Short Communication

BLOOD BIOMARKERS OF ALCOHOL ABUSE

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The biological, clinical and social effects of alcohol abuse call for objective and specific biomarkers of alcohol-related diseases and early detection of alcohol consumers at risk. Alcohol abusers may exhibit several clinical and/or chemical changes. Changes in parameters such as gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and mean corpuscular volume (MCV) may serve as biomarkers of chronic alcoholism. All available biomarkers have two drawbacks. The first is that they indicate adverse effects in a particular organ, but tell little about their aetiology. The second is that they are not sensitive enough to detect abuse before it results in organic impairment. The 1990s have seen the introduction of a new diagnostic biomarker, carbohydrate-deficient transferrin (CDT). Reduced concentrations of this biomarker are found in serum after regular high alcohol intake. Relying on literature and their own clinical experience, the authors conclude that CDT seems to meet the clinical criteria of reliability and specificity.

KEY WORDS: *alanine amino transferase, aspartate amino transferase, carbohydrate-deficient transferrin, gamma-glutamyl transpeptidase, liver enzymes, mean corpuscular volume*

Early detection of excessive alcohol use is very important due to its harmful effects on a human organism and a person's psychosocial life. It is not surprising that a number of scientists have persistently been searching for an ideal diagnostic biomarker that could confirm the diagnosis of alcohol abuse. Such an indicator should include clinical and biochemical parameters which would specifically correlate with the presence of ethanol or its metabolites in the organism, be directly related with the quantity of alcohol consumed, be susceptible enough to correlate the quantity of consumed alcohol and the psychosomatic risk, and finally, feature certain kinetics during abstinence (1, 2). However, no such biomarker has yet been discovered. Transferrin with a reduced carbohydrate share, carbohydrate-deficient transferrin (CDT) found in blood serum of examined persons comes the closest to meeting the required criteria (1).

Transferrin is a glycoprotein synthesised in the liver, and is responsible for the transport of iron in plasma. Transferrin carbohydrates are olygosacharides, linked to transferrin in two complex chains. Every chain contains bonds between N-acetylglucosamine, mannose, galactose and sialic acid. The terminal of the chain is concluded by a negatively charged sialic acid, which defines the isoelectric point (pI) of the protein. Various isoforms of transferrin can contain between 0 and 5 sialic acids (asialo- to pentasialotransferrin). The most common isoform is the tetrasialotransferrin with the pI of 5.4.

The blood of persons who consume at least 60 g of pure alcohol per day (e. g. 750 ml of wine, 1,750 ml of beer or 180 ml of brandy) 14 days in a row, contains isoforms of transferrin with a reduced number of sialic acids (asialo-, disialo- and trisialotransferrin), called CDT. The methodology for determining the CDT content is based on the differences in charge between the desialised forms of transferrin. The formation of CDT is associated with the impact of acetaldehyde, the first metabolite of ethanol, on glycosiltransferase activity in the liver, which affects the transfer of the

sialic acid to the transferrin molecule. In turn, the liver produces transferrin molecules with the reduced share of carbohydrates, that is CDT, in addition to regular trasferrin.

More recent biomarkers of alcohol abuse include the assessment of the mitochondrial aspartate aminotransferase (mAST), serotonin metabolites, acetaldehyde and acetaldehyde adducts, as well as the application of tests for early detection of alcohol consumption (EDAC). However, none of these biomarkers meets all requirements. Human serum contains two AST isoenzymes: mAST and cytosolic AST (cAST). The enzyme activity in the serum of healthy persons mostly involves cAST, while in persons who consume too much alcohol the liver mitochondria are selectively damaged. This results in an increased activity of mAST. The proportion between mAST and overall AST activities helps to differentiate between alcohol-induced liver changes and changes of other aetiology (2).

Serotonin usually metabolises into 5-hydroxytriptofol-3-acetic acid (5-HIAA) and 5-hydroxytriptofol (5-HTOL). The major part is represented by the 5-HIAA metabolite. Alcohol intake tends to stimulate the metabolism of serotonin into 5-HTOL. Elevated concentrations of this metabolite can be traced in blood six to fifteen hours after the alcohol levels return to normal (2).

