UDC 616.71-007.234-001.56616.153.478.6 doi: https://doi.org/10.15407/ubj94.03.053

DYNAMICS OF HOMOCYSTEINE LEVEL IN PATIENTS WITH OSTEOPOROTIC FRACTURE

N. A. HASANOVA

Azerbaijan Medical University, Biochemical Department, Baku, Azerbaijan;
e-mail: hasanovanaila@yahoo.com

Received: 04 June 2022; **Revised:** 01 August 2022; **Accepted:** 29 September 2022

The research was carried out in order to investigate the blood serum level of homocysteine (HCY) which is involved in bone metabolism and has prognostic significance in the monitoring of the regenerative processes in osteoporosis and osteoporotic fractures. The study was carried out on patients 45-83 years old divided into 3 groups: group I – 14 patients with osteoporosis confirmed by densitometry or X-ray examination, group II – 15 patients with non-osteoporosis fractures, group III – 25 patients with osteoporotic fractures. The control group consisted of practically healthy 14 people. In patients with various fractures osteosynthesis with Ilizarov apparatus or with metal plates was performed. After the operation, the patients were treated in an inpatient setting for a week, then sent for outpatient treatment and prescribed calcium and vitamin D supplements to accelerate the bone regeneration process. A blood sample was taken at 3 stages to monitor the dynamics of HCY level by Elisa test: on the 1st day before treatment, on the 10th day of treatment and 1 month after it. The results showed that on the 1st day before the treatment HCY concentration was statistically increased 2.7 times in group I, 5.6 times in group II, and 6.5 times in group III compared to the control group. In the month of recovery, a significant decrease in HCY level was observed in all treated groups but it still remained higher than in the control indicating the need to recommend additional therapeutic prescriptions.

K e y w o r d s: level of homocysteine, osteoporosis, osteoporotic fractures.

The osteoporosis is known to be a serious health and economic problem, especially for women in the postmenopausal period [1]. The bone mass is affected by the environmental, metabolic and genetic factors. There is evidence suggesting that the health of the bones is affected by homocysteine (HCY) [2, 3]. Its level is inceasing depending on the age day by day [3, 4]. The patients who had the osteoporotic fractures need more care. In the United States osteoporotic fractures occur in approximately 2.5 million patients each year [1-4]. Homocysteine is one of the main parts of the methylation cycle, which is an important biochemical pathway in our cells involved in many critical body functions. The research that was carried out recently showed that the increase of the HCY level may be associated with the osteoporotic fractures [5, 6]. Thus, HCY accelerates oxidative stress, causing osteoblast damage and apoptosis. As a result, the function of osteoblasts is impaired, and bones lose strength. At the same time, HCY enhances oxidative stress in osteocytes and causes their apoptosis. Thus, dysfunction of osteoblasts and osteocytes leads to disruption of bone formation and remodeling. The people who had the elevated level of serum HCY have a 2-4 times higher risk of fracture than those with low levels [5-7]. Homocysteine concentration in humans varies with age and gender. Plasma concentrations of homocysteine increase with age and normal HCY levels are higher in males aged 30-40 than in females [8].

The purpose of this to study examine of the homocystein level in the blood serum of patients with osteoporosis and osteoporotic fractures.

Materials and Methods

In 2018-2019, 54 people who applied to the Research Institute of Traumatology and Orthopedics in Azerbaijan (Baku) participated in the study. The average age of patients was 61.2±0.8 (45-83 years). These patients collapsed at home and were traumatized, and the diagnosis of osteoporosis was confirmed by densitometry or X-ray examination. The patients with non-osteoporotic fractures were injured for various reasons, including auto accident. Another 14 people included in the study were pa-

tients with osteoporosis, however, without fractures. The control group consisted of practically healthy 14 people. Thus, 54 patients were divided into 3 groups: I group included 14 patients with osteoporosis, II group included 15 patients with non-osteoporotic fractures, and III group included 25 patients with osteoporotic fractures.

All patients were initially informed and blood samples were taken from patients upon ethical consent (Protocol No 07, 27.06.2019), with the permission of the Ethics Committee of Azerbaijan Medical University.

After a detailed history, blood samples were taken from patients on an empty stomach. Blood sample were taken from all three groups of patients in 3 stages, to monitor the dynamics of the serum HCY levels: on the 1st day before starting treatment, on the 10th day of treatment and after 1 month.

