BRIEF REPORT

The characteristics of transferrin variants by carbohydrate-deficient transferrin tests using capillary zone electrophoresis

Gilsung Yoo | Juwon Kim | Kap Joon Yoon | Jong-Han Lee

Departments of Laboratory
Medicine, Yonsei University Wonju College
of Medicine, Wonju, Korea

Correspondence

Jong-Han Lee, Department of Laboratory
Medicine, Yonsei University Wonju College
of Medicine, Wonju-si, Korea.
Email: cello425@yonsei.ac.kr

Background: Transferrin is the major plasma transport protein for iron. We aimed to investigate the characteristics of transferrin variant by carbohydrate-deficient transferrin (CDT) test using capillary zone electrophoresis.

Methods: We retrospectively analyzed the CDT tests of 2449 patients from March 2009 to May 2017 at a tertiary hospital in Korea. CDT was quantified using a Capillarys 2 system (Sebia, Lisses, France) by capillary zone electrophoresis. The characteristics of variant transferrin patterns using electropherogram of CDT tests were analyzed.

Results: Seventy-seven (3.1%) patients were classified as variant transferrin. Mean age of these patients was 51.8 years, and the male-to-female ratio was 3.5:1. The most common variants were the BC variants (n = 37), followed by the CD variants (n = 27), unclear patterns (n = 7), BD variants (n = 3), CC variants (n = 2), misclassification (n = 1). In the variant Tf group, the most common disease was alcoholic liver cirrhosis (n = 22, 28.6%), followed by the toxic effects of substances (n = 17, 22.1%), and mental and behavioral disorders attributable to alcohol (n = 11, 14.3%). Nonvariant group showed a predominance of the toxic substance effects (n = 880, 37.1%), a personal history of suicide attempts (n = 634, 26.7%), and mental and behavioral disorders due to alcohol (n = 336, 14.2%).

Conclusion: We analyzed the basic characteristics of variant transferrin by CDT tests using capillary zone electrophoresis. The prevalence of variant transferrin was 3.1% of the study subjects. Male patients, alcohol abusers, and liver cirrhosis patients predominated in the variant transferrin population. Further prospective studies are warranted to elucidate variant transferrin in clinical practice.

KEYWORDS

alcohol abuse, capillary zone electrophoresis, carbohydrate-deficient transferrin (CDT), liver cirrhosis, variant transferrin

1 | INTRODUCTION

Herein, we described the characteristics of transferrin (Tf) compositional variants as determined by carbohydrate-deficient transferrin (CDT) tests. Tf is an iron-transporting glycoprotein with two iron binding sites and two N-linked carbohydrate chains harboring N-acetylglucosamine, mannose, galactose, and terminal sialic acid residues.¹ Variations in the number of terminal sialic acid

residues influence the electrophoretic mobility of the Tf molecule and thus permit analytic separation of the different isoforms.² Among the human Tf isoforms, which vary from asialo-Tf and octasialo-Tf, the most common distribution observed in nonalcohol drinkers is tetra-Tf (79.9%), followed by pentasialo-Tf (14.0%), trisialo-Tf (4.02%), disialo-Tf (1.14%), and hexasialo-Tf (0.73%) in order.^{3,4} In contrast, chronic alcohol abusers show an increase in the disialo-Tf and asialo-Tf fractions, which constitute the so-called

carbohydrate-deficient transferrin (CDT). The CDT has been used as a marker of chronic alcohol abuse.⁵ Although it is not fully understood why chronic alcohol consumption increases CDT, it might be that the ethanol alters glycoprotein metabolism, particularly in terms of protein transport and enzyme activity.⁶ Different analytical methods have been developed and employed to quantify CDT in human serum, these include isoelectric focusing followed by immunofixation, anion exchange chromatography fractionation followed by turbidimetry, HPLC, direct nephelometry, and capillary zone electrophoresis (CZE).⁷ Of these methods, HPLC and CZE can perform the separation and quantification of the full spectrum of Tf isoforms and allow changes in Tf glycosylation due to acquired liver disease or congenital disorders of glycosylation (CDG) and various genetic Tf variants to be detected.⁸ Of the three main Tf variant groups, that is, Tf-B, Tf-C, and Tf-D, Tf-C encompasses 16 distinct subtypes and is the most common phenotype in all populations.¹ However, the majority of previous studies on Tf variants have been conducted in Western populations and relatively few have been conducted in Asia. Therefore, we examined the characteristics of Tf variants in Korean through CDT tests.

