

PREVALENCE OF GAMMA-GLUTAMYLTRANSFERASE AND MEAN CORPUSCULAR VOLUME LABORATORY MARKERS IN ALCOHOL DEPENDENT PATIENTS

CZĘSTOŚĆ WYSTĘPOWANIA LABORATORYJNYCH MARKERÓW GAMMA-GLUTAMYLOTRANSFERAZY I WSKAŹNIKA ŚREDNIEJ OBJĘTOŚCI KRWINKI CZERWONEJ U PACJENTÓW UZALEŻNIONYCH OD ALKOHOLU

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Abstract

Introduction: DSM-5/APA indicates three sensitive laboratory markers of heavy drinking useful in the diagnosis of alcohol use disorders: mean corpuscular volume (MCV), gamma-glutamyltransferase (GGT) and carbohydrate-deficient transferrin (CDT). The study aim was to identify the proportion of the occurrence of the abnormal values of the readily available laboratory tests for MCV and GGT among the patients with alcohol dependence at the time of their admission for treatment.

Material and methods: There were 543 patients included in the study, with average age 45 (SD ± 12.3) years, 72% were males. Clinical diag-

Streszczenie

Wprowadzenie: DSM-5/APA wskazuje trzy wrażliwe laboratoryjne markery intensywnego picia, które są przydatne przy diagnozowaniu zaburzeń związanych z używaniem alkoholu: wskaźnik średniej objętości krwinki czerwonej (MCV), gamma-glutamylotransferaza (GGT) i transferyna z niedoborem węglowodanów (CDT). Celem badania było ustalenie odsetka nieprawidłowych wartości łatwo dostępnych testów laboratoryjnych dla MCV i GGT wśród pacjentów uzależnionych od alkoholu w momencie przyjęcia do leczenia.

Materiał i metody: Badaniem objęto 543 pacjentów, których średnia wieku wynosiła 45 lat (SD ± 12,3 roku); 72% stanowili mężczyźni. Rozpo-

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nosis of alcohol dependence was assessed according ICD-10/WHO criteria.

Results: Increased values of MCV were detected in 44% of patients and GGT in 50%. At least one marker was increased in 64% of the patients.

Conclusions: Significant proportion of patients with alcohol dependence had normal values of MCV and GGT at the time of treatment admission. The findings indicate the important, but only supportive role of these laboratory markers in the alcohol use disorders assessment. Clinical assessment of patient's condition according to valid diagnostic criteria has a crucial role in the diagnosis of alcohol dependence.

Keywords: Mean corpuscular volume, Gamma-glutamyltransferase, Breathalyzer test, Alcohol dependence

znane kliniczne uzależnienia od alkoholu określano według kryteriów ICD-10/WHO.

Wyniki: Podwyższone wartości MCV wykryto u 44% pacjentów, a GGT u 50% z nich. Co najmniej jeden marker był zwiększony u 64% pacjentów.

Wnioski: Znaczny odsetek pacjentów uzależnionych od alkoholu miał prawidłowe wartości MCV i GGT w momencie przyjęcia do leczenia. Wyniki wskazują na istotną, ale tylko wspierającą rolę tych markerów laboratoryjnych w ocenie zaburzeń używania alkoholu. W rozpoznawaniu uzależnienia od alkoholu kluczową rolę odgrywa kliniczna ocena stanu pacjenta według aktualnych kryteriów diagnostycznych.

Słowa kluczowe: wskaźnik średniej objętości krwinki czerwonej, gamma-glutamylotransferaza, badanie alkomatem, uzależnienie od alkoholu

■ INTRODUCTION

To assess the novel World Health Organization (WHO) risk drinking levels the National FINRISK Study [1] analysed biomarkers of liver status, inflammation and lipid profiles from a population based survey. Serum liver enzymes gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), C-reactive protein (CRP) and lipid profiles were measured. Alcohol risk category was roughly linearly related with the occurrence of elevated values for GGT, ALT and CRP. Alcohol drinking also significantly influenced the incidence of abnormalities in serum lipids. An umbrella-shaped association was observed between total alcohol consumption and changes in high-density lipoprotein (HDL) cholesterol concentrations [2]. Already in 1986, it was noted by Wehr and Woronowicz [3] that HDL cholesterol level was markedly elevated in most of heavy drinkers shortly after alcohol abuse and diminished gradually following abstinence. DSM-5 [4] indicates three sensitive laboratory markers of heavy drinking, which are useful at diagnosis of alcohol use disorders. Indicators of heavy drinking are: mean corpuscular volume (MCV), gamma-glutamyltransferase (GGT) and carbohydrate-deficient transferrin (CDT). Although an extensive amount of previous literature is available on biomarkers of alcohol consumption, the information on the sensitivities and specificities