Blood levels of homocysteine (HCY) were determined at a wavelength of 450 nm by the enzyme-linked immunosorbent assay (Cloud Clone Corp. Elisa kits, USA).

The statistical evaluation was performed by using SPSS 26.0 program (IBM SPSS Inc., USA) The comparison of numerical variables was conducted with dispersion ANOVA, and Kruskal-Wallis H test, the statistical accuracy of the change of variables in dynamics – with Wilcoxon test. To compare categorical variables, Pearson Chi-square and Fisher Exact tests were performed using exact results. All data were provided as mean ± SD. The significance level of the difference between the indicators was considered statistically reliable when it was at least $P < 0.05$.

Results and Discussion

According to the results, there was no statistically significant difference between the age and sex groups in the concentration of HCY in the blood samples ($P > 0.05$). The results showed that on the 1st day the concentration of HCY was statistically significant increased by 2.7 times ($P_U = 0.108$) in group I, by 5.6 times ($P_U < 0.001$) in group II, and by 6.5 times in group III ($P_U < 0.001$) compared with the control group. Thus, the average value of HCY in group I was 1.76 ± 0.56 μg/ml; in group II – 3.57 ± 0.62 μg/ml; in group III – 4.2 ± 0.50 μg/ml. The results show that the serum HCY level increases more sharply after fractures, especially in osteoporotic patients. Thus, during osteoporotic fractures (group III), the concentration of HCY increased by 2.4 times ($P_U = 0.005$) when it was compared with the patients who had osteoporosis not accompanied by fractures (group I). It turned out that an elevation in HCY levels increases the risk of fractures. The Figure shows that the maximum level of HCY was observed in non-osteoporotic fractures, and the maximum mean value was observed in osteoporotic fractures.

The comparison of minimum and maximum HCY levels, their mean values ($M \pm m$), and its initial level for each group on the 10th day and a month later has been given in the Table.

When determining the level of HCY in the dynamics of the treatment in patients who had osteoporosis (group I), after 10 days, its concentration increased in 8 people compared to the results before treatment, and decreased in 6 people. Thus, after 10 days, the concentration of HCY decreased by 15.0% and averaged 2.01 ± 0.66 μg/ml ($P_W = 0.875$). However, the concentration of HCY is still 3.0 times higher ($P_U = 0.141$) than in the control group. After a month of the treatment, the results change very little compared to 10 days after treatment ($P_W = 0.158$). As it can be seen from the Table, the mean concentration of HCY after a month was 2.03 ± 0.67 μg/ml.

In the dynamics of the treatment, the concentration of HCY decreased by 15.0% after 10 days in the group with non-osteoporotic fractures (group II) compared with the 1st day and averaged 3.11 ± 0.54 μg/ml ($P_W = 0.009$). In this group, the concentration of HCY decreased in almost all the majority of patients – in 14 people, and increased only in one person. As it can be seen from the results, the concentration of HCY remained by 4.8 times ($P_U < 0.001$) higher after 10 days compared to the control group. A month after the starting treatment, the concentration of HCY in the majority of patients (14 people) decreased by 43.0% compared to pre-treatment results ($P_W = 0.003$). Thus, the concentration of HCY in this group averaged 2.50 ± 0.41 μg/ml a month after treatment. Although the result decreased by 25.0% compared to 10 days ago, it remained 3.9 times higher ($P_U < 0.001$) than in the control group.

After 10 days of treatment for osteoporotic fracture (Group III), it was established that the concentration of HCY significantly decreased by 46.0% compared to pre-treatment ($P < 0.001$). This decrease was observed in the majority of patients (23 people), and in 2 people, on the contrary, the HCY content was increased in comparison with the results before

