

Determination of Carbohydrate-deficient Transferrin Levels by Using Capillary Electrophoresis in a Korean Population

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Background: Carbohydrate-deficient transferrin (CDT) levels have rarely been determined in an Asian population. We evaluated the analytical performance of a test for measuring CDT levels by using capillary electrophoresis (EP).

Methods: We determined the precision of CDT measurement by using capillary EP and nephelometry and compared the CDT values obtained using both the methods. We included healthy control subjects, abstinent patients with liver disease, and individuals consuming varying amounts of alcohol.

Results: The CDT measurement by using capillary EP were correlated well with those CDT measurement by using nephelometry, N Latex CDT assay, $Y=0.5706X+1.581$, $R=0.930$. The results obtained from both methods showed good qualitative agreement with each other (κ coefficient=0.61). Genetic variants of transferrin isoforms were detected in 4.1% of the tested population. Both the CDT and γ -glutamyl transpeptidase (GGT) levels in the abstinent patients with liver disease were significantly higher than those in healthy abstinent individuals (0.9% vs. 0.5%, 109.5 mg/dL vs. 28.5 mg/dL, respectively), but the difference in CDT values in the 2 groups was less pronounced for the CDT values. Individuals who had a mean daily alcohol intake of more than 60 g/day showed significantly higher CDT levels than those who had a mean daily alcohol intake of less than 60 g/day (1.9% vs. 0.7%, $P=0.03$).

Conclusions: The CDT test using capillary EP showed good performance, and this method has several advantages such as automation and detection of variant forms. Thus, CDT can be a more useful marker than GGT for monitoring alcohol abstinence, especially in patients with liver disease. (*Korean J Lab Med* 2010;30:477-84)

Key Words : CDT, Capillary electrophoresis, Liver disease

INTRODUCTION

Carbohydrate-deficient transferrin (CDT) is reported to be an accurate biomarker for the detection of chronic

alcohol abuse and for monitoring abstinence [1, 2]. Transferrin (Tf) is the most important iron transport protein. Tf is synthesized primarily in the hepatocytes and consists of 3 substructural domains. These domains include a single polypeptide chain, 2 independent metal ion-binding sites, and 2 N-linked complex glycan chains [2]. Tf exists in several glycoforms, which differ in the degree of branching of the N-linked glycan chains. The glycan chains have bi, tri, and tetraantennary structures [2, 3]. The primary Tf isoform is tetrasialo-Tf (64–80%). Other less abundant isoforms found in nonpathologic conditions include pentasialo-Tf, trisialo-Tf, and disialo-Tf

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[2, 4, 5].

Stibler and Kjellin [6] first reported that Tf isoforms, with an isoelectric point (pI) greater than 5.7, were present in alcoholic subjects. These Tf isoforms correspond to asialo-Tf, monosialo-Tf, and disialo-Tf and are collectively referred to as CDT [1, 2]. Sustained alcohol consumption causes a change in the glycoform pattern and an increase in CDT levels. The half-life of CDT is 1-1.5 weeks. Generally, it takes more than 2 weeks of abstinence for CDT levels to return to normal [1, 4, 7, 8]. Measurement of CDT levels has not been routinely performed over the years because traditional methods for measuring CDT levels, such as isoelectric focusing, are laborious and time-consuming. Several commercial immunoassay kits have been developed for the measurement of CDT levels, but the inclusion of trisialo-Tf in the CDT fraction can result in false high CDT values when these tests are used [2, 9, 10]. Recently, new methods, including capillary zone electrophoresis (EP) [11], high-performance liquid chromatography (HPLC) [3, 10, 12], and direct immunoassay using monoclonal antibodies [4] have been developed. These methods are automated and produce more reliable data.

To date, the measurement of CDT levels has not been performed routinely for monitoring chronic alcohol abuse in Korea. To our knowledge, CDT levels have rarely been measured in an Asian population. In this study, we evaluated the analytical performance of a test for measuring CDT levels by using capillary EP (Sebia, Every, France) and determined the cut-off values for CDT in a Korean population.

