Forensic Aspects of Postmortem Serum Carbohydrate-Deficient Transferrin Analysis as a Marker of Alcohol Abuse

Vesna Popović[1], Tatjana Atanasijević[1], Slobodan Nikolić[1], Nataša Božić[2], Zoran Vujčić[3], Jelena Micić-Labudović[1]

[1]Institute of Forensic Medicine, University of Belgrade – School of Medicine, Belgrade, Serbia;
[2]Centre of Chemistry – IHTM, Belgrade, Serbia;
[3]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

SUMMARY

Introduction Carbohydrate-deficient transferrin (CDT) has been suggested as one of alcohol abuse indicators having produced good results in forensic medicine for years.

Objective The aim of the study was to identify correlation between present methodology of alcohol abuse diagnosis at autopsy (macroscopic and microscopic findings) and CDT examination using the method of isoelectrofocusing (IEF) in polyacrylamide gel electrophoresis (PAGE). We also analyzed if the time interval between the moment of death and blood sample collection influences CDT findings.

Methods The method used for CDT analysis was IEF-PAGE. Sera of 49 males and 11 females aged 14-87 years, average age 46.85±18.53, were used in this study. Control group consisted of five patients who died after medical treatment that lasted longer than 15 days, and five patients who started Disulfiram therapy in controlled hospital environment.

Results The results obtained in CDT examination in dead bodies' sera showed sensitivity 59% and specificity 71%. A high incidence of falsely positive CDT result was noticed in liver failure and cirrhosis of non-alcoholic origin. CDT analysis is also possible to be done in samples collected postmortem up to 76 hours.

Conclusion In forensic medicine, the method of CDT determination is reliable for the diagnosis of alcohol abuse.

Keywords: alcoholism; carbohydrate-deficient transferrin (CDT); postmortem analysis; specificity; sensitivity

INTRODUCTION

Pathological changes caused by chronic alcohol abuse could considerably contribute to the cause of death and, on the other hand, changes in psychic sphere of an alcoholic could initiate accidents, homicides and suicides. Furthermore, withdrawal-related death is an entity that must be considered in cases of sudden death in the alcoholic [1, 2].

Changes related to chronic alcohol abuse occur either indirectly, due to alcohol abusers accidental falls (prominent body part injuries, chronic subdural hematoma, etc.) or directly due to toxic effect of alcohol as well as its highly reactive metabolite – acetaldehyde. In postmortem diagnosis, the traditional methods used to identify alcoholism are autopsy findings, histological examination, medical records, heteroanamnestic data and blood alcohol concentrations. Alcohol related pathological changes are nonspecific and they could be found in numerous diseases. The same changes of the liver could be found in obesity, defects in fat metabolism, various kinds of hepatitis, hypoxic damage of hepatocytes, as well as toxic liver damage (chlorophorm, arsenic, carbo-tetrachlorid, bacterial toxins, etc). Because of relatively non-specific pathological features, the difficulty in interpreting blood alcohol levels for the purpose of an alcoholic and the lack of valid heteroanamnestic records, it is necessary to establish a reliable biological marker for alcoholism identification in forensic practice [3].

Various biological markers such as methanol, phosphatidylethanol or fatty acid ethyl esters (FAEE) are found to be usable even on dead body material [4, 5]. Carbohydrate-deficient transferrin (CDT) has been suggested as one of alcohol abuse indicators. Experience with CDT on dead body material is still humble [1, 2, 3, 6-9].

Serum trasfferin (Tf) occurs in at least seven isoforms: hexa-, penta-, tetra-, tri-, di-, mono- and asialo-trasferrin with different pIs (isoelectronic points). In healthy person, the tetrasialo isoform accounts for about 80% of all circulating transferrin. The term CDT refers to isoforms with pIs≥5.7, i.e. asialo-Tf, monosialo-Tf and disialo-Tf [8]. CDT increases to above normal values after more that 50-80 g (males) and 40 g (females) of alcohol daily intake and for the period longer than one and up to four weeks [4, 9-12]. After a few days, CDT values show a fast increase in the serum of the alcohol abuser, to decrease towards normal values after the discontinuation of alcohol intake [13, 14, 15]. During abstinence, CDT serum concentration returns to normal values again after one

Correspondence to:
Vesna POPOVIĆ
Institute of Forensic Medicine
School of Medicine
Deligradska 31a, 11000 Belgrade
Serbia
gatanasijevic@sbb.rs

and a half to two weeks [10, 15-18]. Raynard et al. [19] stated that the CDT half-time is 17±4 days.