of even the most commonly used markers has remained controversial. Based on study findings of authors Lieb *et al.* and Tavakoli *et al.* [5, 6] sensitivity and specificity of GGT as heavy drinking indicator varies from 40% up to 80%. The same authors refer to low sensitivity of MCV values under 50%, but the specific range is broad according to different studies. However, the specificity of abnormal values of MCV for diagnosis of heavy drinking is as high as 90%. For example, Wurst *et al.* [7] indicate for detected sensitivity 40% for MCV and 73% for GGT in patient with alcohol dependence. According to Moravcová *et al.* [8], CDT has shown the highest sensitivity and specificity of values from the abovementioned three markers among chronic drinking patients.

The mechanisms underlying the formation of macrocytes are diverse and complex. In Vitamin B₁₂/folate deficiencies, there is a defect involving nuclear maturation, which affects cell division. Hemoglobin synthesis proceeds normally while nuclear division lags behind, thereby resulting in larger than normal red cells. Macrocytosis in alcoholism is related to the direct toxic effect of alcohol on the red cell membrane [9, 10]. According to Liangpunsakul [11], heavy alcohol use causes the alteration in the lipid structure of red cell membrane, leading to the increase in the MCV values. There seems to be a dose-dependent response between erythrocyte size (MCV) and ethanol intake [12].

In heavy drinkers without co-morbidities, high MCV values are typically seen without anaemia. Upon abstinence, normalisation of red cell indices may require 2-4 months [13].

The liver is a major target of ethanol toxicity due to its primary role in ethanol metabolism. Therefore, unexpected abnormalities in liver enzyme activities, GGT or ALT, are frequently the first clinical signs of excessive alcohol consumption. Non-alcoholic fatty liver disease (NAFLD) associated with obesity is the most common non-alcoholic cause of increased GGT and ALT activities. Alcohol use and obesity often co-exist and create toxicity in a synergistic manner. Alcoholic liver disease (ALD) and NAFLD can also be overlapping phenomena [13]. GGT is a membrane-bound glycoprotein enzyme, which has long been used as a marker of excessive alcohol intake [14, 15]. GGT is sensitive to changes in alcohol consumption, but, due to lack of specificity, it is not suitable for screening among populations with non-alcoholic liver diseases, obesity or hospitalised patients [16, 17]. In alcoholics, increased activities usually return to normal within 2-3 weeks upon abstinence, whereas persistently abnormal values may suggest liver disease. In heavy drinkers without co-morbidities, high MCV values are typically seen without anaemia, whereas in patients with ALD and a concomitant folate deficiency, megaloblastic bone marrow alterations and haemolysis, high MCV and anaemia usually co-exist [15].

Because of these dynamics, elevated levels of GGT and CDT are good indicators of actual heavy drinking and acute relapse to drinking in the process of abstinence from alcohol, so called "state markers". Elevated MCV is associated frequently with prolonged heavy drinking and points to the suspected dependence even if abstinence from drinking lasts for more days or even a month after a period of heavy drinking [4, 13]. The goal of the study was to discover the occurrence of the abnormal values in readily available laboratory tests for MCV and GGT in the patients who were seeking treatment for alcohol dependence at the time of treatment entry.

■ MATERIAL AND METHODS

The study was retrospective and based on descriptive analysis of the data from medical records of all patients who were asking for alcohol relat-

ed problem treatment at the Centre for Treatment of Drug Dependencies (CTDD) in Bratislava in the years 2014 and 2015. Only those with diagnosis of alcohol dependence (F10.2) according to ICD-10/WHO [18: 225-227] diagnostic criteria were included. The assessment was conducted after examination by experienced psychiatrist in the field of dependence. The whole group consisted of 597 patients. Only the patients who volunteered were included for further analysis. Those on court orders for mandatory treatment were excluded as were patients whose blood sample could not be taken for various reasons. Subjects were excluded if they had active and serious medical diseases (such as congestive heart failure, chronic obstructive pulmonary disease, cancer, uncontrolled diabetes and chronic renal failure). From the overall group, the study sample of 543 patients was formed for further statistical analysis. The average age of the patients was 45 years ($SD \pm 12.3$). 72% were males. Blood samples were taken for hematologic and biochemical examination of MCV and GGT in a standard procedure, realised in certified laboratory. Breathalyzer test (BT) on alcohol was conducted during the treatment entry examination with calibrated detector Alco-sensor IV CM. Two subgroups according to positive and negative presence of alcohol were created for comparison of biological markers. Comparison of biological markers was also conducted between the subgroups of males and females.