MATERIALS AND METHODS

1. Patients

To evaluate the relationship between CDT levels and alcohol consumption, we included 45 alcoholic patients who had liver disease (39 men and 6 women, age: 18-71 yr: 35, alcoholic liver disease: cirrhosis or fatty liver or pancreatitis and 10, nonalcoholic liver disease: hepatitis

A or B) and 48 healthy individuals were included in the study. Among the 48 healthy individuals, 42 control subjects (22 men and 20 women, age: 25-55 yr, median age: 40 yr) were selected to validate the cut-off value. All of the 42 individuals were either totally abstinent or consumed not more than 25 g of alcohol per day. None of the patients showed clinical and laboratory evidence of chronic alcohol abuse. To evaluate the effect of liver disease on CDT levels, 28 abstinent patients who had liver disease (13 men and 15 women, median age: 41 yr (range, 26-63 yr); 3, acute hepatitis A; 22, chronic hepatitis B; 2, chronic hepatitis C; and 1, primary biliary cirrhosis) were also enrolled. The diagnosis of liver disease was performed by determining abnormal levels of liver enzymes, serological markers such as hepatitis B virus, hepatitis C virus, and hepatitis A virus, and radiological findings. Information regarding alcohol consumption (average daily consumption of alcohol, duration of alcohol consumption, and date and dose of recent drinking) was collected during an interview. The daily intake of each beverage was expressed in grams of pure ethanol, and mean daily alcohol consumption was calculated using the frequency of drinking and daily intake of each drinking event. In order to analyze the relationship between CDT and alcohol consumption, we excluded patients who had genetic variants of the Tf isoforms. We also excluded patients who gave incomplete information regarding their alcohol habit or if the last day they had a drink before more than 2 weeks. This study was approved by the Institutional Review Board of Konkuk University Medical Center (Seoul, Korea).

2. Determination of CDT levels

1) Capillary electrophoresis

In a capillary zone electrophoresis system (Sebia), the charged molecules are separated on the basis of their electrophoretic mobility in an alkaline buffer, according to pH and electro-osmotic flow. Transferrin glycoforms are directly detected at 200 nm from the cathode end of the capillary. Using a basic pH buffer, transferrin iso-

forms are detected in the following order: asialo-Tf, disialo-Tf, trisialo-Tf, tetrasialo-Tf, and pentasialo-Tf. CDT quantification includes di-Tf and asialo-Tf. Procedures were performed according to the manufacturer's instructions. Samples were first automatically diluted with an iron saturation solution and then aspirated and subjected to EP.

2) Nephelometric measurement of CDT levels

We measured CDT levels using an N Latex CDT assay (Dade Behring, Marburg, Germany). This is a direct method and is based on the direct nephelometric measurement of disialo-Tf and asialo-Tf by using specific antibodies. We used a BN II nephelometer system (Dade Behring, Marburg, Germany) for this assay, with a reference interval of 1.19–2.47%.

3. Performance of the capillary electrophoresis method for measuring CDT levels

1) Precision

Intra-run precision and total precision were determined as described in the CLSI protocol EP5-A2 using control material [13]. Each control was analyzed in duplicate over 5 days with 2 runs.

2) Comparison between the two methods

In total, 40 samples covering a wide range of CDT values were analyzed using the capillary EP and nephelometric methods. The values from the 2 methods were compared using Pearson's correlation coefficient for regression analysis.

4. Statistics

Relationships between the variables were analyzed using Student's *t*-test and Mann-Whitney test for continuous variables. Pearson's correlation and Bland-Altman plots were used for the comparison studies. Agreement between the tests was assessed using the κ coefficient as follows: κ coefficient greater than 0.81 was con-

sidered as very good agreement; 0.6–0.8, good agreement; 0.41–0.6, moderate agreement; 0.21–0.4, fair agreement; and κ coefficient less than 0.4, poor agreement. Statistical analysis was performed using SPSS software, version 12.0 (SPSS Inc., Chicago, IL, USA) and MedCalc Statistical software 9.3.9.0 (Mariakerke, Belgium). *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

1. Precision

The precision within each run had a low of 6.9% (normal control, mean value 0.51%) and high of 1.8% (abnormal control, mean value 6.96%). The total precisions of normal and abnormal control were 6.6% and 4.5%, respectively (Table 1).

