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N-ACETYL-β-HEXOSAMINIDASE B, THE SPECIFIC MARKER OF POST ALCOHOLIC LIVER DAMAGE

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Abstract

Introduction: There is still a lack of proper markers for monitoring post-chronic alcohol drinking liver injury. The aim of our research was to compare the usefulness of the serum HEX B, AST and ALT activities as markers of liver damage in ALD.
Material and Methods: The study group consisted of 31 persons with alcohol abstinence syndrome; control group consisted of 33 blood donors. The activity of ALT, AST and HEX B were determined by spectrophotometric methods in the serum of all participants.
Results: In the serums of the control group, levels of ALT, AST and HEX B were in the normal range and amounted: 22.0 ±11.4 U/L; 20.8 ±9.9 U/L and 64.4 ±48.0 nKat/L respectively, whereas in the study group, activities were significantly higher than in the controls, and amounted: ALT – 75.3 ±82.4 U/L, AST – 96.8 ±102.6 U/L, and HEX B - 226.0 ±134.5 nKat/L.
Conclusion: In persons with post-alcoholic liver injury, serum HEX B activity is a better marker of liver injury than ALT and AST.

Key words: alcohol dependence, liver injury markers, ALT, AST, HEX B.

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Introduction

The World Health Organization (WHO) reports increasing ethanol drinking as one of the main causes for the increase in morbidity and disability among high developed countries (particularly in Eastern Europe) [1]. Ethanol, which is soluble in water and lipids, is absorbed and metabolized mostly in the gastrointestinal tract and liver. In the liver, ethanol activates the Microsomal Ethanol Oxidizing System (MEOS) resulting in an increase of acetic aldehyde and free radicals toxic actions, and creates qualitative alimentary deficits (Fig. 1).

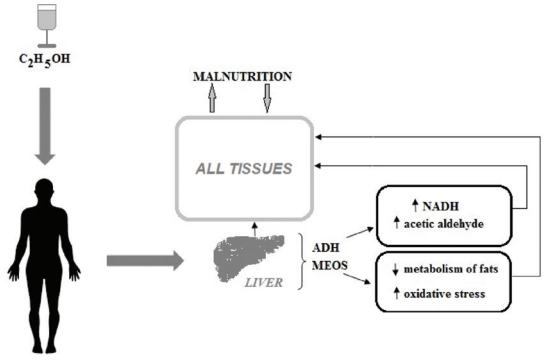

Fig. 1. Alcohol abuse; ADH-alcohol dehydrogenase; MEOS - Microsomal Ethanol Oxidizing System; NADH – Nicotinamide adenine dinucleotide, reduced form.

The risk of alcoholic liver disease (ALD) appears after daily consumption of more than 80g ethanol [2,3], and women are more vulnerable than men [4]. The increased susceptibility for alcoholic liver injury among women probably results from lower water contents and lower activity of alcohol dehydrogenase (ADH), which is associated with higher production of toxic acetaldehyde [5]. Prevalent forms of alcoholic liver disease include: liver steatosis, alcoholic liver inflammation and hepatic cirrhosis [6,7,8]. Alcoholic liver damage depends on one's sex, the amount of alcohol consumed, period of time of alcoholic drinking and nature of condiments and drugs. Harm from alcohol use is significantly diminished by early diagnosis and proper therapy [4]. Unfortunately, alcohol dependence is recognized in less than 50% of the drinking people, but far fewer alcohol dependent people are properly treated [9,10]. Failure in the diagnosis and treatment of ALD largely stems from a lack of cooperation among alcohol dependent people with medical doctors. Therefore, blood and salivary laboratory tests that detect alcoholic metabolic changes play a crucial role in diagnosis and treatment of ALD. Waszkiewicz et al. [11,12,13] distinguished "traditional" and "new" biomarkers of alcohol dependence. "Traditional" biomarkers of alcohol dependence included blood serum concentration of: ethanol, carbohydrate-deficient transferrin (CDT), high density

lipoprotein (HDL), uric acid; activity of gamma-glutamyl--transpeptidase (GGTP), aspartate (AST) and alanine (ALT) transferases, and mean corpuscular volume (MCV).

"New" markers of alcoholic disease, according to Waszkiewicz et al. [12], included serum activity of isoenzyme B of the N-acetyl-β-hexosaminidase (HEX B). An increase HEX B activity in the serum of alcohol drinkers precedes an increase in activity of GGTP, AST and ALT. Sensitivity to serum HEX B activity determination amounted to 70-90%. The activity of serum HEX B activity is two times higher in heavy drinkers as compared to serum HEX B activity of people drinking alcohol sporadically. Heavy drinker's serum HEX B activity was significantly diminished after two weeks of abstinence. Serum HEX B activity significantly increases after a single intake of more than 2g of ethanol/kg of body weight, while serum ASP and ALT activities remain in normal ranges, and only de Ritis index is slightly increased [14]. However, we did not find any data concerning the direct comparison of AST, ALT and HEX B activities in the same serums derived from heavy drinkers during post alcohol drinking period.