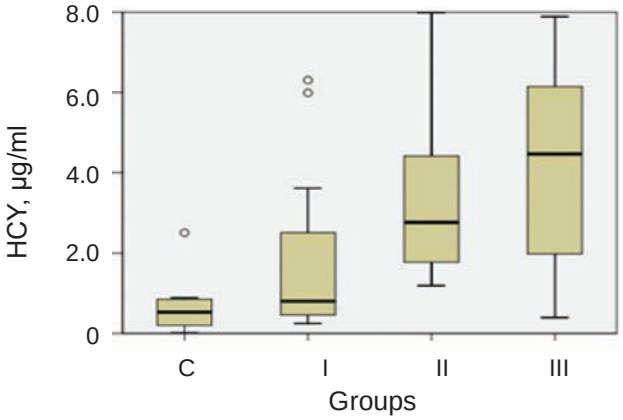

Fig. The levels of HCY. (C – control group, I – patients with osteoporosis, II – patients with non-osteoporotic fractures, III – patients with osteoporotic fractures)

treatment. Thus, after 10 days of treatment, HCY's concentration was 2.88 ± 0.40 µg/ml. Apparently, HCY's concentration remained statistically significantly higher by 4.5 times ($P_U < 0.001$) than in the control group. One month after treatment, a decrease in HCY concentration by 96.0% compared to pretreatment ($P_W < 0.001$) was observed. In the studied group, a decrease in the concentration of HCY was recorded in 22 patients. HCY concentration in this group was 10 days after treatment, with an average of 2.14 ± 0.35 µg/ml and ranged from 0.19-5.98 µg/ml within the group.

The results showed that HCY levels decreased in the first month of the recovery period in 92% of patients with osteoporotic fractures, 93% of patients with non-osteoporotic fractures, but increased in 57% of patients with osteoporosis.The difference was statistically significant compared to the control group ($P < 0.05$).

Also, after 1 month, X-ray examination revealed that the recovery process in non-osteoporotic fractures proceeds faster than in osteoporotic fractures.

Bone remodeling is a very complex process. Homocysteine is known to modulate this process through several known mechanisms such as an increased osteoclast activity, decreased osteoblast activity, and direct action of HCY on bone matrix [9]. According to a study by Rotterdam and Lasa, they did not determine the association of the elevated homocysteine levels with Ca and vitamin D treatment. İncreased homocysteine levels could lead to an increase in the risk of fracture through the interference in collagen cross-linking. We therefore speculate that homocysteine interferes with the development of the microarchitecture of bone independently of the amount of mineral components in the bone [12]. Elevation of HCY weakens the absorption of calcium

T a b l e . Dynamics of homocysteine level by groups (M ± m), µg/ml

Groups	*n*	*M ± m*	Min	Max	P_W
		I stage			
Control	14	0.65 ± 0.17	0.01	2.51	
I	14	1.76 ± 0.56	0.25	6.31	
II	15	3.57 ± 0.62	1.20	7.99	
III	25	4.20 ± 0.50	0.40	7.89	
		II stage			
I	14	2.02 ± 0.66	0.26	7.59	0.875
II	15	3.11 ± 0.54	1.14	7.71	0.009
III	25	2.88 ± 0.40	0.23	6.59	0.000
		III stage			
I	14	2.03 ± 0.67	0.28	7.92	0.510
II	15	2.50 ± 0.41	0.99	5.56	0.003
III	25	2.14 ± 0.35	0.20	5.98	0.000

*P_W – Statistical accuracy of the difference according to the Wilcoxon criterion. I – patients with osteoporosis, II – patients with non-osteoporotic fractures, III – patients with osteoporotic fractures. I stage – samples were taken on the 1st day , II stage – samples were taken after 10 days, and III stage – samples were taken after a month of treatment

and has a negative effect on bone mineralization. An increase in HCY leads to an uneven distribution of collagen in the bone matrix,and, as a result, chondrocytes cannot receive extracellular signals from the environment for differentiation and mineralization [10, 11].

We can see similar results in the literature. Van Meurs and co-authors assessed the level of HCY in old patients (55 years and older) in Amsterdam and Rotterdam, and observed a relationship between the risk of fracture and the level of HCY, independent of bone mineral density and other potential risk factors for fracture [12, 13].

Similar results were also observed by McLean and co-authors in a subgroup of the Framingham Study [14]. However, Périer et al. obtained different results. They carried out over 671 women, who were in the postmenopausal period for 10 years in the prospective studies. They concluded that HCY was not an independent risk factor for postmenopausal osteoporosis fractures in healthy women with a wide age range [15].