2. Comparison of CDT levels measured by capillary EP and those measured by nephelometry

The CDT values measured using capillary EP correlated well with those obtained by the nephelometric N Latex CDT assay ($Y=0.5706X+1.581$; $R=0.930$, Fig. 1). The %CDT levels by capillary EP were generally lower than those obtained by the nephelometric N Latex CDT assay. The mean bias (capillary EP–nephelometric N Latex CDT assay) was -0.9 (SD, 1.0; 95% CI, -3.0–1.2), with a median (range) difference of -1.1 (-2.59–3.88). Qualitative agreement between the 2 methods was 82.8%, and the 2 methods showed good agreement (κ coefficient=0.61; 95% CI, 0.32–0.89).

Table 1. Precision of %CDT using capillary electrophoresis

Analyte	Mean (%)	Within-run precision SD (CV, %)	Total precision SD (CV, %)
Normal control	0.51	0.04 (6.9)	0.03 (6.6)
Abnormal control	6.96	0.13 (1.8)	0.31 (4.5)

Abbreviation: CDT, carbohydrate-deficient transferrin.

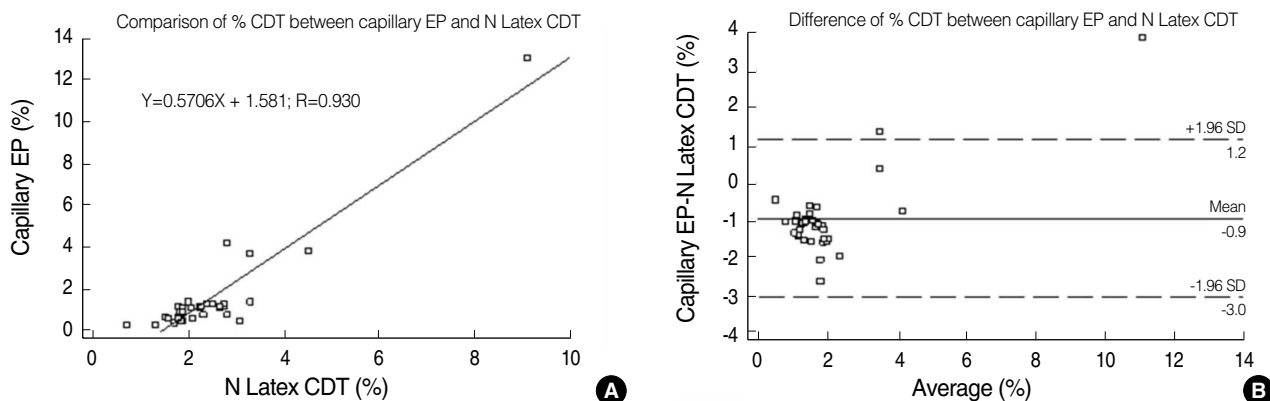


Fig. 1. Correlation (A) and difference (B) between %CDT levels by capillary electrophoresis and N Latex CDT method. Abbreviation: CDT, carbohydrate-deficient transferrin.

3. Frequency of genetic variants of the transferrin isoforms

Among the 121 subjects, five individuals (4.1%) had genetic variants of the transferrin isoforms (B and D variants). CDT values can be calculated in these cases, since approximately twice the CDT peak of 1 isoform can correctly estimate the masked CDT peak of the other isoform.

4. Validation of the reference interval and baseline CDT levels in abstinent individuals

None of the 42 healthy control subjects (men, 22 and women, 20) had a CDT level above the cut-off value that was provided by manufacturer (1.3%). The average CDT value for the healthy subjects was 0.52% (SD, 0.18; median, 0.5; range, 0.3-1.0). The CDT levels in men (mean, 0.57; SD, 0.19) were significantly higher than those in women (mean, 0.46; SD, 0.15; $P=0.02$).

5. Comparison of CDT and γ -glutamyl transpeptidase levels in abstinent patients with liver disease and those in healthy controls

Both CDT and γ -glutamyl transpeptidase (GGT) levels were significantly higher in abstinent patients with liver disease than in healthy abstinent individuals (0.9% vs. 0.5%, P value=0.046; 109.5 mg/dL vs. 28.5 mg/dL, P value <0.001, respectively) (Table 2, Fig. 2). However, the dif-

Table 2. Distribution of %CDT using capillary electrophoresis and GGT in healthy abstinent individuals and abstinent patients with liver disease

