between alcohol intake and %CDT values in a population with a continuous distribution of alcohol intake in the range 0–70 g/day.

MATERIALS AND METHODS

We designed a multicentre study over 3 months. The study was approved by the ethics committee of Clermont-Ferrand University Hospital in 2002.

Population

The study included 183 healthy subjects (143 men, 40 women) undergoing a routine medical check-up at their workplace. There were no exclusion criteria concerning any particular health disorder or biological abnormalities. The presence of the subjects at their workplace at the time of the clinical examination and blood collection excluded patients with acute or serious chronic diseases. Alcohol consumption was calculated using a self-declared frequency/quantity questionnaire with multiple choice items covering the 4 weeks preceding the blood collection. This system makes the questionnaire independent from the examiner. Patients were informed that their questionnaire and sample residues, after routine examination and blood collection excluded patients with acute or chronic diseases. Alcohol consumption was calculated using a self-declared frequency/quantity questionnaire with multiple choice items covering the 4 weeks preceding the blood collection. This system makes the questionnaire independent from the examiner. Patients were informed that their questionnaire and sample residues, after routine determinations, could be used for anonymous epidemiological studies, in conformity with the relevant legislation. Seven sub-groups were made up from this population, according to daily ethanol intake and by increments of 10 g from 0 to 70 g/day. These groups were used to assess the relationship between the amount of alcohol intake and %CDT value.

In accordance with the World Health Organisation (WHO) recommendations setting the limit of hazardous alcohol consumption to two drinks per day for women and three per day for men, we constituted a ‘normal consumers group’ (NC) with men consuming <45 g/day and women consuming <30 g/day. These values were chosen assuming that one standard drink is, in France, ~12–14 g (7 doses in 0.75 l of 12% alcoholic wine or 14 g in 0.25 l of 5.5% alcoholic beer).

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In the same way, a ‘hazardous consumers’ group (HC) was formed with men and women whose alcohol intake exceeded the aforementioned limits. One additional sub-group of abstinent alcoholics (AA) was made up from 37 (26 men, 11 women), previously heavy drinkers who had stopped drinking for >2 months. A reference group (RG) consisted of 133 healthy teetotallers (74 men, 59 women) was recruited by occupational medicine in the same conditions as the 183 subjects of the study. This group was used to validate the absence of sex difference in %CDT values with this method.

Blood sampling and analytical methods
The subjects were sampled after an overnight fast. The samples were centrifuged within the 4 h following blood collection, and sera were stored for at the most 1 month at ~20°C until CDT was measured. CDT concentration was measured by a nephelometric immunoassay after ion exchange chromatography using the kit recently developed by Axis Shield, %CDT TIA (Oslo, Norway) and distributed by Bioread (Hercules, USA). This modified assay, unlike the former one from Axis, measures all the transferrin variants with 0–2 sialic acid residues, but not the trisialotransferrin fraction. Concentration of %CDT is expressed as a percentage of the total transferrin. Measurements of %CDT and transferrin were carried out on an Immage nephelometer (Beckman Coulter, Brea, USA). Cut-off value of %CDT was set at 2.6% of the total transferrin, as proposed by the manufacturer. The intra- and inter-assays coefficients of variation of this method are, respectively, 4.3 and 8.2%.

Table 1. Descriptive values of %CDT and GGT in the reference group (RG)

<table>
<thead>
<tr>
<th>Number</th>
<th>Mean age*</th>
<th>%CDT Mean</th>
<th>Range</th>
<th>95% CL</th>
<th>GGT Mean</th>
<th>Range</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>74</td>
<td>42.7</td>
<td>2.04</td>
<td>1.20–2.97</td>
<td>1.97–2.11</td>
<td>27.7</td>
<td>4–125</td>
</tr>
<tr>
<td>Women</td>
<td>59</td>
<td>34.4</td>
<td>2.02</td>
<td>1.02–3.57</td>
<td>1.90–2.14</td>
<td>19.9</td>
<td>3–235</td>
</tr>
</tbody>
</table>

*P < 0.05 between men and women.

RESULTS

We first analysed the results by considering the different sub-groups. Mean age was significantly lower (P < 0.05) in sub-group 1 as compared to three of the other occupational medicine sub-groups (sub-groups 4, 5, and 7) and to the AA sub-group. No other age difference was observed. The sex ratio (ratio number of men/whole population) progressively increased with ethanol intake from sub-group 1 (0.64) to 7 (1.0) (P < 0.05 between sub-groups 1 and 7). This point is of no consequence because no sex difference in %CDT values was found using this technique (Table 1). Values of %CDT increased with alcohol intake from sub-group 1–7 (Table 2). This process was moderate for alcohol intake <40 g/day and showed a markedly progression when the daily ethanol intake exceeded this value. A significant threshold was observed in %CDT values between sub-groups 4 and 5 (P < 0.05) (Figure 1). Such a variation in consecutive sub-groups was observed only between sub-groups 6 and 7 (Table 2). Importantly, mean %CDT values were under the cut-off in sub-groups 1–4, and over this limit in sub-groups 5–7.