Dhonukshe-Rutten and co-authors observed the high levels of bone formation and resorption biomarkers in patients with hyperhomocysteinemia (HHCY) [12, 16]. Herrman and co-authors conducted a study among post-and premenopausal women and reported a positive correlation between HCY concentration and deoxypyridinoline, a bone-resorption marker in the urine, but not associated with osteocalcin, a bone-resorption marker in the serum. Under the influence of HCY, bone metabolism is directed to the enhancement of resorption [16]. The acceleration of resorption has been confirmed in patients with HHCY [17]. The available results suggest that HHCY may affect osteoclast activity, but these data are not sufficient to conclude that osteoclasts are the primary target of HCY in human bone [18, 19].

There is evidence that an increase in homocysteine levels is associated with a decrease in bone mineral density (BMD) [20]. However, in the scientific literature available to us, there is no systematized information on the effect of hyperhomocysteinemia on the growth, structure and shaping of the bones of the skeleton in different age periods with trauma to the musculoskeletal system [21].

Although there is sufficient information in the literature on the relationship between homocysteine and bone fractures and its role in the repair of bone fractures, these data are quite contradictory and require further extensive research. In our study, the role of homocysteine in the pathogenesis of fractures of various origins, as well as in the dynamics of recovery, was comparatively studied. The results show that people with high levels of homocysteine have an increased risk of fractures as a result of trauma. This risk was higher in patients with osteoporosis, which may be primarily due to vitamin D deficiency in osteoporosis.

Conclusions. Thus, according to the results, a statistically significant difference in the level of HCY in the groups compared to the control group was observed (P <0.05). High concentrations of HCY resulted in a high risk of fracture. In the first month of recovery, a significant decrease in HCY concentrations was observed. A slight increase in the level of HCY in the dynamics of treatment in patients with osteoporosis not accompanied by fractures indicates that the treatment is not fully effective. This is evidenced by the fact that the content of HCY in the dynamics of fracture recovery remains higher than in the control. It may be useful to monitor the serum dynamics of HCY in order to control metabolic processes in the bone during the recovery process of osteoporotic fractures. More extensive and long-term research is needed to monitor the dynamics of HCY during the full recovery period and to select new treatments.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

ДИНАМІКА РІВНЯ ГОМОЦИСТЕЇНУ У ПАЦІЄНТІВ З ОСТЕОПОРОТИЧНИМ ПЕРЕЛОМОМ

N. A. Hasanova

Azerbaijan Medical University, Biochemical Department, Baku, Azerbaijan
e-mail: hasanovanaila@yahoo.com

Дослідження проводили з метою вивчення у сироватці крові рівня гомоцистеїну (HCY), який бере участь у метаболізмі кісткової тканини та має прогностичне значення в моніторингу регенеративних процесів за остеопорозу та остеопоротичних переломів. Пацієнтів віком 45-83 років було розподілено на 3 групи: I група – 14 пацієнтів з остеопорозом, підтвердженим

денситометрією або рентгенівським обстеженням, II група – 15 пацієнтів із неостеопорозними переломами, III група – 25 пацієнтів з остеопоротичними переломами. Контрольну групу склали 14 практично здорових осіб. Пацієнтам із різними переломами проводили остеосинтез апаратом Ілізарова або металевими пластинами. Після операції протягом тижня хворих лікували в стаціонарі, потім направляли на амбулаторне лікування і призначали препарати кальцію і вітаміну D для прискорення процесу регенерації кісткової тканини. Для моніторингу динаміки рівня HCY методом ELISA відбирали кров у 3 етапи: за один день до лікування, на 10-й день лікування та через 1 місяць після нього. Результати показали, що за один день до лікування концентрація HCY була статистично більше в 2,7 раза в I групі, в 5,6 раза в II групі і в 6,5 раза в III групі порівняно з контрольною групою. Через один місяць після лікування рівень HCY значно знизився в усіх групах, але все ще був вищим, ніж у контрольній, що вказує на необхідність рекомендувати додаткові терапевтичні призначення.

Ключові слова: рівень гомоцистеїну, остеопороз, остеопоротичні переломи.

