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Original Article

Acute exogenous intoxications and homocysteine

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Abstract

Inroduction: Oxidative stress is an important pathogenetic factor in a number of socially significant diseases, including the acute exogenous poisoning. Homocysteine is a sulfur-containing amino acid synthesized on the basis of methionine, which plays an important role as an oxidizing agent in the human body. As such a factor, it was the monitored subject of this study.

Aim: To measure the level of homocysteine in acute exogenous poisoning with alcohol, heroin and cerebro-toxic drugs.

Materials and methods: This is a prospective longitudinal study including 118 patients with moderate or severe acute poisoning with cerebro-toxic drugs (n=45), alcohol (n=40), heroin (n=33) and a "control group" (n=35). Clinical laboratory tests were performed according to the standards of a clinical laboratory. In the statistical analysis we used alternative and variance analysis, parametric methods for hypothesis assessment, and nonparametric methods for normal distribution.

Results and discussion: The results showed that for the three groups of intoxications, the average homocysteine levels were higher than those of the control group ($p<0.001$). The intergroup comparison criterion for normal distribution showed that the changes in patients with alcohol intoxication ($u=3.39$; $p<0.001$) and heroin intoxication ($u=2.00$; $p<0.001$) were highly statistically significant without correlating with the severity of the poisoning ($p>0.05$).

Conclusion: There is a risk of oxidative stress in intoxication with alcohol and narcotics. A reliable marker for the complex evaluation of oxidative stress in people is monitoring the serum level of homocysteine and its careful interpretation.

Keywords

homocysteine, oxidative stress and acute exogenous intoxications

INTRODUCTION

Homocysteine is defined as an indisputable factor – an oxidant that causes oxidative stress in the body.[1-6]

In recent years, reports have emerged in the scientific literature about the role of oxidative stress as a major pathogenetic factor in a number of socially significant diseases, including some exogenous intoxications.[7,8]

Homocysteine is an essential sulfur-containing amino acid discovered in 1932 by Vegnaund. Its metabolism stands at the intersection of two pathways: remethylation and trans-sulfuration. Under physiological conditions, the remethylation is a major pathway for the metabolism of homocysteine to methionine. Its exchange is closely related to that of methionine and depends on the amount of vitamins B6 and B12, and folate in the body, as well as on a number of enzymes. A primary role plays the 5,10-methylenetetra-