2 | MATERIALS AND METHODS

All the results of the CDT assay along with the corresponding electronic medical records of patients in Wonju Severance

Christian Hospital, tertiary teaching hospital were collected retrospectively from March 2009 to May 2017. The data collected included the basic demographics, the diagnosis by the physician and the laboratory values, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet, total bilirubin, international normalized ratio of prothrombin time (INR), creatinine, and sodium.

This study was approved by the institutional review board of Yonsei University Wonju College of Medicine (Approval no. CR317321).

Carbohydrate-deficient transferrin was tested by a Capillarys 2 system (Sebia, Lys, France) according to the manufacturer's instructions. Briefly, the Capillarys 2 systems use the principle of capillary zone electrophoresis in free solution. Charged molecules are separated according to the electrolyte pH and electroosmotic flow. The separation is proceeded on their electrophoretic mobility at a specific pH in an alkaline buffer.

The CDT profile is identified as a variant Tf if two criteria are met: There are two peaks of similar optical density on the profile and the distance between these two peaks does not exceed the threshold.

To compare the parameter between variant group and nonvariant group, an independent sample t test was performed for quantitative variables and chi-squared test and Fisher's exact test were performed for the categorical data. A *P*-value < .05 was considered statistically significant. All statistical analyses were performed using SPSS, version 23 (IBM SPSS, Chicago, IL, USA).

	Variant, N = 77	Nonvariant, N = 2372	<i>P</i> *
Gender (n, %)			
Male	60 (77.9)	1514 (63.8)	.016^a
Female	17 (22.1)	858 (36.2)	
Age (mean ± SD, range)	51.8 ± 15.1 (24-88)	49.9 ± 15.7 (12-93)	.287 ^b
CDT% (mean ± SD)	1.7 ± 1.9	1.7 ± 2.3	.915 ^b
Alcohol abuser (n, %)	44 (57.1)	732 (30.9)	<.001^a
Liver disease (n, %)			
Hepatitis	11 (14.3)	238 (10.0)	.306 ^a
Cirrhosis	33 (42.9)	443 (18.7)	<.001^a
Hepatocellular carcinoma	4 (5.2)	84 (3.5)	.356 ^c
	Mean ± SD)	(Mean ± SD)	
AST (U/L)	180 ± 274.8 (n = 33)	95 ± 564.6 (n = 1283)	.387 ^b
ALT (U/L)	67 ± 104.5 (n = 52)	81 ± 361.3 (n = 1775)	.786 ^b
Platelet (10 ⁹ /L)	171 ± 113.6 (n = 62)	217 ± 102.3 (n = 2027)	.002^b
Total bilirubin (mg/dL)	5.3 ± 8.59 (n = 26)	1.3 ± 2.86 (n = 987)	.027^b
INR	1.3 ± 0.67 (n = 41)	1.1 ± 0.66 (n = 1483)	.038^b
Cr (mg/dL)	0.8 ± 0.12 (n = 11)	1.2 ± 5.74 (n = 442)	.808 ^b
Na (mmol/L)	133 ± 24.9 (n = 62)	130 ± 30.9 (n = 1884)	.294 ^b

^aAnalyzed by chi-squared test

^bAnalyzed by unpaired *t* test

^cAnalyzed by Fisher's exact test.

*The *P*-values in bold are statistically significant at *P* < .05.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CDT, carbohydrate-deficient transferrin; INR, international normalized ratio of prothrombin time.

TABLE 1 Baseline characteristics of study population

3 | RESULTS

A total of 2449 CDT results of patients were analyzed. Among them, a total of 77 (3.1%) patients were classified as the Tf variant using Capillarys 2 software, as shown in Table 1. The mean age of the Tf variants group (51.8 ± 15.1 years) was not statistically different from the nonvariant group (49.9 ± 15.7 years) ($P = .287$). The proportion of males was significantly higher in the variant group than the nonvariant group (77.9% vs. 63.8%, $P = .016$). Interestingly, the incidence of alcohol abuse (57.1% vs. 30.9%, $P < .001$) and liver cirrhosis (42.9% vs. 18.7%, $P < .001$) was relatively higher in the variant group than in the nonvariant group. The variant group showed higher total bilirubin ($P = .027$) and INR levels ($P = .038$), but lower platelet counts ($P = .002$) than those of nonvariant group.