	Healthy abstinent individuals (N=42)	Abstinent liver disease (N=28)	P value
%CDT			
Mean (SD)	0.5 (0.2)	0.9 (0.7)	0.002
Positive rate	0/42 (0.0%)	1/28 (3.5%)	0.217
GGT (mg/dL)			
Mean (SD)	28.5 (15.1)	109.5 (75.7)	<0.001
Positive rate	0/42 (0.0%)	8/27 (29.6%)	<0.001

Abbreviations: CDT, carbohydrate-deficient transferrin; GGT, γ -glutamyl transpeptidase.

ference between the 2 groups was more pronounced for the GGT values. Moreover, the positive rate of GGT, according to the cut-off, was significantly higher in the abstinent patients with liver disease than in healthy individuals ($P<0.001$). In contrast, the positive rate of CDT was very low in both groups (Table 2).

6. CDT levels in relation to alcohol consumption

The amount of daily alcohol intake significantly affected CDT levels. The distributions for the CDT and GGT levels according to mean daily alcohol consumption are presented in Table 3. Individuals who had a mean daily alcohol intake of more than 60 g/day showed significantly higher CDT levels than those who consumed less than 60 g/day ($P=0.034$). CDT values were lower in groups who had a longer duration of alcohol abstinence, although this finding was statistically insignificant (Table 4).

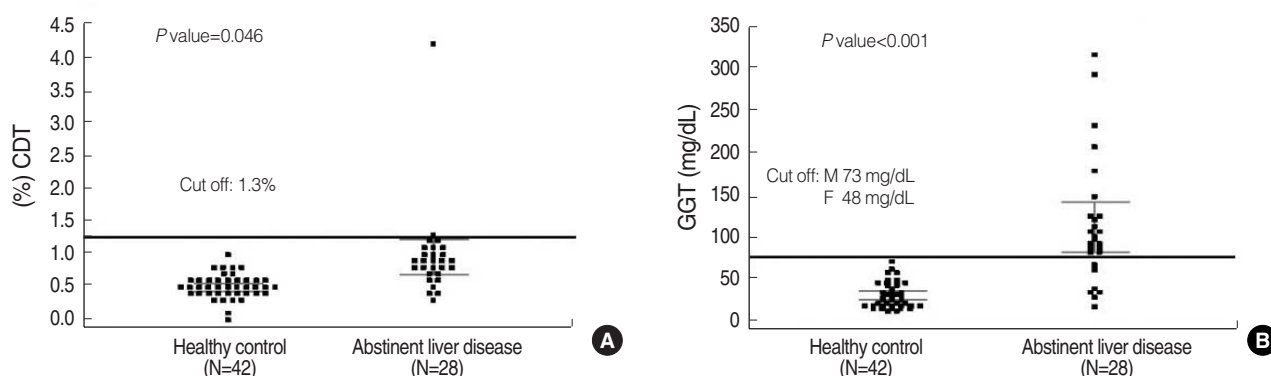


Fig. 2. Distribution of %CDT using capillary electrophoresis (A) and GGT (B) in healthy abstinent individuals and abstinent patients with liver disease.

Abbreviations: CDT, carbohydrate-deficient transferrin; GGT, γ -glutamyl transpeptidase.

Table 3. Distribution of %CDT values according to mean alcohol consumption

Alcohol consumption (g/day)	N of subjects (N=83)	%CDT	
		Mean (SD)	Median (range)
≤ 30	46	0.5 (0.2)	0.5 (0.0-1.0)
31-60	8	1.2 (0.5)	1.0 (0.8-2.1)
61-120	15	1.2 (0.9)	1.1 (0.3-3.8)
>120	14	2.1 (3.2)	1.2 (0.6-13.0)
≤ 60	54	0.6 (0.4)	0.6 (0.0-2.1)
>60	29	1.7 (2.4)	1.2 (0.3-13.0)
		* $P=0.034$	

*Statistical analysis was performed between two groups according to the mean alcohol consumption, ≤ 60 g/day vs. >60 g/day.

Abbreviation: CDT, carbohydrate-deficient transferrin.

7. Sensitivities and specificities of CDT using capillary EP

Receive operating characteristic (ROC) curves and sensitivities and specificities of CDT measurement using capillary EP at different cut-off limits for each mean alcohol consumption value are shown in Table 5 and Fig. 3. The area under the ROC curves (AUC) was larger for mean alcohol consumption detection, which was greater than or equal to 40 g/day, than smaller volumes. The sensitivities and specificities were best at the cut-off limit of 0.75–0.95%, depending on the different mean alcohol doses.