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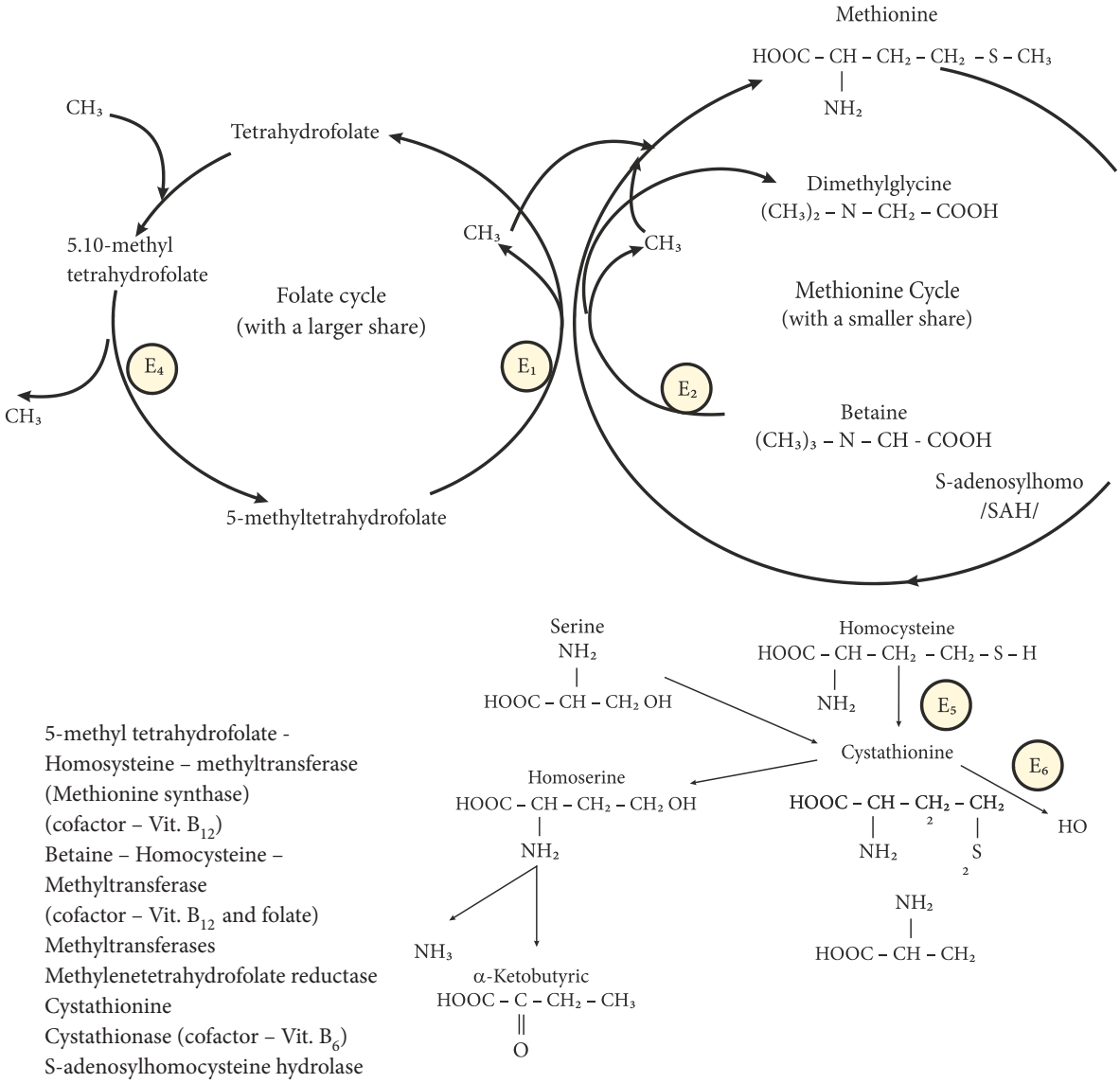
hydrofolate reductase **(Fig. 1)**. In the body it is represented in several forms – reduced, oxidized (5-10%) and mixed (70-80%), as physiologically active is the reduced – 1% of the total.[1] In practice, it is the total homocysteine in serum that is usually determined, as there is a positive correlation with the reduced form and it is a significant oxidative factor in the body.[1,9,10]

Numerous epidemiological and clinical studies over the past two decades in different countries have shown that the hyperhomocysteinemia is an independent risk factor in the development of atherosclerosis, ischemic heart disease, cerebrovascular disease, diabetes mellitus, renal failure, venous thrombosis, etc. by enhancing the oxidative processes.[2,6,9,11-13] Various pathogenetic mechanisms are discussed – direct toxic effect on the endothelium (endothelial dysfunction), oxidative damage to various cellular structures, activated platelet aggregation, increased smooth muscle proliferation, etc.[10,14,15] Undisputed is the role of oxidative damage as a key factor among the mechanisms.[3-5]

In the field of experimental and clinical toxicology, hyperhomocysteinemia is mainly associated with the systematic alcohol dependence (SAD).[16-23] In many countries, including Germany and France, it is used as a marker for chronic alcoholism.[24]

Chronic alcoholics often suffer from specific deficiencies of micronutrients, including the following vitamins: folate, vitamin B-6 and vitamin B-12. The possible link between homocysteine and alcoholism stems from the fact that the metabolism of homocysteine is closely related to the metabolism of these three vitamins. In fact, homocysteine stands at the intersection of two pathways: methylation and trans-sulfuration. Therefore, hyperhomocysteinemia is the result of aggression of the toxic factor on structures responsible for impaired methionine metabolism due to enzymatic or vitamin deficiency.