Each variant Tf case could be classified as a specific genetic variant based on the shifting in the Tf peak compared with the CC homozygote, which is the most common phenotype in all populations considered as normal pattern. In other words, variant Tf-B has lower isoelectric point (pI) values and migrates to the anode compared to variant Tf-C, whereas the variant Tf-D has higher pI values

and cathodic migration than variant Tf-C.¹ In addition, each variant Tf was assumed to be composed of two alleles showing similar amounts. Of the 77 variant Tfs (60 males and 17 females), the most common variants were the BC variants ($n = 37$), followed by the CD variants ($n = 27$), BD variants ($n = 3$), and CC variants ($n = 2$), except for seven unclear patterns (Figure. 1). The remaining one patient showed no Tf peak shift, suggesting a nonvariant (CC homozygote), whereas Capillarys 2 software assigned the patient as a variant case. However, this patient did not show variant Tf in CDT test 2 months ago. This discordance might be caused by the disialo-Tf- trisialo-Tf bridging phenomenon, which is observed frequently in liver cirrhosis patients.⁹ Indeed, this patient had a history of hepatitis B virus-related cirrhosis, which was believed to be the reason for this misidentification as a variant Tf.

In the variant Tf group, the most common disease was alcoholic liver cirrhosis ($n = 22$, 28.6%), followed by the toxic effects of substances ($n = 17$, 22.1%), and mental and behavioral disorders attributable to alcohol ($n = 11$, 14.3%). In contrast, the nonvariant group showed a predominance of the toxic substance effects ($n = 880$, 37.1%), a personal history of suicide attempts

FIGURE 1 The electropherogram of normal and various variant transferrin patterns. (X-axis, migration point; Y-axis, relative optical density %) A, Transferrin (Tf)-CC homozygote (normal) pattern. B, Tf-CC heterozygote pattern ($n = 2$). C, Tf-BC pattern ($n = 37$). D, Tf-BD pattern ($n = 3$). E, Tf-CD pattern ($n = 27$). F, Unclear pattern ($n = 7$)

(n = 634, 26.7%), and mental and behavioral disorders due to alcohol (n = 336, 14.2%).

4 | DISCUSSION

Monitoring of Tf isoforms is important in clinical and forensic purpose for the determination of carbohydrate-deficient transferrin (CDT) and the assessment congenital disorders of glycosylation.¹⁰ In the present study, we described the characteristics of variant Tf by CDT tests by capillary zone electrophoresis. The incidence of variant Tf was about 3.1%. Also, we presented the clinical differences of variant Tf group and nonvariant Tf group. Although some biases could exist due to many CDT tests requested when drunken patients visited the emergency room, the differences between Tf variant group and non-Tf variant group should be more considered.

Several studies have suggested the associations between some Tf variants and various disease entities. For example, Zambenedetti et al¹¹ showed that the Tf C2 allele was associated significantly with Alzheimer's disease in APOE4-negative subjects. Sikström et al¹² reported that the Tf-C3 variant confers a protective effect on smoking-related (squamous and small cell) types of lung cancer. In a Croatian study including 153 schizophrenia and 23 schizoaffective disorder patients, the TF-C2 variants were not high-risk genetic variants for schizophrenia/schizoaffective disorder.¹³ Although existing studies have suggested various meaning of variant Tfs, that the potential mechanism of variant Tf might be explained by the altered iron metabolism of variant Tf or other associated genetic factors regarding to linkage disequilibrium.¹⁴ Therefore, when the variant Tf is detected or the patient is suspicious of congenital disorders of glycosylation (CDG), the patient should be considered to refer additional specialized tests, usually genetic testing which allows to identify the enzyme whose deficiency leads to CDG or atransferrinemia. Recently, the capillary electrophoresis methods such as capillary zone electrophoresis for the monitoring of CDT or capillary isoelectric focusing have been represented as effective tools to assess unusual transferrin patterns, including genetic variants.¹⁰

The present study had some limitations. Because the CDT results were collected retrospectively, a detailed history of the drinking habits and the unconsidered medical history in the electronic medical records could not be fully analyzed. In addition, the possible misclassifications of Tf variants could not be thoroughly excluded because each variant was assigned based on the shape of the electropherogram without genetic analysis. However, we surveyed the overall characteristics of variant Tf by CDT test using capillary zone electrophoresis. This approach might be helpful to screen the rare genetic disorder of glycosylation.

In conclusion, we reported the incidence of variant Tf was approximately 3.1% in Gangwon Province, South Korea. The most common pattern of variant Tf was BC variant pattern, followed by CD variant and BD variant pattern. The percentage of male patients, alcohol abusers, and liver cirrhosis patients were higher in the variant Tf group than those of nonvariant Tf group. Further prospective studies will be warranted to elucidate the clinical meaning of variant Tf.

ORCID

Jong-Han Lee <http://orcid.org/0000-0003-4036-8443>

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