Serum Homocysteine and Reactive Species Levels in Fragility Fractures of the Pelvis

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Although there are studies showing the link between homocysteine and bone quality, the relationship remains controversial. The purpose of the study was to investigate homocysteine levels in patients with fragility fractures of the pelvis and their association with serum Total Antioxidant Status.The study included a group of 60 patients, aged >65years, with pelvic fragility fractures and osteoporosis admitted in our trauma department between January 2015 and January 2018 and a control group of 30 patients, aged >65years without fragility fractures or osteoporosis.The total antioxidant status (TAS) and homocysteine (Hcy) concentrations were determined in serum of all the patients. TAS levels had a significantly lower values in the group with pelvic fragility fractures compared with the control group ($p<0.001$).We noticed a significant higher mean of Hcy levels ($p<0.001$) in patients with pelvic fragility fractures compared with the control group. As conclusion this study shows that patients with fragility fractures of the pelvis have moderately elevated levels of homocysteine and reduced serum Total Antioxidant Status.

Keywords: fragility fracture, homocysteine, osteoporosis, oxidative stress

Osteoporosis and associated fractures are a major public health concern due to morbidity, mortality, loss of function and decrease the quality of life. Osteoporosis is characterized by bone demineralisation and loss of resistance that leads to fractures due to fragility. Although bone tissue contains tree cell types, osteoblasts and osteoclasts are key players in bone remodeling.The result of remodeling depends on their coordinated interaction. In osteoporosis the regulation favors osteoclast activity and bone tissue is lost.

Fragility fractures of the pelvis are the result of a low-energy impact but they can also occur spontaneously in elderly people with osteoporosis [1]. Most of the basic fragility fractures, occur due to minor trauma, show minimal displacement and does not usually require surgical treatment. Some authors [2] believe that functional treatment consisting of rest, pain relief and physical rehabilitation, has the disadvantage of prolonged immobilization with physical and psychological consequences [3, 4]. Depending on the personality of the fracture and the patient's comorbidities, fragility fractures can be treated also surgically [5-9].

Homocysteine (Hcy) is a non-proteinogenic amino acid, being synthesized in the body as an intermediate in the metabolism of methionine. The structure of the homocysteine is shown in the figure 1.

An elevated level of this amino acid in the blood is called hyperhomocysteinaemia (hHCy). Depending on the

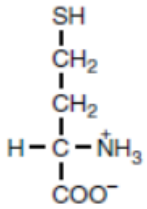

Fig. 1. Chemical structure of homocysteine

homocysteine levels, three types of hHCy are distinguished: mild hHCy (16 - 30 μmol/L), intermediate hHCy (31 - 100 μmol/L) and severe hHCy (over 100 μmol/L). The common cause of mild hHCy is polymorphism in the methylene-tetrahydrofolate reductase gene, manifested particularly as a result of insufficient folic acid intake from food. In addition the homocysteine level increases also as a result of renal failure and smoking. Severe hHCy is determined genetically and it is accompanied by thrombosis of the venous and arterial systems, thromboembolic disease and atherosclerosis complications. The homocysteine level can be usually easily decreased by the administration of vitamins: pyridoxine, B12 and folic acid [10]. Elevated serum levels of homocysteine have deleterious effects on vascular endothelial cells, bone, neural cells [11]. Recent studies indicate that homocysteine is involved in bone matrix degradation and altered biomechanical bone properties through osteoclasts activity [12]. A possible mechanism by which homocysteine is involved in bone

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metabolism is the destruction of the cross-linking of collagen molecules and the increase in the level of advanced endoglicant products. Another mechanism is activation of osteoclastgenesis and causing detrimental effects on bone via oxidative stress induced metallo-proteinase-mediated extracellular matrix degradation and decrease in bone blood flow.

The purpose of the study was to investigate homocysteine levels in patients with fragility fractures of the pelvic ring and their association with serum Total Antioxidant Status.

Experimental part

We enrolled in the present study 60 patients, aged >65years, with fragility fractures of the pelvic ring admitted in our trauma department between January 2015 and January 2018. We excluded the patients with a history of pelvic fracture and those taking certain anti-osteoporotic drugs. Pelvic fractures that occurred after high energy trauma or on tumorous bone were also excluded.The control group included 30 patients, aged >65years, without fragility fractures or osteoporosis. Patients were divided into 2 groups: non-osteoporosis control group (C) had normal bone mineral density (BMD) and osteoporosis patient (F) with fragility fractures of the pelvic ring had low BMD.

Diagnosis of osteoporosis

Total hip, femoral neck and lumbar spine BMD was measured in all subjects included in the study by dual-energy X-ray absorptiometry (DXA) using the Hologic®DXA scanner and was expressed in absolute values as grams of mineral content per square centimeters of bone area (g/cm^2). Coefficients of variation of DXA measurements were 1%. Osteoporosis in post-menopausal women and men over 50 is defined as a T-score less than 2.5 SD (standard deviation minus average density). Reduced bone mass corresponds to the T-score -2.4 to -1.0 SD, and normal bone density is defined as the T-score between -1.0 and +1.0 SD [13]. BMD results were classified according to World Health Organization criteria [14].

Determination of TAS

The serum TAS levels was determined using the TAS Assay Kit from Sigma Aldrich for research and manual use, according to the instructions.