There is a direct correlation between the level of homo-



E_1 – 5-methyl tetrahydrofolate - Homosysteine – methyltransferase (Methionine synthase) (cofactor – Vit. B_{12})
E_2 – Betaine – Homocysteine – Methyltransferase (cofactor – Vit. B_{12} and folate)
E_3 – Methyltransferases
E_4 – Methylenetetrahydrofolate reductase
E_5 – Cystathionine
E_6 – Cystathionase (cofactor – Vit. B_6)
E_7 – S-adenosylhomocysteine hydrolase

Figure 1. Homocysteine exchange in the human organism (by Zdr. Taralov, T. Tzvetkova, 2000; Miner, Jacobsen, D'Angelo modification).

cysteine in the serum and the amount of methionine in the food and vice versa – in regards to the vitamins contained in it, which are enzymatic cofactors in the exchange of homocysteine (B-12, B-6, and folate).

In SAD, impaired homocysteine remethylation (folate and B-12 dependent) is a predictive cause of homocysteine elevation, whereas vitamin B-6 dependent trans-sulfonation is less important, despite the proven effect of selective vitamin B-6 deficiency on homocysteine trans-sulfonation. The homocysteine levels, whether elevated or within reference values, correlate inversely with those of folate (stronger) and vitamins B-12 and B-6 (weaker).

These dependencies are particularly pronounced in patients with alcohol intoxication, and especially in chronic alcohol abuse. Folate deficiency is thought to be the most important cause of hyperhomocysteinemia in SAD.

Over the last few years, studies have been conducted to determine the place and role of homocysteine in various acute toxicological conditions (including alcohol, drug addictions, etc.).[10,17,18] In the accessible literature, these studies are limited and too contradictory, so we set ourselves the following goals:

1. Tracking the level of homocysteine in some acute exogenous poisonings – alcohol, narcotics (heroin) and cerebro-toxic drugs.

2. Determining the correlation between the type of toxic substance and the severity of the intoxication.

Namely, as an important oxidant factor, the homocysteine was the subject of monitoring in this study.

MATERIALS AND METHODS

A prospective longitudinal study of 118 patients with moderate and severe acute exogenous poisoning with alcohol, narcotics and cerebro-toxic drugs, treated in Department of Clinical Toxicology of the St George University Hospital, Plovdiv over a period of 2 years. Thirty-five healthy sex- and age-matched men and women were included in the control group.

Distribution:

- By sex: 38 women and 80 men
- By age: from 17 to 72 years old

Severity: The 3-step classification of Al. Monov (1996) was used.

Clinical and laboratory tests were performed at the Central Clinical Laboratory of St George University Hospital Plovdiv.

Statistical analysis of data was performed using SPSS ver. 13.0, at a level of significance $p<0.05$. The following analyses were used:

1. Alternate and variance analysis – standard deviation, minimum, maximum and range;

2. Parametric methods for assessment of hypotheses – ANOVA, F-criteria;

3. Nonparametric methods for normal distribution

Methodology for determining the serum level of homocysteine

Reagents: FPIA (Fluorescence polarization immunoassay) – kit by Abbott Laboratories USA.

Analyser: AxSYMTM system.

Principle: homocysteine from the sample forms immune complex with the homocysteine-monoclonal antiserum and the homocysteine-fluorescein conjugate. The polarized light excites the fluorescein. The resulting polarization is inversely proportional to the concentration of the tested component.

Analytical reliability: sensitivity – 0.8 μmol/l; non-reproducibility in series – CV<4.5%; non-reproducibility in time – CV<4.6%; recovery: no dilution – 96.6-105.0%; with a dilution of 1:2 or 1:32 – 86.4-102.2%; the method is specific, no cross-reaction with L-cysteine (100.0 mM), with adenosine (5.0 mM), with glutathione (100.0 mM) was detected.

Reference interval: men: 5.90–16.0 μmol/l, women: 3.36–20.44 μmol/l

RESULTS AND DISCUSSION

Analysis of data from monitoring serum homocysteine traceability showed that in all three observed groups of patients with acute poisoning with cerebro-toxic drugs, alcohol and heroin, the average homocysteine levels were significantly higher than those of the control group **(Table 1)**. This necessitated an intergroup comparison criterion for normal distribution. The comparison showed the existence of statistically significant changes between the control group and the groups of drug addicts patients and patients with acute alcohol poisoning (u=2.00 control group/heroin and u=3.39 control group/alcohol) – $0.05>p<0.001$.