As opposed to %CDT, GGT values did not parallel the increase of daily ethanol intake. Thus no threshold was observed in GGT values, with no group having a GGT value significantly different from the flanking sub-groups.

We next performed a group analysis. According to their alcohol intake and following the WHO recommendations,
125 subjects (97 men, 28 women) constituted the NC group, and 58 (46 men, 12 women) the HC (High Consumers) group. NC and HC subjects did not differ in age ($P = 0.407$), and HC subjects had a sex ratio slightly higher than those from NC. Both %CDT and GGT values were significantly higher in HC as compared to NC ($P < 0.01$) (Table 3). However, the 95% confidence limit of GGT mean in HC subjects overlapped the upper limit for the reference (45 IU/L). In addition, at 45 g/day (men) and 30 g/day (women), respectively, of alcohol intake, %CDT appears much more selective than GGT, combining higher sensitivity and specificity (Table 3).

The comparison of NC, HC, and AA groups (Table 3) revealed that %CDT values were, in both sexes, identical in NC and AA groups. GGT values were higher in NC compared to AA, but we observed no difference between AA and HC groups.

DISCUSSION

The two main goals of this study were to clarify the dose–effect relationship of alcohol intake on %CDT values, and also to validate the %CDT cut-off value proposed by the manufacturer for this commercially available kit.

Few studies have been dedicated to this aspect since it has been released on the market. Previous studies confirmed its diagnostic relevancy by comparing alcohol-dependent patients to social drinkers or abstinent subjects (Anton et al., 2001; Helander et al., 2001; Anttila et al., 2003; Schwan et al., 2004). Two other studies correlated %CDT turbidimetric immunoassay (TIA) values obtained using different analytical systems (Helander, 2002; Schwarz et al., 2003). These studies demonstrated the homogeneity of the results, with ‘r’ values ranging from 0.960 to 0.985, and slopes ranging from 0.98 to 1.11. Using the previous %CDT technique, from the same manufacturer, and studying a population divided in three groups (0–19, 20–29, ≥30 g/day) Carlsson et al. (2003) observed a correlation between %CDT and alcohol consumption. In our study, the progressive rise of %CDT in parallel to alcohol intake is reported, for the first time, using a wider distribution of alcohol intakes. This observation highlights the clinical relevance of the technique that allows a follow-up of unselected populations.

The second objective of this study was to validate the cut-off proposed by the manufacturer. Previous studies had proposed to set the cut-off at 2.5% (Fleming and Mundt, 2004) or 3% (Helander et al., 2001). Our results confirm that the manufacturer’s cut-off is suitable for a diagnostic use, based on the WHO definition for at-risk alcohol consumption. We observed a significant threshold in %CDT values corresponding to alcohol intake at the limit between accepted consumption and at-risk consumption. Figure 1 shows the evolution of %CDT and GGT with alcohol consumption. The narrow distribution of %CDT values compared to GGT in each group may be attributed to the higher specificity of %CDT, whereas GGT can be increased by a number of other factors. This can explain the overlapping of GGT values observed in Figure 1. The question of sex differences for %CDT reference values remains unclear. So far, no study had checked whether or not the same sex difference was observed for %CDT values as was the case with the CDTect method. As shown in Table 1, we observed the absence of sex difference in teetotallers (RG). This result is in agreement with results obtained using a very similar method (Schellenberg et al., 1996) for CDT measurement, and more recent data (Schwan et al., 2004) obtained with this %CDT method. This finding confirms the validity of one unique cut-off value for men and women, as proposed by the manufacturer.

The sensitivity we obtained for heavy drinkers is higher than those previously published (Anttila et al., 2003). Lastly, the specificity that we report here for groups NC and AA (Table 2) is slightly lower than in other studies (Anton et al., 2001). This might result from the inclusion of subjects who stated an alcohol intake at 40 g/day, whereas usual control populations are frequently based on alcohol intake <20 g/day.
CONCLUSION

Despite a huge number of publications dedicated to examine CDT as a bio-marker, its diagnostic merit is still a matter of controversy. We show here that the new %CDT TIA kit provides results reflecting alcohol consumption even for moderate alcohol intakes. In addition, a threshold is observed for %CDT measured in patients with an alcohol intake exceeding the limit defined by the WHO to distinguish hazardous drinkers. This threshold confirms the validity of the cut-off specified by the manufacturer and the absence of sex related difference for %CDT values.

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REFERENCES