The aim of our research was to compare the usefulness of the serum HEX B, AST and ALT activities as markers of liver damage in ALD.

Material and methods

Study Group

The control group (C) consisted of 33 (10♀, 23♂) blood donors from the Regional Blood Donors and Hemotherapy Centre in Poznań, Poland. Blood donors were verified by Regional Blood Donors Centre and signed formal consent for participation in the study. Exclusion criterion included chronic diseases, and chronically taking drugs, such as pain-killers. The study group (S) consisted of 31 patients (18-65 years old) of the Wanda Błeńska Toxicological Department with Center of Toxicological Information of Franciszek Raszeja, City Hospital in Poznań. The patients were treated for alcohol-withdrawal syndrome. Some of them were also dependent on benzodiazepines, and all patients received benzodiazepine before blood collection for biochemical determinations. Qualification for the research group was based on standard anamnesis and written consent of each patient to participate in the study, according to the local Bioethical Commission.. The patients, in terms of their treatment for alcohol-withdrawal syndrome, were classified according to Clinical Institute Withdrawal Assessment for Alcohol, Revised (CIWA - AR) scale [15], constituting enquiry form for evaluating the symptoms of alcoholic abstinence syndrome [15]. Depending on scoring of the CIWA - AR scale, the following degree of abstinence syndrome were distinguished:

- below 8 points – mild,
- 8-15 points – moderate,
- above 15 points – substantial degree of symptoms and / or complications of alcohol abstinence syndrome.

The patients that qualified for the research group had mild to moderate symptoms of alcohol abstinence syndrome.

Laboratory Tests

Blood was collected from each participant into disposable standard vacuum aspiration sets (S-Monovette, Sarstedt, Poland). Collected blood samples were stored for 15-20 min in aspiration sets at laboratory temperature, centrifuged 10 min at 3,000 rev/min with a centrifuge (MPW-350R, MPW Medical Instruments, Poland), transferred to plastic tubes, and stored (-80°C) until testing.

Serum ALT was determined by enzymatic method of Wróblewski et al. [16]; AST was determined by Karmen et al. method [17] in Bergmayer's et al. modification [18] at Cobas Integra® 400 plus (Switzerland). Both methods were based on changes in absorbency at 340 and 405 nm reflected transformation NADH to NAD+ proportional to ALT and AST activity. Serum HEX B activity was determined according to Chojnowska et al. [19]. Enzymatic determinations were performed in duplicates at microplates (Greiner 96-U Transparent, Germany) with the microplate reader Infinite ® 200 PRO (TECAN, Switzerland).

Statistical analysis

The results were analyzed using Statistica 10 (StatSoft, Poland). Normality and Kruskal- Wallis tests were used for determining significant differences between groups. The area under an ROC curve was used for evaluation the usefulness of tests (the greater the area means a more useful test). Statistical significance was set at $p < 0.05$.

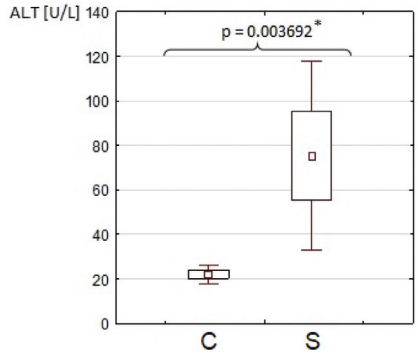

Fig. 2: Serum ALT activity in post-alcoholic syndrome; C - control, S - study group post--alcoholic syndrome.

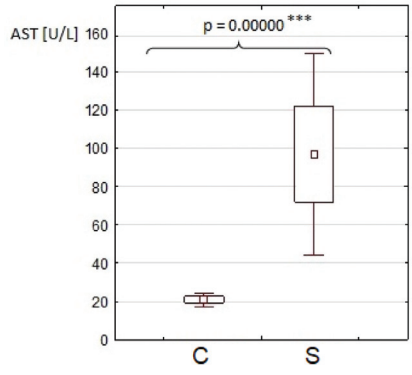

Fig. 3. Serum AST activity in post-alcoholic syndrome; C - control, S - study group of post--alcoholic syndrome.