Acetaldehyde is the first ethanol metabolite, but it is not considered a good biomarker of alcohol abuse, since it metabolises into acetate within five hours from alcohol intake. Forming Schiff bases with amines, acetaldehyde readily binds to a protein in an irreversible reaction, producing acetaldehyde-protein adduct. Many proteins generate adducts with acetaldehyde, among them albumins and haemoglobin. Abnormal blood concentrations of these adducts were found after acute alcohol intoxication, as well as after chronic alcohol abuse (2).

Early detection of alcohol consumption (the so called EDAC test) relies on the results of routine biochemical and haematological examinations (usually 35 parameters) analysed by the linear discriminant function (LDF) using the Statistical Package for the Social Sciences programme. The analysis yields a numerical value pointing to a heavy drinker or a person at risk of alcohol abuse (3-5).

Apart from the recent biomarkers mentioned above, diagnosing alcohol abuse should rely on the results of routine biochemical and haematological tests such as gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), high density level (HDL)-cholesterol, and mean corpuscular volume (MCV), as well as on the concentrations of ethanol and methanol in blood (2, 6-10).

All liver disorders such as holestase and cyrrhosis of various origin, neoplasia and toxic damages caused by alcohol, drugs or tobacco are accompanied by increased GGT activity in serum. This enzyme is not limited only to the liver, but can also be found in the kidneys, pancreas and prostate. Normal serum GGT values in men are nearly 50 % higher than in women, mostly due to prostate GGT (11). A single excessive alcohol intake has no effect on GGT activity. However, in chronic alcohol consumption, the activity of the enzyme increases and varies from person to person (the sensitivity of the method is only 50 %) (9). The kinetics of increased GGT activity and the return to normal levels largely depend on how strong was the induction of the enzyme and significantly correlate with the liver disorder. A two to five week abstinence from alcohol usually suffices for GGT to return to normal activity (7, 9).

The first sign of the liver cell decay is the increase in catalytic activities of serum AST and ALT. It is well known that a liver disease can be caused by a variety of agents. Moreover, changes in other organs may also increase the levels of AST and ALT in serum. Therefore the activities of both enzymes are relatively unspecific for diagnosing alcoholism. In case of alcohol-related liver disease, only an activity ratio of AST:ALT higher than 2 is of diagnostic importance.

Research has shown that one of the nonspecific indicators of alcohol abuse is elevated HDL-cholesterol. High concentrations of HDL-cholesterol in the serum may be indicative of chronic alcohol abuse (7 ,9).

Increased MCV can be due to the antagonistic effect of alcohol on folate. However, higher MCV also accompanies vitamin B_{12} or folic acid deficiencies, liver disease, some haematologic diseases, hypothyroidism, tobacco smoking or antiepileptic intake. If the increase in MCV levels is caused by alcohol drinking, it is reasonable to expect that the concentrations will return to normal values after three to six months of abstinence from alcohol (due to the corpuscle life expectancy) (7, 9).

The assessment of ethanol concentration in blood, urine or exhaled air is particularly useful in acute alcohol intoxication. Since alcohol disintegrates quickly after consumption, a one-time identification

of high ethanol blood levels tells very little of the frequency and duration of alcohol abuse. If alcohol concentrations in the blood are higher than the clinical assessment of drunkenness shows it is reasonable to suspect high alcohol tolerance and dependence (7, 9).

In addition to ethanol, the testing may include methanol as the most frequent accompanying alcohol found in almost all alcoholic beverages. Methanol absorption in the body is slightly slower than that of ethanol, and it can be found in all body fluids and organs. Alcoholdehydrogenase (ADH) is important for methanol metabolism. As ethanol shows greater affinity to the ADH-NAD complex, methanol is metabolised only after ethanol blood levels drop below 0.4 g/kg. Nevertheless, alcohol abstinence of approximately 24 hours suffices for the complete disintegration of methanol (12).

The aim of this study was to evaluate the usefulness of standard blood biomarkers in detecting alcohol abuse and in assessing its harmful effects in our patients performed during regular preventive medical check-ups. We especially focused on the applicability of carbohydrate-deficient transferrin in diagnosing alcohol abuse.