Determination of homocysteine

Total plasma homocysteine was determined by the immunoassay technique using an Axis Homocysteine EIA kit, according to the instructions.

Informed consent from all patients included in this study was obtained. The current research has been conducted in accordance to the ethical principles set out by the Helsinki Declaration.

Statistical analysis

Statistical analysis was carried out using SPSS version18. Data were presented as mean±standard deviation for normally distributed variables (age, height, weigh).

Results and discussions

A total of 90 subjects was included in this study. The mean ±SD age of patients was73±6.75 years for the C group and 78±8.35 years for the F group.General characteristics of the patients included in the study are presented in table 1.

Data given as mean±SD

There were no significant differences in age, height, weight, current smoking and coffee consumption between control group and patients with osteoporosis.

World Health Organization defines osteoporosis as a systemic bone disease characterized by a decrease in bone mass and damage to the bone tissue microarchitecture, with a subsequent increase in bone fragility and proneness to fractures [14].

Osteoporosis is a disease of etiology that undergoes the pathogenic pattern of association of several risk factors that do not clinically determine an alarming symptom. Risk factors like gender, ethnicity, physical activity, drinking, smoking, estrogen, Ca and vitamin D have a negative impact on bone mineral density and increase the chances of fractures of fragility.

For these risk categories, early identification of osteopenia or osteoporosis is mandatory. The sooner the therapy starts, the lower the risk of fracture. To prevent bone loss some studies support the concept of using nitric oxide donors which can have anti-inflammatory effects [15,16].

The TAS levels varied between 1.111 µmol Trolox equivalent/L and 1.396 µmol Trolox equivalent/L in patients with fragility fracture of the pelvic ring and osteoporosis. In non-osteoporosis control group , TAS values ranged between 1.532 µmol Trolox equivalent/L and 1.745 µmol Trolox equivalent/L with a mean of 1.632 µmol Trolox equivalent/L (table 2).

TAS levels had a significantly lower values in the group F compared with the group C($p<0.001$).

Oxidative stress is involved in many diseases, disturbing the oxidant-antioxidant balance [17]. Recent studies showed that oxidative stress may cause osteoporosis by involving in bone remodeling [18].Altindag et all. [19] observed that increased osteoclastic activity and decreased osteoblastic activity may be associated with an imbalance between the oxidant and antioxidant status in osteoporosis.

Homocysteine has been suggested to be an oxidizing factor [20, 21]. Tyagi N et al.[22] reported that homocysteine caused the production of reactive oxygen species by autooxidation.

Table 1
BASELINE CHARACTERISTICS OF THE STUDY PARTICIPANTS

Characteristic	Control (n=30)	Osteoporosis(n=60)	p
Age(years)	73±6.75	78±8.35	0.324
Height(cm)	169±11.70	167±12.30	0.124
Weight(kg)	81±9.85	85±11.15	0.431
Current smoking(%)	31.21	30.10	0.612
Coffee consumption(>3cups/d, %)	43.51	39.14	0.321
Femoral neck BMD(g/cm^2)	0.81±0.03	0.72±0.04	<0.001
Lumbar spine BMD(g/cm^2)	1.12±0.15	0.82±0.09	<0.001
Total hip BMD(g/cm^2)	0.96±0.08	0.81±0.06	<0.001

Data given as mean±SD

Table 2

STATISTICAL DESCRIPTION OF TAS VALUES a) p<0.001 ns) p>0.05

Parameter	Group C	Group F
Number	30	60
Mean	1.632	1.241[a)]
Standard deviation	0.086	0.062
Variance	0.051	0.032
Skewness Test	-0.024	0.018
Std.Error of Skewness	0.327	0.561
Kurtosis	-1.243	-1.532
Std.Error of Kurtosis	0.621	0.945
Minimum	1.532	1.111
Maximum	1.745	1.396

The Hcy levels varied between 13.215 ìmol/l and 16.927 ìmol/l in patients with fragility fracture of the pelvic ring and osteoporosis (F). In non-osteoporosis control group (C) , Hcy values ranged between 10.421 ìmol/l and 12.314 ìmol/l with a mean of 11.321 ìmol/l (table 3).

Plasma Hcy levels are significantly higher in the group F compared with the group C(p<0.001). Similarly, researchers in another study [23] found that Hcy level in elderly patients with osteoporotic fracture is higher than that of non-osteoporotic patients. Numerous studies show that although high levels of homocysteine are a risk factor for fractures, they are not correlated with bone mineral density.

Conclusions

We show that the osteoporosis patients with fragility fractures of the pelvic ring had reduced TAS compared with control group. As conclusion this study shows that patients with fragility fractures of the pelvis have moderately elevated levels of homocysteine**.** Further investigations are needed to examine the relationship of oxidative stress and homocysteine in patients with fragility fractures.

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Table 3

STATISTICAL DESCRIPTION OF Hcy VALUES a) p<0.001 ns) p>0.05

Parameter	Group C	Group F
Number	30	60
Mean	11.321	14.892[a)]
Standard deviation	0.962	0.714
Variance	0.095	0.052
Skewness Test	-0.037	0.029
Std.Error of Skewness	0.327	0.561
Kurtosis	-1.134	-0.762
Std.Error of Kurtosis	0.733	0.872
Minimum	10.421	13.215
Maximum	12.314	16.927

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