Transferrin itself is glycoprotein stable at room temperature, resistant to spontaneous and microbiological decomposition [20]. These features are desirable in forensic work because it enables analysis even after longer postmortem time.

OBJECTIVE

The aim of the study was to identify correlation between the present methodology of alcohol abuse in the diagnostics of dead bodies (macroscopic and microscopic findings) and CDT examination using the method of isoelectrofocusing (IEF) in polyacrilamid gel electrophoresis (PAGE) with samples prepared by Rivanol according to Sagan [21]. At the same time, the application of IEF-PAGE method in forensic work was also evaluated. We examined if the time interval between the moment of death and blood sample collection influences CDT findings.

METHODS

In this study 60 dead bodies were initially examined at the Institute of Forensic Medicine of the Belgrade School of Medicine. Since the records of one case were not available, a total of 59 dead bodies were analyzed (47 males and 12 females) aged 14-87 years.

For each subject the data about age, sex and alcohol abuse were collected as heteroanamnestic data from the closest relative, or from medical records and/or police reports. For 10 of 57 subjects it was proven that they have not consumed alcohol during past 15 days. This group was assigned as a control group and included five patients treated at the Emergency Center due to trauma, and who died after medical treatment that lasted longer than 15 days, but were without pathoanatomic findings or anamnestic alcohol abuse data, and five patients who started Disulfiram therapy in controlled, hospital environment.

The cases were subjected to postmortem macroscopic and microscopic analysis with a special attention to the changes specific for alcoholism such as fatty liver, cirrhosis or hepatitis, atrophy and fibrosis of the pancreas, brain atrophy and fibrosis and hypertrophy of the heart [1].

For measurement of alcohol and CDT concentration, a total of 57 blood samples were drawn from the femoral vein during autopsy. Alcoholemia was established using the standard method of gas chromatography. The sera were derived by centrifugation in a very short period of time after collecting (up to 1 hour), then stored at -20°C. CDT concentration was analyzed by IEF-PAGE. It is well known that by using IEF (as well as capillary electrophoresis and HPLC), the fractions of asialo- and monosialo-transferin (Tf) are not identified in the serum of healthy subjects. This makes this technique adequate for CDT analysis [8].

Transferrin standard was made of serum pool of blood donors.

The data obtained were analyzed by SPSS software (average values, mediana, modus, χ^2), while specificity and sensitivity were used for evaluating the model.

RESULTS

Fifty-nine subjects were included in the study with complete data (47 male and 12 female), of the average age 46.85±18.53 years; the youngest was 14 and the oldest 87. The average age of males and females was 47.96±18.81 and 42.05±17.51, respectively (t=1.016; p>0.10).

In 38 cases blood samples were collected in the interval shorter than 36 hours postmortem, while 19 blood samples were collected after that period (the longest interval was 76 hours). In 24 analyzed cases CDT values were positive; in 18 cases up to 36 hours postmortem and in 6 cases after 36 hours. There was no significant difference between these groups (χ^2=1.29, p>05).

In 22 of 31 subjects for whom it was undoubtedly stated that they had not consumed alcohol in the last 15 days of life CDT was not found. CDT was found in 14 of 26 subjects who had consumed alcohol in the last 15 days of life (χ^2=5.342, p>05) (Table 1). This shows that the presence of CDT in the analyzed sample was not accidental but was the result of drinking habits. According to these results, it is concluded that the sensitivity of CDT analysis was 59%, and specificity 71%.