References

1. Kanis JA, Cooper C, Rizzoli R, Reginster JY. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int*. 2019; 30(1): 3-44.
2. Saito M, Marumo K. The effects of homocysteine on the skeleton. *Curr Osteoporos Rep*. 2018; 16(5): 554-560.
3. Périer MA, Gineyts E, Munoz F, Sornay-Rendu E, Delmas PD. Homocysteine and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int*. 2007; 18(10): 1329-1336.
4. Herrmann M, Kraenzlin M, Pape G, Sand HM, Herrmann W. Relation between homocysteine and biochemical bone turnover markers and bone mineral density in peri- and postmenopausal women. *Clin Chem Lab Med*. 2005; 43(10): 1118-1123.
5. Weber DR, Coughlin C, Brodsky JL, Lindstrom K, Ficicioglu C, Kaplan P, Freehauf CL, Levine MA. Low bone mineral density is a common finding in patients with homocystinuria. *Mol Genet Metab*. 2016; 117(3): 351-354.
6. Leboff MS, Narweker R, LaCroix A, Wu L, Jackson R, Lee J, Bauer DC, Cauley J, Kooperberg C, Lewis C, Thomas AM, Cummings S. Homocysteine levels and risk of hip fracture in postmenopausal women. *J Clin Endocrinol Metab*. 2009; 94(4): 1207-1213.
7. Narvaez J, Maldonado G, Intriago M, Cardenas J, Guerrero R, Neyro JL, Rios C. Role of homocysteine and vitamin B in bony metabolism. *Rev Colomb Reumatol*. 2020; 27(4): 278-285.
8. Selhub J. Public health significance of elevated homocysteine. *Food Nutr Bull*. 2008; 29(2 Suppl): S116-S125.
9. Vacek TP, Kalani A, Voor MJ, Tyagi SC, Tyagi N. The role of homocysteine in bone remodeling. *Clin Chem Lab Med*. 2013; 51(3): 579-590.
10. Behera J, Bala J, Nuru M, Tyagi SC, Tyagi N. Homocysteine as a pathological biomarker for bone disease. *J Cell Physiol*. 2017; 232(10): 2704-2709.
11. Kim JI, Moon JH, Chung HW, Kong MH, Kim HJ. Association between homocysteine and bone mineral density according to age andsex in healthy adults. *J Bone Metab*. 2016; 23(3): 129-134.
12. Van Meurs JB, Dhonukshe-Rutten RAM, Pluijm SMF, van der Klift M, de Jonge R, Lindemans J, de Groot LC, Hofman A, Witteman JC, van Leeuwen JP, Breteler MMB, Lips P, Pols HAP, Uitterlinden AG. Homocysteine levels and the risk of osteoporotic fracture. *N Engl J Med*. 2004; 350(20): 2033-2041.
13. Van Meurs JB, Uitterlinden AG. Homocysteine and fracture prevention. *JAMA*. 2005; 293(9): 1121-1122.
14. McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples LA, Kiel DP. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med*. 2004; 350(20): 2042-2049.
15. Périer MA, Gineyts E, Munoz F, Sornay-Rendu E, Delmas PD. Homocysteine and fracture risk in postmenopausal women: the OFELY study. *Osteoporos Int*. 2007; 18(10): 1329-1336.
16. Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B_{12} status relate to

bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. *J Bone Miner Res*. 2005; 20(6): 921-929.
17. Pooneh Salari P, Larijani B, Abdollahi M. Association hyperhomocysteinemia with osteoporosis: a systematic review. *Therapy*. 2008; 5(2): 215-222.
18. Lim JS, Lee DH. Changes in bone mineral density and body composition of children with well-controlled homocystinuria caused by CBS deficiency. *Osteoporos Int*. 2013; 24(9): 2535-2538.
19. Wang P, Liu L, Lei SF. Causal effects of homocysteine levels on the changes of bone mineral density and risk for bone fracture: A two-sample mendelian randomization study. *Clin Nutr*. 2021; 40(4): 1588-1595.
20. Yang J, Hu X, Zhang Q, Cao H, Wang J, Liu B. Homocysteine level and risk of fracture: A meta-analysis and systematic review. *Bone*. 2012; 51(3): 376-382.
21. Enneman AW, Swart KM, Zillikens MC, van Dijk SC, van Wijngaarden JP, Brouwer-Brolsma EM, Dhonukshe-Rutten RA, Hofman A, Rivadeneira F, van der Cammen TJ, Lips P, de Groot CP, Uitterlinden AG, van Meurs JB, van Schoor NM, van der Velde N. The association between plasma homocysteine levels and bone quality and bone mineral density parameters in older persons. *Bone*. 2014; 63: 141-146.