Table 1. Serum level of homocysteine (μmol/l) in the observed groups

Indicators Groups	Number	0 ± S0	Sx	F	p
Heroin	33	13.76±2.41	13.62		
Alcohol	40	19.78±2.75	17.37		
Drugs	45	14.61±1.67	11.22	4.75	<0.01
Control group	35	8.91±0.23	1.41		

The obtained results are logical, given that in the field of toxicology hyperhomocysteinemia is associated with SAD.[16-23] In many countries (e.g. Germany, France), it is used as a marker for chronic alcoholism.[24] The latter disturbs the transmethylation and trans-sulfuration of homocysteine, resulting in doubling its level in the blood.[6,25] In the case of alcohol and wine abusers, it is higher than that for the beer consumers.[24]

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Twenty patients with acute alcohol intoxication had SAD dating back more than 3.5 years. The analysis of our results on homocysteine levels in acute poisoning without SAD and with SAD, showed a significant increase in homocysteine in the second group **(Table 2)**.

Table 2. Homocysteine levels (μmol/l) in acute poisoning with SAD and without SAD

Acute alcohol poisoning	Number	Indicators		
		0 ± S0	t	p
Without SAD	20	10.36±0.80	3.69	< 0.001
With SAD	20	29.21±4.59		

Table 3 shows that homocysteine levels in individuals without SAD ('real' alcohol poisoning) were not significantly higher than those in the control group.

Table 3. Homocysteine levels (μmol/l) in acute poisoning without SAD and control group

Acute alcohol poisoning	Number	Indicators		
		0 ± S0	t	p
Without SAD	20	10.36±0.80	2.12	> 0.05
Control group	35	8.91±0.23		

Significant hyperhomocysteinemia is also established in SAD individuals compared with the control group **(Table 4)**.

Table 4. Homocysteine levels (μmol/l) in acute poisoning with SAD and control group

Acute alcohol poisoning	Number	Indicators		
		0 ± S0	t	p
With SAD	20	29.21±4.59	5.88	< 0.001
Control group	35	8.91±0.23		

This data outlines a tendency for expressed hyperhomocysteinemia in patients with SAD. The presence of a statistically significant change in the level of homocysteine in the total number of cases of acute alcohol poisoning is due to the presence of a significant number of patients with SAD (50%) among them.

Similar results were reported also by other authors who found an increased level of homocysteine in patients with SAD.[16-23]

There are just a few studies of homocysteine in acute poisoning without SAD. After a review of the relevant literature, we found only one similar study, B. Hultberg[26] who also found no hyperhomocysteinemia in "pure" acute alcohol poisoning.

To assess the dependence between the type of toxic substance and the severity of intoxication, we used cross tabulation on these categories **(Table 5)**.

We did not find a statistically significant correlation between the severity of acute poisoning and the type of intoxication.

The analysis of our data showed a statistically significant increase of homocysteine in patients with heroin intoxication compared to that in the control group (u=2.00, p<0.001), without it correlating with the severity of acute poisoning.

Hyperhomocysteinemia in heroin poisoning is confirmed by the studies of some researchers (RS Williams et al.[27,28]), which is associated with a more severe degree of these intoxications.

The topicality of the study of this essential oxidant is due to the fact that homocysteine is considered to be an important factor in determining cardiac and thrombotic risk in patients with narcotic addiction (severe cocaine and heroin intoxications) predisposing to toxic cardiomyopathy.

The test we performed demonstrate that the average homocysteine levels in acute poisoning with cerebro-toxic drugs are higher than those of the control group, but fail to reach statistical significance **(Table 1)**. This can be most likely accounted for by the fact that the majority of acute

Table 5. Homocysteine (μmol/l) - type of toxic substance and severity of acute poisoning

Group	Severity	Number	0 ± S0	Sx	t	p
Heroin	moderate	13	11.58±0.71	2.46	0.90	> 0.05
	severe	20	15.08±3.84	17.15		
	all	33	13.76±2.41	13.62		
Alcohol	moderate	25	18.62±4.00	20.01	0.61	> 0.05
	severe	15	21.71±3.14	12.17		
	all	40	19.78±2.75	17.37		
Medications	moderate	30	15.28±2.37	13.00	0.68	> 0.05
	severe	15	13.29±1.69	6.56		
	all	45	14.61±1.67	11.22		
Total	moderate	68	15.86±1.84	15.07	0.25	> 0.05
	severe	50	16.53±1.90	13.45		
	all	118	16.15±1.33	14.35		

poisoning with drugs is not toxicomania.