The presence of alcohol was found in 10 subjects; alcoholemia was above 1‰ in three subjects (which means that they must have taken over 70 g of alcohol) while CDT was positive. All this allows the conclusion that they were chronic alcoholics. In three of seven other subjects who had alcoholemia lower than 1‰, CDT was also positive.

In our analyzed sample, 12 subjects had severe liver disease and in eight subjects CDT was positive (three subjects were drunk having 1-2.55‰ of alcohol).

Table 1. Number of nonconsumers and consumers that were CDT positive, had liver disease on autopsy and increased alcohol concentration of over 1‰

Parameter	Nonconsumers (n=31)		Consumers
	Control group (n=10)	Heteroanamnestic data (n=21)	Heteroanamnestic data (n=26)
CDT positive	0	9	14
Liver disease autopsy	0	0	3
Increased blood alcohol concentration of over 1‰	0	0	3

n – number of subjects

DISCUSSION

To evaluate CDT analysis values better, it was necessary to determine if the patients had consumed alcohol in the past 15 days. The knowledge of this was important in the study, as half-life of CDT is 15 days and its significant concentrations in circulation could not be expected after that period of time [10, 15, 16, 17, 19]. In all cases with medical treatment longer that 15 days (based on Emergency Center records of patients' histories), these data were valid. The problem appeared with those died at the spot (except for ten cases of acute drunkenness) thus, in all others, we used heteroanemnestic data regarding alcohol consumption and drinking habits.

In the literature, we have found that valid CDT results are obtained 36-72 hours postmortem [2, 3, 6, 7, 22, 23]. Our results are coherent with the data of transferrin resistance to spontaneous and bacterial decomposition that makes it adequate for alcohol detection in dead bodies even when the interval from the moment of death up to blood samples collection is longer. Some blood samples drawn from dead bodies showed a lower or higher level of hemolysis. However, hemoglobin does not disturb the identification of CDT focused in further pH area gel.

We have to point out that it was a hard task to obtain valid data on the following: if the subject had consumed alcohol within 15 days prior to death on the spot or after the survival time shorter than 15 days. Cases, where we could not obtain valid data of drinking habits on the basis of heteroanamnestic data, were grouped as those who had not consumed alcohol in the past 15 days. Thus, the obtained values of specificity and sensitivity can be regarded as the possible lowest values. The analysis of the obtained results led to the conclusion that CDT analysis on dead body material is the valid diagnostic procedure in alcohol abuse determination.

It can be found in the literature that CDT values are not in strong correlation with the quantity of alcohol intake. Grouping of the samples according to alcohol content are based on literature knowledge of transferrin change and CDT occurrence due to 50-80 g of alcohol daily intake during at least a week. At the same time, the same quantity of alcohol causes alcoholemia a little above 1‰ when alcohol is taken within a short period of time [4, 10, 11, 12]. The presence of CDT along with alcoholemia under 1‰ proves that those persons were chronic alcoholics [9, 24].

Severe diseases of the liver (cirrhosis, hepatitis B and C) influence CDT concentration according to the disease stage [4, 16, 17]. CDT rises more often in early stage of alcoholic liver disease when transferrin synthesis is high, while in further stages, CDT reduces below normal values correlating the reduction of the number of functional hepatocites [16]. A high incidence of falsely positive CDT result has been noticed in liver failure and cirrhosis of non-alcoholic origin [13, 15, 25]. Our analysis confirmed this, because eight subjects of 12 with severe liver disease were CDT positive but three were with high alcoholemia.

CONCLUSION

The results obtained in CDT examination in the sera of dead bodies (sensitivity 59% and specificity 71%) make the method of CDT determination reliable for the diagnosis of alcohol abuse in forensic medicine. CDT analysis is also possible to be performed in samples collected postmortem up to 76 hours.

NOTE

This paperwork is a part of PhD dissertation of the first author "The molecule variants of the carbohydrate-deficient transferrin as a marker of alcohol abuse", defended at the School of Medicine, University of Belgrade.