SUBJECTS AND METHODS

The control group (Group 1) consisted of 43 healthy subjects (mean age 47.1 years, range 32-56 years) who had never consumed alcohol. These control subjects were analysed for serum AST, ALT, GGT, and MCV, and their values kept within the normal range.

Group 2 consisted of 45 subjects (mean age 46.7 years, range 39-54 years) who were suspect of alcohol abuse and dependence. Each subject was taken a blood sample for testing with biomarkers of alcohol abuse.

Catalytic activities of AST, ALT and GGT were determined according to procedures recommended by the International Federation of Clinical Chemistry, using a biochemical analyser BM/HITACHI 902 produced by Roche Diagnostics Corp. (IN), USA, while the MCV results were obtained using the COBAS MICROS haematological analyser, also produced by Roche Diagnostics.

The per cent of CDT in blood serum was determined using the homogeneous immunologic method (Tina-quant® %CDT, Roche Diagnostics). The set consisted of columns with ionic exchangers for the separation of transferrin isoforms and of reagents for turbidimetric determination of transferrin isoforms and the overall transferrin concentration. The analytical parameters for defining turbidimetric measurements of transferrin were set on the BM/HITACHI 902 biochemical analyser device according to manufacturer's instructions. After determining separate isoforms of transferrin (CDT) and the overall transferrin concentration, the CDT percentage was calculated according to the formula defined by the reagent manufacturer.

Data were analysed using standard methods of parametric statistics. The differences between the mean values of individual groups were tested using the Statistical Package for the Social Sciences computer programme for variance analysis and Pearson's correlation.

RESULTS

The ratability of %CDT determination expressed as relative standard deviation (RSD) was established by human serum (N=10 in a series) and commercial native control serums (N=10 in consecutive days) analyses (Table 1).

Table 1 *Ratability of per cent carbohydrate-deficient transferrin (%CDT) determination in human serum and in control material*

Sample	Mean	SD	RSD (%)
[a]Human serum	12.2	0.729	6
[b]Native control serum			
Level I	4.53	0.377	8.3
Level II	10.2	0.57	5.6

[a]In series (N=10)

[b]On consecutive days (N=10)

Table 2 shows the mean values of the measured parameters and variance analysis and Table 3 the relationships between CDT and other measured parameters. In Group 1, only one subject showed abnormal AST activity (over 0.60 μkat/L) and four subjects had the border %CDT values (between 5 and 6).

In Group 2, only one GGT finding was within the normal range (below 0.62 μkat/L), and the highest measured activity was impressive 25.3 μkat/L. Activities of ALT and AST were within the normal range (<0.70 μkat/L and <0.60 μkat/L, respectively) in 21

Table 2 *Measured parameters in controls (Group 1) and subjects with alcohol abuse (Group 2)*

Variable	Group 1	Group 2	Variance analysis	
			F	P
Gamma-glutamyltransferase (GGT)	0.284±0.113	3.99±5.67	18.322	0.000
Alanine aminotransferase (ALT)	0.312±0.168	0.841±0.653	26.353	0.000
Aspartate aminotransferase (AST)	0.279±0.058	0.654±0.447	28.959	0.000
Mean corpuscular volume (MCV)	90.1±3.572	94.8±7.37	24.783	0.000
Carbohydrate-deficient transferrin (%CDT)	4.37±0.725	5.65±1.89	17.308	0.000

Results are presented as means ± standard deviations

Table 3 *Correlation between per cent carbohydrate-deficient transferrin (%CDT) and other parameters measured in the examined subjects*

Variable	Group 1		Group 2		All	
	r	P	r	P	r	P
Gamma-glutamyltransferase (GGT)	-0.092	0.558	0.280	0.063	0.388	0.000
Alanine aminotransferase (ALT)	-0.109	0.485	0.166	0.307	0.334	0.002
Aspartate aminotransferase (AST)	0.117	0.462	0.468	0.002	0.571	0.000
Mean corpuscular volume (MCV)	-0.202	0.193	0.693	0.000	0.575	0.000

r – Pearson correlation coefficient
Group 1 – control; Group 2 – subjects with alcohol abuse

subjects and 23 subjects, respectively, whereas MCV was normal in 15 subjects (<94 fL). In 17 subjects %CDT was below 5, in 11 between 5 and 6 and in 17 above 6 (the highest was 12.1).