DISCUSSION

Methods to determine CDT levels, such as isoelectric focusing, anion-exchange chromatography, and HPLC,

Table 4. Distribution of %CDT values according to number of abstinent days

Abstinent day	N of patients	%CDT		P value
		Mean (SD)	Median (range)	
≤ 3 d	20	2.0 (2.7)	1.1 (0.5-13.0)	0.110*
≤ 7 d	12	1.0 (0.4)	1.0 (0.3-1.4)	0.314 [†]
>7 d	12	0.9 (0.3)	1.1 (0.5-1.3)	

Statistical analysis was performed for comparison of number of abstinent day * ≤ 3 d vs. >3 d and [†] ≤ 7 d vs. >7 d.

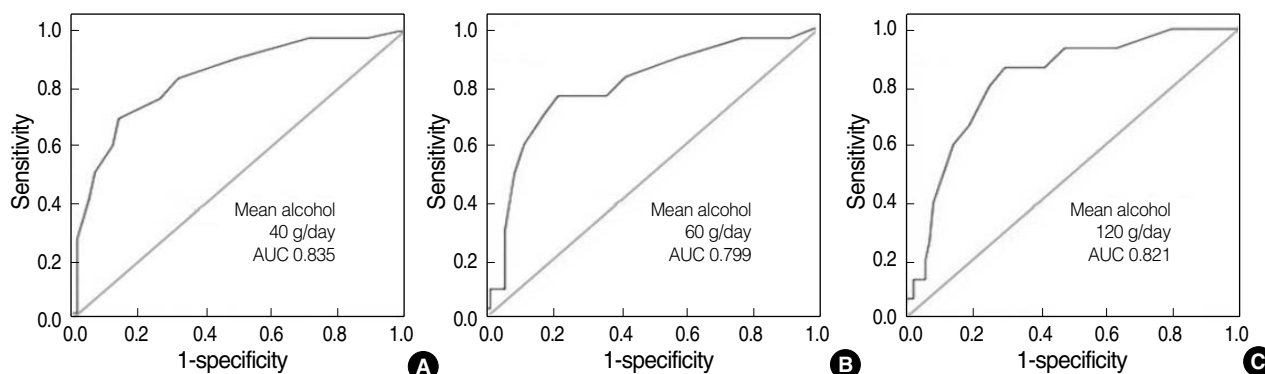
Abbreviation: CDT, carbohydrate-deficient transferrin.

are complex, time-consuming, and laborious [1, 8, 14]. Capillary EP for CDT testing is fully automated, time-saving, and adaptable for use with all of the transferrin glycoforms. In the present study, total precision for capillary EP (6.6% and 4.5% at the low and high levels of CDT, respectively) was better than that of other methods [14–16]. The results were comparable to those of other studies that used capillary EP [11, 14, 17, 18]. CDT results from the capillary EP method correlated well with those from the nephelometric N Latex CDT assay, another available routine method ($R=0.930$). Inclusion of more samples with high CDT levels would have yielded a higher correlation. Although CDT values from the nephelometric assay were higher than those from capillary EP, the qualitative agreement of the two methods, according to the cut-off (83.3%), was acceptable. At least 38 genetic variants of human Tf cause amino acid substitutions [2, 19]. Most individuals express allele C. Other common allele types are the anodal B and cathodal D alleles. The BC or CD variants may cause abnormal CDT results.

Table 5. Sensitivities and specificities of %CDT using capillary electrophoresis at different cut-off limits for detecting each amount of mean alcohol consumptions (N=83)

%CDT cut off	Target amount of mean alcohol consumption					
	40 g/day or more		60 g/day or more		120 g/day or more	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
0.75	76.7%	73.2%	76.7%	63.8%	86.7%	63.3%
0.85	69.8%	85.7%	76.7%	78.3%	86.7%	70.2%
0.95	60.5%	75.0%	70.0%	82.6%	80.0%	75.0%

Abbreviation: CDT, carbohydrate-deficient transferrin.

**Fig. 3.** Receiver operating characteristic curves of %CDT using capillary electrophoresis for detection of alcohol consumptions according to each target amount of daily alcohol consumption (A: mean alcohol 40 g/day or more, B: 60 g/day or more, C: 120 g/day or more, N=83). Abbreviation: AUC, area under ROC curves.