We could not compare this indicator with studies by other authors due to the lack of data from the scientific literature on homocysteine testing in cases of poisoning with cerebro-toxic drugs.

We can conclude that the increased levels of homocysteine in the respondents are due to the disturbed metabolism of methionine or due to processes that inhibit the homocysteine metabolism in acute exogenous alcohol and narcotics.

FINDINGS

1. A significant increase of homocysteine was found in individuals with acute alcohol intoxication, particularly expressed in those with SAD and absent in the "pure" alcoholic intoxication.
2. High levels of homocysteine are also registered in acute poisoning with heroin.
3. Hyperhomocysteinemia was found in people with chronic administration of toxic agents – alcohol and heroin.
4. The homocysteine level in the three acute poisoning groups is not dependent on the severity of the intoxication.

CONCLUSION

Exogenous poisonings are aggressive "agents" that create oxidative stress in the body. Experimental and clinical observations have shown that high levels of free radicals or the decreased activity of the antioxidant system is a serious risk factor in relation to the rate of development and severity of organ damage in acute poisoning. That is why the study of markers of oxidative stress (such as homocysteine) is of great practical value in exogenous intoxication.

The literature expresses opposing views on the use of oxidants or a package of such, as a reliable marker for assessing oxidative stress in humans. It is logical to assume that the most in-depth understanding gives a simultaneous study of more oxidant indicators, which is laborious, expensive and practically impossible. All this points to the obvious need to measure single oxidants and their careful interpretation, which would allow a complex assessment of oxidative stress in the body.

Based on this study, we can conclude that:

– Exogenous toxins are a significant factor causing aggressive oxidative stress in the body.

– Hyperhomocysteinemia as an oxidative factor may worsen some acute exogenous intoxications.

REFERENCES

1. Taralov Z, Tsvetkova T. Hyperhomocysteinemia and its clinical manifestations. Contemporary Medicine (2001) LII, Book 3: 26-32
2. Frinkelsteine JD. Homocysteine: a history in progress. Nutr Rev 2000; 58:193–204.
3. Kanani PM, Sinkey CA, Browning L, et al. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocysteinemia in humans. Circulation 1999; 100:1161–8.
4. Loscalzo J. The oxidant stress of hyperhomocysteinemia. J Clin Invest 1996; 98(1):5–7.
5. Lin L, Du H. An anthraquinone compound and its protective effects against homocysteine-induced cytotoxicity and oxidative stress. Spectrochim Acta A Mol Biomol Spectrosc 2018; 202: 314–8.
6. Kumar A, Palfrey HA, Pathak R, et al. The metabolism and significance of homocysteine in nutrition and health. Nutr Metab (Lond) 2017;14:78.
7. Betteridge DJ. What is the oxidative stress? Metabolism 2000;49(2): 3-8.
8. Sies H. Oxidative stress: introduction. In: Sies H, editor. Oxidative stress. New York: Academic Press; 1985. p. 1–8.
9. Miner S, Ewroski J, Cole D. Clinical chemistry and molecular biology of homocysteine metabolism: an update. Clin Biochem 1997; 30(3):189–201.
10. Esse R, Barroso M, Tavares de Almeida I, et al. The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art. Int J Mol Sci 2019; 20(4): p. E867.
11. Wu X, Zhang L, Miao Y, et al. Homocysteine causes vascular endothelial dysfunction by disrupting endoplasmic reticulum redox homeostasis. Redox Biol 2019; 20: 46–59.
12. Moretti R, Caruso P. The controversial role of homocysteine in neurology: from labs to clinical practice. Int J Mol Sci 2019;20(1): p. E231.
13. Bhatia P, Singh N. Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. Fundam Clin Pharmacol 2015;29(6):522–8.
14. Chambers JC, McGregor A, Jean-Marie J, et al. Acute hyperhomocysteinemia and endothelial dysfunction. Lancet 1998;351:36–7.
15. Tawakol A, Omland T, Gerhard M, et al. Hyperhomocysteinemia is associated with endothelium dependent vasodilatation in humans. Circulation 1997;95:1119–21.
16. Bleich S, Degner D, Wiltfang, et al. Elevated homocysteine levels in alcohol withdrawal. Alcohol Alcohol 2000;35(4):351–4.
17. Cravo M, Camilo ME. Hyperhomocysteinemia in chronic alcoholism: relation to folate, vitamin B12 and vitamin B6 status. Nutrition 2000;16(4):296–302.
18. Stickel F, Choi SW, Kim YI, et al. Effect of chronic alcohol consumption on total plasma homocysteine level in rats. Alcohol Clin Exp Res 2000;24(3):259–64.
19. Trotti R, Carratelli M, Bo P, et al. Pro-oxidant activity and methionine metabolism in chronic abusers: relationships to alcohol withdrawal and folate administration. Panminerva Med 2004; 46(3):161–4.
20. Bayerlein K, Hillemacher T, Reulbach U, et al. Alcoholism-associated hyperhomocysteinemia and previous withdrawal seizures. Biol Psychiatry 2005;57(12):1590–3.
21. Lutz UC. Alterations in homocysteine metabolism among alcohol dependent patients--clinical, pathobiochemical and genetic aspects. Curr Drug Abuse Rev 2008;1(1):47–55.
22. Karagülle D, Heberlein A, Wilhelm J, et al. Biological markers for alcohol withdrawal seizures: a retrospective analysis. Eur Addict Res 2012;18(3):97–102.
23. Prior PL, Vaz MJ, Ramos AC, et al. Influence of microelement con-