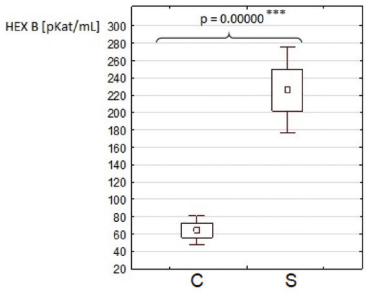

Fig. 4. Serum HEX B activity in post-alcoholic syndrome; C - control, S - study group of the post-alcoholic syndrome.

The results from the serum determination of ALT, AST and HEX B in control and post-alcoholic syndrome group did not overlap, but the differences between the control and study group were the largest in HEX B.

There were no significant differences between area under the ROC curve (AUC) between HEX B (0.942) and AST (0.966) and ALT (0.856), however HEX B had a slight tendency to have better accuracy than ALT (p=0.212, CI95% 0.048-0.220). We found a better accuracy of AST than ALT (p=0.04, CI95% 0.003-0.215) (Fig. 5).

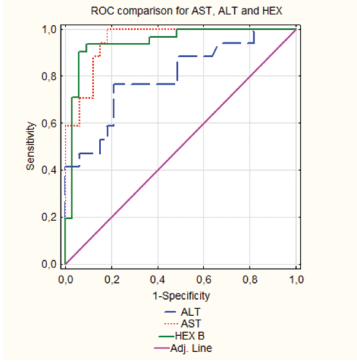

Fig. 5. The accuracy values for AST, ALT and HEX B activity in alcohol dependent persons.

Analyzed ROC data shown that AST had excellent sensitivity and good specificity, ALT and AST had fair sensitivity and specificity, and HEX B and AST had excellent sensitivity and specificity (Table 1).

Table 1. The cut-off, sensitivity and specificity values for AST, ALT and HEX B activity. aCut-off values for enzyme activities were obtained for alcohol dependent persons by ROC analysis.

	Marker	Cut-off value[a]	Sensitivity	specificity
Activity	AST	26	100%	81%
	ALT	31	76%	78%
	HEX B	102	90%	93%

Discussion

It was reported that alcoholic beverages are prominent legal drugs in the world, and alcohol abuse is the most frequent cause of liver injury [20,21]. It was estimated that in Poland (38,434 million inhabitants), about 2-2.5 million people abuse alcohol and about 800,000 people are addicted to alcohol [22]. Alcoholic liver disease covers a whole spectrum of acute and chronic diseases, including alcoholic fatty liver, alcoholic liver inflammation, and liver cirrhosis. The proper classification of ALD is based on anamnesis, or clinical examination and results of the laboratory tests. However, up till now, none of the laboratory tests used are specific to alcoholic liver damage. In ALD, we noted a significant increase in serum AST and somewhat smaller, but significant, increase in serum ALT activity (Figs. 2 & 3), which is in agreement with other research [11,12,13,14]. A very sensitive and promising marker of alcohol - induced liver injury is serum HEX B activity [12,13]. Serum HEX B activity significantly increases in chronic alcohol drinking, and return to normal values after 7-10 days of abstinence [12]. In our research group, all patients, that chronically used alcohol, stopped drinking from a few to several hours before collecting blood for laboratory tests, which explained significantly increased serum activity of AST (Fig. 2), ALT (Fig. 3) and HEX B (Fig. 4). The most significant differences between the data of the studied and control group were found in the cases of AST (Fig. 2) and HEX B (Fig. 4). Our results confirmed the conclusion of Waszkiewicz et al. [12,13] that HEX B is a promising and sensitive marker of alcohol addiction. Differences between serum HEX B activity of chronic alcohol drinkers and the control group were the highest in HEX B (Fig. 4), lesser in AST (Fig. 3), and the least in ALT (Fig. 2). In the study group, serum HEX B activity (Fig. 4) had the lowest coefficient of variation in comparison to AST (Fig. 3) and ALT (Fig. 2).

During evaluation of our results it should be taken into consideration that all our patients were treated with benzodiazepines before the collection of blood, and some of them were benzodiazepine dependent. It was reported [23,24] that benzodiazepines cause liver damage resulting in an increase in the serum aminotransferase activities. In order to confirm specificity of HEX B increase in ALD, further investigation is necessary to distinguish if the liver damage accompanied by significant increases in blood ALT, AST and HEX B activity were caused by ethanol, benzodiazepines, or a combined action of ethanol and benzodiazepines.

Conclusions

We showed, that HEX B has excellent accuracy as shown in the literature for AST and slightly better accuracy than ALT. HEX B had the best specificity of analyzed enzymes. Therefore, HEX B may be treated as an excellent alcohol dependence marker, better than AST and ALT.

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