All biochemical parameters in Group 1 were within the normal limits, but in Group 2 they were considered pathological. The two groups show a statistically significant difference in means ($P<0.001$).

The percentages of CDT in both groups taken together show a statistically significant correlation with all measured parameters. If the correlation is assessed for group 1 only, it can be seen that there is none. Group 2, shows a significant correlation ($P<0.01$) between %CDT and AST and %CDT and MCV. The correlation between %CDT and GGT is weak, and no correlation is visible for %CDT and ALT.

DISCUSSION

In the last decade, a number of studies investigated the importance of %CDT as a biomarker of alcohol abuse. Due to the fact that its origin directly depends on ethanol or its metabolites, it has been considered of high diagnostic importance with sensitivity 73 % and specificity 96 % (11).

False positive %CDT was found in persons suffering from primary biliary cyrrhosis, chronic active hepatitis, genetic D-forms of transferrin, and carbohydrate-deficient glycoprotein syndrome. False negative results were found in persons who, before blood sampling, were had been consuming less than 60 g of ethanol per day or had been consuming this daily quantity for less than two weeks (1). Since the increased CDT is independent of the liver status, determining %CDT helps to distinguish alcohol-induced changes in the liver from changes of other origin (12-14).

This %CDT assessment in serum with the aim to ascertain alcohol abuse has been supported by the US Food and Drug Administration (FDA). In turn, US FDA refers to liver enzymes GGT, AST, and ALT, which have served as biochemical biomarkers of alcohol abuse for decades, as biomarkers of liver damage. It recommends that GGT, AST and ALT findings should be considered with %CDT results, which increases the diagnostic sensitivity from 73 % to 90 % (11).

In our study, we used a homogenous immunologic method with Roche reagents (Tina-quant® %CDT) to determine blood serum %CDT. The repeatability of %CDT determination corresponded to the recommendations of the reagent manufacturers.

The manufacturer explains the reference range as follows: in persons who abstain from alcohol or consume it in "normal" quantities, the values for %CDT are below 5. All the %CDT above 6 indicate that the person has been consuming more than 60 g of ethanol for 14 days minimum. When abstinence

from alcohol is total, the values of %CDT are expected to reach the reference range in one or two months (half-life of approximately 15 days).

Roche Diagnostics has already prepared the 2nd generation of reagents for %CDT determination which are expected to increase the clinical sensitivity of the test. This means that the reference values range will also change.

Despite all the advantages of %CDT over classical laboratory tests (GGT, AST, ALT, and MCV), alcohol abuse assessment should not rely on %CDT alone. Research has shown that only a combination of several laboratory parameters can increase the diagnostic sensitivity and help the physician to determine alcohol abuse (8, 11). The diagnosis should be made by a physician who is trained to assess a variety of alcohol-related diseases and damage to individual organs. The physicians usually apply four basic diagnostic methods (15, 16): case history, clinical check-up, laboratory tests, and psychiatric examination.

It is essential that the physician never forgets that the diagnosis of alcohol abuse or dependence is based on a well-prepared case history and physical check-up. Laboratory tests alone (biochemical, haematological, urine analysis), are insufficient, and they should serve to support case history and physical check-up of the patient. Biochemical parameters provide additional diagnostic material but they are not specific by themselves. In the identification process of alcohol abuse among the population they have a greater importance than placing the diagnosis of individual cases. These processes still do not provide a specific result in the group of alcohol-related health deficiencies (16).