■ RESULTS

Increased values of GGT had 272 (50%) patients and abnormal MCV value was found in 239 (44%). At least one laboratory marker was increased in 348 (64%) patients at the time of their admission to treatment (Figure 1).

There was a positive BT result in 119 (22%) patients. The two subgroups were equal in terms of gender and age. The subgroup with positive BT consisted of 71% males, average age 44 ($SD \pm 11.4$) years. In the subgroup with negative BT were 72% males, average age 45 ($SD \pm 12.3$) years. Abnormal values of GGT were found in 79 (66%) and MCV in 67 (56%) of the patients in the subgroup with positive BT. At least one increased marker was found in 93 (78%) patients (Figure 2).

Comparison of biological markers between the subgroups of males and females revealed

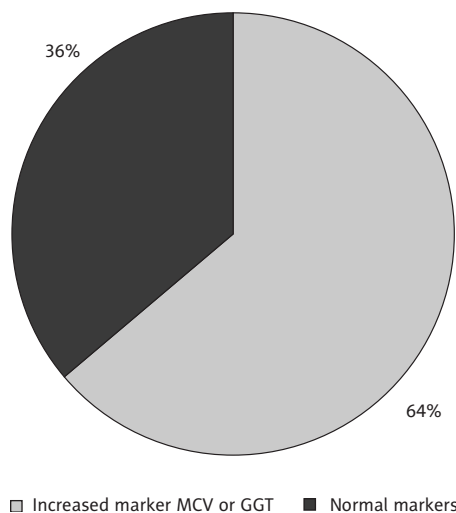


Figure 1. Increased laboratory markers at treatment admission – whole sample

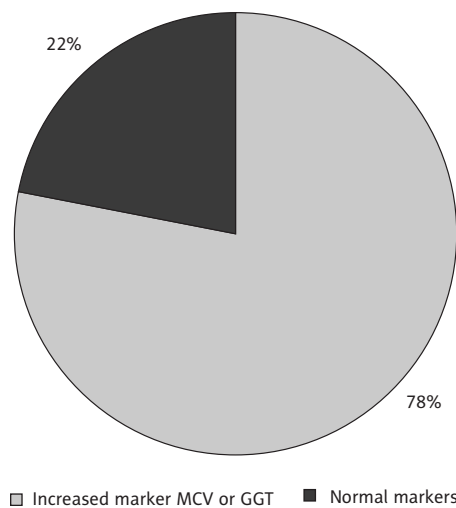


Figure 2. Increased laboratory markers in patients with positive breathalyzer test

that GGT was increased in 194 (50%) males and 74 (49%) females. The difference was statistically nonsignificant. MCV was increased in 154 (40%) males and 85 (56%) females. The difference was statistically significant ($p \leq 0.01$).

■ DISCUSSION

Heavy alcohol consumption with or without signs of alcohol dependence is not only the primary cause of alcohol-specific medical conditions like alcoholic liver disease but contributes

to the development and worsening of many other medical conditions and hampers treatment effectiveness. Physiological macrocytosis is seen in pregnancy, new-borns and infants. Macrocytosis without anaemia may sometimes be a normal variant found in members of the same family, suggesting a genetic predisposition. Studies have further shown a relationship between GGT levels and a variety of extra hepatic chronic diseases, which are associated with oxidative stress, including cardiovascular diseases, diabetes, metabolic syndrome, cancer, neurodegenerative diseases and rheumatoid arthritis [19-21]. While the specific role of alcohol as a possible trigger for such morbidity has remained unknown. Patients visiting doctors are reluctant to self-report and minimise harmful drinking. In alcohol use disorders, biomarkers should be used not only to confirm the aetiology but also to help the interactions between physicians and patients on raising the issue of alcohol use as a possible cause of adverse health outcomes. Therefore recommended biological laboratory markers are useful screening tool in this respect. GGT and MCV of erythrocytes are easy to be tested in daily out-patient practice by general practitioners and specialists. The knowledge of their sensitivity is important for the interpretation and decision-making in medical practice.