These variants can be detected using capillary EP, but the measured CDT levels would be inaccurate. The rate of genetic variations is low in Caucasians (<1%) but is reported to be high in African-Americans, Black Africans, and natives of South America [14, 19]. In our study, 5 of 121 individuals (4.1%) had a genetic variant of the Tf isoforms (BC or CD variant).

Although CDT tests are known to have the highest specificity for alcohol abuse, as compared to other laboratory tests such as GGT, the reported sensitivities and specificities of CDT ranged broadly [18, 20]. Sensitivities were reported to vary from <20% to 100%. Specificities varied from 75% to 100%. This variability was due to differences in the populations, CDT methods, CDT cut-off points, and definitions of alcohol abuse. The sensitivity and specificity were higher in studies that focused on completely contrasting groups, such as alcohol abstainers and heavy alcohol drinkers, but were much lower in clinical settings that included many patients who had liver disease and were moderate alcohol drinkers [18, 20, 21].

In the present study, we evaluated the performance of CDT testing using capillary EP in a population of patients who were alcoholic, had non-alcoholic liver disease, or consumed various amounts of alcohol. The mean daily alcohol consumption positively affected the CDT level significantly. However, these results required validation with a more controlled, prospective study, including many samples with high values. The results in this study included a few samples with high results, thus statistical significance was not high. One individual with very high %CDT (13.0%) was a patient with alcoholic liver cirrhosis. His mean alcohol intake was 160 g/day and recent alcohol intake was 160 g 2 days before blood collection for CDT test, therefore, this high value was considered a compatible result.

CDT values seemed to be affected by the duration of abstinence, although this relationship was statistically insignificant. Many conditions can relate to CDT values. These conditions include drinking patterns, amount of daily alcohol consumption, amount of alcohol consumed

per drinking session, the duration of abstinence before blood sampling, and body weight. In this study, we could not accurately assess drinking habits because our assessment was based on a retrospective interview. However, prospective experiments of long-term drinking may create ethical problems and such studies are difficult to perform [2, 19, 22]. Many studies have reported an increase in CDT levels in patients who have liver disease [19, 23, 24]. In those studies, CDT levels of up to 40% of patients with end-stage liver disease were increased. Evaluation of CDT levels in patients who have liver disease is important, because in Korea, many heavy alcohol drinkers have alcoholic liver disease. We evaluated CDT and GGT levels in abstinent patients who had various liver diseases. Both CDT and GGT values were significantly higher in abstinent patients with liver disease than in healthy abstinent individuals. Different mechanisms may be involved in the increase of GGT and CDT levels. The elevation of CDT levels in patients who have liver disease may be related to changes in Tf levels and variations in the rate of glycosylation [19]. However, in patients who have liver disease, the CDT levels increased to a lesser extent than GGT levels. In addition, the positive rate of CDT, according to the cut-off value of 1.3%, was very low in patients who had liver disease in contrast to GGT. Thus, CDT can be more useful than GGT for monitoring alcohol consumption in patients who have liver disease. Using a ROC curve, the appropriate cut-off and best sensitivities and specificities can be estimated. The AUC was larger for the detection of mean alcohol consumptions of 40 g/day or more than 60 or 120 g/day. The sensitivities and specificities in the present study were comparable to studies which included moderate drinkers [3, 21], but were lower than studies that included extreme groups [18]. The sensitivity and specificity were best at the cut-off limit of 0.75–0.95%. This was lower than the suggested cut-off limit of 1.3%. As previously mentioned, a cut-off of 1.3% may be suitable if many patients with liver disease are included. A lower cut-off would increase sensitivity, which is useful for screening or detection of alcohol consumption. Many

factors are considered in determining the cut-off. These include the characteristics of the sample population, detection dose, and purpose of the CDT test. To avoid the numerous biases that can be made during the biochemical diagnosis of alcoholism, evaluating CDT with other biomarkers has been recommended [18, 20].

In summary, the capillary EP method for measurement of CDT levels showed good performance and had several advantages. CDT can be a more useful marker than GGT for monitoring abstinence, particularly in patients who have liver disease. Further studies are required to elucidate pre-analytic variables, determine cut-off values under various conditions, and standardize the assays before this test can be routinely used.

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