REFERENCES

1. Berkowicz A, Wallerstedt S, Wall K, Denison H. Carbohydrate-deficient transferrin in vitreous humor: a marker of possible withdrawal-related death in alcoholics. Alcohol Alcohol. 2001; 36(3):231-4.
2. Berkowicz A, Wallerstedt S, Wall K, Denison H. Analysis of carbohydrate-deficient transferrin in vitreous humor as a forensic tool for detection of alcohol misuse. Forensic Sci Int. 2003; 137:119-24.
3. Rainio J, De Paoli G, Druid H, Kauppila R, De Giorgio F, Bortolotti F, et al. Post-mortem stability and redistribution of carbohydrate-deficient transferrin (CDT). Forensic Sci Int. 2008; 174:161-5.
4. Musshoff F, Daldrup T. Determination of biological markers for alcohol abuse. J Chromatography B. 1998; 713:245-64.
5. Hansson P, Varga A, Krantz P, Alling C. Phosphatidylethanol in post-mortem blood as marker of previous heavy drinking. Int J Legal Med. 2001; 115:158-61.
6. Malcolm R, Anton RF, Conrad SE, Sutherland S. Carbohydrate-deficient transferrin and alcohol use in medical examiner cases. Alcohol. 1999; 17(1):7-11.
7. Simmonet C, Dumestre-Toulet V, Kintz P, Gromb S. Review of factors susceptible of influencing post-mortem carbohydrate-deficient transferrin. Forensic Sci Int. 1999; 106:7-17.
8. Osuna E, Perez-Carceles M, Moreno M, Bedate A, Conejero J, Abenza J, et al. Vitreous humor carbohydrate-deficient transferrin concentrations in the post-mortem diagnosis of alcoholism. Forensic Sci Int. 2000; 108:205-13.
9. Bortolotti F, Paoli G, Tagliaro F. Carbohydrate-deficient transferrin (CDT) as marker of alcohol abuse: A critical review of the literature 2001-2005. J Chromatography B. 2006; 841:96-100.
10. Helander A, Vabö E, Levin K, Borg S. Intra- and interindividual variability of carbohydrate-deficient transferrin, gamma-glutamyltransferase, and mean corpuscular volume in teetotalers. Clin Chem. 1998; 44(10):2120-5.
11. Perret R, Froehlich F, Lavanchy D, Henry H, Bachman C, Pécoud A, et al. Is carbohydrate-deficient transferrin a specific marker for alcohol abuse? A study in patients with chronic viral hepatitis. Alcohol Clin Exp Res. 1997; 21(7):1337-42.
12. Viitala K, Lähdesmäki K, Niemelä O. Comparison of the Axis %CDT TIA and the CDTect method as laboratory tests of alcohol abuse. Clin Chem. 1998; 44(6 Pt 1):1209-15.
13. De Feo TM, Fargion S, Duca L, Mattioli M, Cappellini MD, Sampietro M, et al. Carbohydrate-deficient transfferin, a sensitive marker of chronic alcohol abuse, is highly influenced by body iron. Hepatology. 1999; 29(3):659-63.