centration on the intensity of alcohol withdrawal syndrome. Alcohol Alcohol 2015;50(2):152–6.
24. Schmidt LG, Bleich S, Boening J, et al. Advances in alcoholism research in Germany. Alcohol Clin Exp Res 2005;29(7):1282–7.
25. Bleich S, Hillemacher T. Homocysteine, alcoholism and its molecular networks. Pharmacopsychiatry. 2009;42 Suppl 1:S102–9.
26. Hultberg B. Elevated plasma homocysteine in acute alcohol intoxication. Annual Meeting of the American Congress of Experimental and Clinical Toxicology, Chicago IL, USA, 1995.
27. Williams RS, Maggiore JA, Shah SM, et al. Homocysteine levels in cocaine abusers. J Toxicol Clin Toxicol 1999; 37(5):671.
28. Williams RS, Maggiore JA, Shah SM, et al. Effects of cocaine abuse on free and total plasma homocysteine. Clin Chem 1999 45(suppl 6):

Острые экзогенные интоксикации и гомоцистеин

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Резюме

Введение: Окислительный стресс является важным патогенетическим фактором в ряде социально значимых заболеваний, включая острое экзогенное отравление. Гомоцистеин является серосодержащей аминокислотой, синтезированной на основе метионина, который играет важную роль в качестве окислителя в организме человека. В качестве такого фактора он является наблюдаемым объектом данного исследования.

Цель: Измерить уровень гомоцистеина при остром экзогенном отравлении алкоголем, героином и церебротоксическими препаратами.

Материалы и методы: Это проспективное долгосрочное исследование с участием 118 пациентов с умеренным или тяжёлым острым отравлением церебротоксическими препаратами (n=45), алкоголем (n=40), героином (n=33) и контрольной группой (n=35). Клинические лабораторные исследования проводились в соответствии с клиническими лабораторными стандартами. Для статистического анализа использовались альтернативный и вариационный анализ, параметрические методы оценки гипотез и непараметрические методы нормального распределения.

Результаты и обсуждение: Результаты показали, что в трёх группах интоксикации средние уровни гомоцистеина были выше, чем в контрольной группе ($p<0.001$). Критерий межгруппового сравнения нормального распределения показал, что изменения у пациентов с алкогольной интоксикацией ($u=3.39$; $p<0.001$) и героиновой интоксикацией ($u=2.00$; $p<0.001$) были более статистически значимыми, не коррелируя с тяжестью отравления. ($p>0.05$).

Заключение: Существует риск окислительного стресса при алкогольном и наркотическом опьянении. Надёжным маркером для комплексной оценки окислительного стресса у людей является наблюдение уровней гомоцистеина в сыворотке и их тщательная интерпретация.

Ключевые слова

гомоцистеин, окислительный стресс и острая экзогенная интоксикация