Quite often, the diagnosis can be confirmed by a questionnaire, which can help to identify a number of clinical symptoms, obtain history data and data on the drinking habit. Some authors believe that 80-90 % of patients answer truthfully. A well-known questionnaire is the Michigan Alcohol Screening Test (*MAST*). This questionnaire consists of 25 questions, most of them referring to complications caused by alcohol. It focuses on the patients' perception of their own alcohol consumption (17). The second questionnaire in common use is the Severity Alcohol Dependence Questionnaire which focuses on the degree of alcohol abuse. The most common and simple questionnaire is the so called *CAGE* (C for cut down; A for annoyed; G for guilty; E for eye-opener) which initiates the diagnostic procedure for potential alcoholism. The questionnaire consists of four questions and two positive answers is enough to be considered alcohol dependent (18). The Alcohol Use Disorder Identification Test (*AUDIT*) questionnaire is very practical in identifying alcohol abuse. It has the greatest overall validity in the population at large, and is more sensitive in populations with a low prevalence of alcoholism (such as Asian) and more effective in detecting hazardous and harmful drinking. Test sensitivity is 92 % and reliability 93 %; it is simple and takes but a few minutes (19).

The diagnostic procedure is usually concluded by an extensive psychiatric evaluation. The physician makes his final diagnosis on the basis of all tests performed, including the opinion of the psychiatrist. If a patient is diagnosed alcohol abuse/dependence, she or he is immediately invited to enter a recovery programme.

CONCLUSION

The carbohydrate-deficient transferrin (CDT) is a relatively new biochemical parameter. Despite the fact that it does not meet all the criteria for an ideal biomarker of alcohol abuse, it is still recommended in diagnosing alcohol abuse, in combination with other laboratory tests. Companies producing reagents for determining %CDT in blood serum are trying to develop such a reagent mix that would increase the clinical sensitivity of the test. Literature on CDT as an indicator of alcohol abuse suggests that higher clinical sensitivity and standardised laboratory procedures will increase the diagnostic value of blood serum %CDT.

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Sažetak

BIOKEMIJSKI POKAZATELJI U KRVI UŽIVALACA ALKOHOLA

Biološka, klinička i socijalna stanja alkoholičara već dugo iziskuju objektivne i specifične pokazatelje poremećaja uzrokovanih uživanjem alkohola u svrhu što ranijeg otkrivanja rizičnih uživalaca. Uživaoci prekomjernih količina alkohola mogu pokazivati nekoliko kliničko-kemijskih promjena od kojih se neke upotrebljavaju kao pokazatelji alkoholizma. To su ponajprije promjene u aktivnostima jetrenih enzima gama-glutamiltransferaze (GGT), aspartat aminotransferaze (AST) i alanin aminotransferaze (ALT) te promjene srednjeg volumena eritrocita (MCV). Svi ti pokazatelji imaju dvije slabe strane. S jedne strane, mogu odražavati poremećaj ili bolest određenog organa s nepouzdanom specifičnošću prepoznavanja etiologije bolesti. S druge strane, nisu dovoljno osjetljivi za otkrivanje kroničnog alkoholičara prije nastajanja organskih komplikacija. Tijekom posljednjeg desetljeća uveden je u dijagnostiku kroničnog alkoholizma novi biokemijski pokazatelj sniženi ugljikohidratni transferin (*carbohidrate deficient transferrin, CDT*). To je glikoprotein koji prenosi željezo u tijelu i koji se stvara i oslobađa iz jetre. Iako razlozi još nisu sasvim rasvijetljeni, u osoba koje redovito uživaju veće količine alkohola koncentracija sniženog ugljikohidratnog transferina u serumu redovito je povišena.
U radu su dani odabrani relevantni literaturni podaci o biokemijskim pokazateljima uživanja alkohola i dijagnosticiranja kroničnog alkoholizma. Usto, autori su i sami procjenjivali do koje mjere uobičajeni krvni biopokazatelji alkohola odražavaju štetne učinke uživanja alkoholnih pića u skupini vlastitih ispitanika tijekom periodičkih medicinskih pregleda. Pritom su se posebno usredotočili na novi biokemijski parametar postotak CDT-a (%CDT).
Na temelju pregleda literaturnih podataka i vlastitog opažanja, autori su zaključili da %CDT kao biopokazatelj alkohola u krvi u velikoj mjeri udovoljava postavljenim kliničkim kriterijima pouzdanosti i specifičnosti.

KLJUČNE RIJEČI: *alanin aminotransferaza, aspartat aminotransferaza, gama-glutamiltransferaza, jetreni enzimi, sniženi ugljikohidratni transferin, srednji volumen eritrocita*

REQUESTS FOR REPRINTS:

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