The majority of patients with diagnosis of alcohol dependence had at least one of two biological laboratory markers of heavy drinking positive at treatment entry in our study. But their sensitivity was limited, which is consistent with the findings of the others [5-7]. This is supporting the assumption that the alcohol dependence cannot be diagnosed solely based on the abnormal values of laboratory markers: GGT and MCV, and not only because of their limited specificity. One third of the patients with presence of clinical characteristics fulfilling criteria for alcohol dependence had normal values of the abovementioned laboratory markers in that time. Their proportion was smaller (22%), but still significant from clinical perspective in a subgroup with positive BT. The patients reporting for a treatment under the influence of alcohol are mostly those with higher level of alcohol dependence. They are in more serious condition. Here it is important to mention that this was in the situation where patient entries were on voluntary basis. Diag-

nosis was based on their self-reported histories, screening questionnaires, as well as on the clinical expertise, if biological markers were negative. The assessment is particularly difficult in the forensic situations, when alcohol use is underreported by the subject intentionally and no elevation of biological markers is present. Different sensitivity of laboratory markers in alcohol dependent patients is also influenced by other factors, which we were not able to control for in our study. Yokoyama *et al.* [22] discovered the important role of genetic polymorphism of alcohol dehydrogenases in different MCV values among chronic drinkers. Sakutata [23] observed, that chronic consumers of spirits have significantly higher MCV and GGT values comparing to chronic drinkers of fermented spirits (35% vs. 16% for MCV; 38% vs. 27% for GGT). Also sample preselection among patient population plays an important role in biomarkers sensitivity [24]. This can also be influenced by drinking habits and culture in the selected population and by historical setting. In Slovak conditions over a quarter of the century ago Rusnak *et al.* [25] found out that people diagnosed with alcohol dependence manifested increased sensitivity of both markers (GGT 72%, MCV 78%).

The significant difference between male and female patients in the occurrence of abnormal MCV values might be supporting the hypothesis expressed by Mundle *et al.* [26] that bone marrow of females is more sensitive to become damaged by chronic drinking. Only a few data exist on this issue in this respect in the literature. Further research can elicit and validate the finding.

Increased laboratory markers are tool for diagnostics, but most importantly for evaluation of treatment effectiveness, when following the dynamics of their change during treatment of alcohol use disorders [27]. Furthermore, in the detection of relapses the baseline values of CDT and GGT should be measured and compared on individual basis to the pre-treatment values [17]. It is important to plan repeated examinations with regard to time needed for marker normalisation.

■ CONCLUSIONS

A significant proportion of the patients with alcohol dependence has no increased values of MCV and GGT at the time of treatment admission. The findings indicate the important, but only supportive role of these laboratory markers in the assessment of alcohol use disorders and especially in the diagnosis of alcohol dependence. Clinical diagnostic assessment of the patient's condition according to valid diagnostic criteria of ICD-10/WHO has a crucial role in the diagnostic process.

■ HIGHLIGHTS

- Laboratory tests for MCV and GGT are broadly used in diagnosis of alcohol dependence.
- A significant proportion of patients with alcohol dependence had normal values of MCV and GGT at the time of treatment admission.
- Laboratory markers have an important, but only supportive role in alcohol use disorder assessment.

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■ LIST OF ABBREVIATIONS

- ALT – alanine aminotransferase
- APA – American Psychiatric Association
- ALD – alcoholic liver disease
- CDT – carbohydrate-deficient transferrin
- CRP – C-reactive protein
- CTDD – Centre for Treatment of Drug Dependencies
- DSM-5 – The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
- BT – breathalyzer test
- GGT – gamma-glutamyltransferase
- MCV – mean corpuscular volume
- NAFLD – non-alcoholic fatty liver disease
- ICD-10 – International Classification of Diseases
- WHO – World Health Organization

Conflict of interest/Konflikt interesów

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Ethics/Etyka

The work described in this article has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) on medical research involving human subjects, EU Directive (210/63/EU) on protection of animals used for scientific purposes, Uniform Requirements for manuscripts submitted to biomedical journals and the ethical principles defined in the Farmington Consensus of 1997.

Treści przedstawione w pracy są zgodne z zasadami Deklaracji Helsińskiej odnoszącymi się do badań z udziałem ludzi, dyrektywami UE dotyczącymi ochrony zwierząt używanych do celów naukowych, ujednoliconymi wymaganiami dla czasopism biomedycznych oraz z zasadami etycznymi określonymi w Porozumieniu z Farmington w 1997 r.

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