14. Huseby NE, Bjordal E, Nilssen O, Barth T. Utility of biological markers during outpatient treatment of alcohol-dependent subjects: carbohydrate-deficient transferrin responds to moderate changes in alcohol consumption. Alcohol Clin Exp Res. 1997; 21(7):1343-46.
15. Mitchell C, Simpson D, Chick J. Carbohydrate deficient transferrin in detecting relapse in alcohol dependence. Drug Alcohol Depend. 1997; 48:97-103.
16. Keating J, Cheung C, Peters TJ, Przemioslo R, Williams R, Sherwood RA. Carbohydrate deficient transferrin in alcoholic and non-alcoholic liver disease: a comparison of two assay methods. Addiction Biology. 1998; 3:205-11.
17. Schmitt UM, Stieber P, Jüngst D, Bilzer M, Wächtler M, Heberger S, et al. Carbohydrate-deficient transferrin is not a useful marker for the detection of chronic alcohol abuse. Eur J Clin Invest. 1998; 28:615-21.
18. Brinkmann B, Köhler H, Banaschak S, Berg A, Eikelmann B, West A, et al. ROC analysis of alcoholism markers – 100% specificity. Int. J Legal Med. 2000; 113:293-9.
19. Raynaud M, Hourcade F, Planche F, Albuisson E, Meunier MN, Planche R. Usefulness of carbohydrate deficient transferrin in alcoholic patients with normal gamma-glutamyltranspeptidase. Alcohol Clin Exp Res. 1998; 22(3):615-8.
20. De Jong G, Van Noort WL, Eijk HG. Carbohydrate analysis of transferrin subfractions isolated by preparative isoelectric focusing in immobilized pH gradients. Electrophoresis. 1992; 13:225-8.
21. Sagan Z. The application of rivanol for serum transferrin and immunoglobulin G determination. Clin Chim Acta. 1968; 21:225-30.
22. Köhler H, West A, Brinkmann B. Stability of carbohydrate deficient transferrin (CDT) in stored blood samples. Int. J Legal Med. 2000; 113:121-2.
23. Ranio J, Paoli G, Druid H, Kauppila R, De Giorgio F, Bortolotti F, et al. Post-mortem stability and redistribution of carbohydrate deficient transferrin (CDT). Forensic Sci Int. 2008; 174:161-5.
24. Appenzeller BMR, Schneider S, Yelges M, Maul A, Wenning R. Drugs and chronic alcohol abuse in drivers. Forensic Sci Int. 2005; 155:83-90.
25. Murawaki Y, Sugisaki H, Yuasa I, Kawasaki H. Serum carbohydrate-deficient transferrin in patients with nonalcoholic liver disease and with hepatocellular carcinoma. Clin Chim Acta. 1997; 259:97-108.

Форензички аспекти постморталне анализе трансферина с недостатком угљених хидрата као маркера злоупотребе алкохола

Весна Поповић[1], Татјана Атанасијевић[1], Слободан Николић[1], Наташа Божић[2], Зоран Вујчић[3], Јелена Мицић-Лабудовић[1]

[1]Институт за судску медицину, Медицински факултет, Универзитет у Београду, Београд, Србија;
[2]Центар за хемију – ИХТМ, Београд, Србија;
[3]Хемијски факултет, Универзитет у Београду, Београд, Србија

КРАТАК САДРЖАЈ

Увод Последњих година трансферин с недостатком угљених хидрата (енгл. *carbohydrate-deficient transferrin – CDT*) један је од маркера злоупотребе алкохола који је показао најбоље резултате у судској медицини.

Циљ рада Циљ студије је био да се одреди корелација између актуелне методологије дијагнозе злоупотребе алкохола на постморталном материјалу (макроскопски и микроскопски налаз) и одређивања *CDT* коришћењем методе исоелектрофокусирања (енгл. *isoelectric focusing – IEF*) у полиакриламидном гелу (енгл. *polyacrylamide gel electrophoresis – PAGE*). Утврђивано је да ли интервал између времена смрти и узимања узорака за *CDT* анализу утиче на налаз *CDT*.

Методе рада За анализу *CDT* коришћена је метода *IEF-PAGE*. За студију су анализирани серуми 49 мушкараца и 11 жена просечне старости од 46,85±18,53 година (распон 14–87 година). Контролну групу чинило је пет пацијената који су умрли након болничког лечења које је трајало дуже од 15 дана и пет пацијената код којих је у контролисаним болничким условима почело лечење дисулфирамом.

Резултати Добијени резултати показују да ова метода анализе *CDT* на постморталном материјалу има сензитивност од 59% и специфичност од 71%. Висока учесталост лажно позитивних резултата утврђена је код обољења јетре и цирозе неалкохолног порекла. Анализу *CDT* је могуће радити и из узорака узетих до 76 сати након смрти.

Закључак У судскомедицинској пракси ова метода анализе *CDT* може се користити за дијагностиковање хроничне злоупотребе алкохола.

Кључне речи: алкохолизам; трансферин с недостатком угљених хидрата (*CDT*); постмортална анализа; специфичност; сензитивност

Примљен • Received: 26/01/2011 **Прихваћен • Accepted:** 01/11/2011