Serum homocysteine levels in children with fractures and low bone mineral density – a pilot study

Š. KUTÍLEK[1], P. ŘEHÁČKOVÁ[2], V. NĚMEC[1], E. BOČKAYOVÁ[1]

[1]*Department of Pediatrics, Pardubice Hospital and Faculty of Health Studies, University of Pardubice, Czech Republic,*
[2]*CCBR – Center for Clinical and Basic Research, Pardubice, Czech Republic*

SOUHRN

Kutílek Š., Řeháčková P., Němec V., Bočkayová E.: **Sérové hladiny homocysteinu u dětí se zlomeninami a nízkou denzitou kostního minerálu – pilotní studie**

Úvod: Vysoké hodnoty homocysteinu v séru (S-Hcy) jsou u postmenopauzálních žen spojovány s nízkou denzitou kostního minerálu (BMD) a zvýšeným rizikem zlomenin. U dětí dosud neexistují souvislé údaje o S-Hcy a stavu skeletu.

Pacienti, metody: Vyšetřili jsme S-Hcy u 19 dětí a dorostenců (12 chlapců a 7 dívek; průměrný věk 14,9 ± 3,3 let) s anamnézou nízkozátěžových zlomenin (v průměru 4 ± 2,6 fraktur na jednoho pacienta) a/nebo nízkou hodnotou BMD v bederní páteři (L1-L4) (méně než –2SD Z-skóre; DXA Lunar GE). Dále byla vyšetřena aktivita alkalické fosfatázy v séru (S-ALP) a marker kostní resorpce CrossLaps v séru. V době vyšetření děti neužívaly žádné léky ovlivňující kostní metabolismus. Získané výsledky BMD, S-ALP, S-CrossLaps byly vyjádřeny pomocí Z-skóre ± SD (směrodatná odchylka).

Výsledky: V porovnání s referenčními hodnotami byla hladina S-Hcy významně zvýšena (1,4 ± 1,8; p = 0,001), zatímco BMD Z-skóre byla signifikantně nižší (–2,1 ± 1,3; p = 0,0001). S-ALP se nelišila od referenčních hodnot (p = 0,91), ale hladina S-CrossLaps byla vyšší (1,5 ± 1,9; p = 0,001). Byly zjištěny inverzní a významné korelace mezi S-Hcy a L1-L4 BMD (r = –0,66; p = 0,01) a S-Hcy a S-ALP (r = –0,56; p = 0,03). Nezjistili jsme korelaci mezi S-Hcy a počtem prevalentních zlomenin (r = 0,11) či S-Hcy a hodnotami S-CrossLaps (r = –0,14).

Závěr: Na základě uvedených výsledků lze předpokládat negativní vliv vysokých hladin S-Hcy na kostní formaci a BMD u dětí a adolescentů s opakovanými zlomeninami, zároveň je u těchto pacientů zvýšená kostní resorpce. Uvedená problematika vyžaduje další zkoumání.

Klíčová slova: homocystein, denzita kostního minerálu (BMD), zlomeniny, markery kostního obratu

SUMMARY

Kutílek Š., Řeháčková P., Němec V., Bočkayová E.: **Serum homocysteine levels in children with fractures and low bone mineral density – a pilot study**

Background: High serum homocysteine (S-Hcy) levels are associated with low bone mineral density (BMD) and increased fracture risk in postmenopausal women. So far, data on S-Hcy and bone health are lacking in children and adolescents.

Patients, Methods: We assessed S-Hcy levels in 19 children and adolescents (12 boys and 7 girls; mean age 14.9 ± 3.3 years) with prevalent low-energy trauma fractures (mean 4 ± 2.6 per patient) and/or low spinal L1-L4 BMD (below –2SD Z-score; DXA Lunar GE). Other assessments included serum alkaline phosphatase (S-ALP) and serum CrossLaps. At the time of assessment, the children were not taking any drugs known to influence bone metabolism. To eliminate the influence of age, the obtained results were expressed as Z-scores ± SD.

Results: S-Hcy Z-score was significantly higher (1.4 ± 1.8; p = 0.001) and L1-L4 BMD Z-score was significantly lower (–2.1 ± 1.3; p = 0.0001), respectively, in comparison with reference values. S-ALP did not differ from reference values (p = 0.91), while S-CrossLaps were higher (1.5 ± 1.9; p = 0.001). S-Hcy was inversely correlated to L1-L4 BMD (r = –0.66; p = 0.01) and S-ALP (r = –0.56; p = 0.03) and not related to number of prevalent fractures (r = 0.11) or S-CrossLaps (r = –0.14). These results suggest negative influence of elevated S-Hcy on bone formation and BMD in children with fractures, while bone resorption was increased in this group of pediatric patients. In conclusion, our results suggest that elevated S-Hcy is a risk factor of impaired bone health in children and adolescents. Further studies are necessary for more detailed clarification of these issues.

Keywords: homocysteine, bone mineral density, fractures, bone turnover markers

Address: Doc. MUDr. Štěpán Kutílek, CSc., Department of Pediatrics, Pardubice Hospital and Faculty of Health Studies, University of Pardubice, Czech Republic, e-mail: stepan.kutilek@nemocnice-pardubice.cz

Osteologický bulletin 2012;17(2):65–68

Došlo do redakce: 10. 7. 2012
Přijato k tisku: 27. 7. 2012

Introduction

Homocysteine, an amino acid homologous to cysteine, is biosynthesized from methionine by the removal of its terminal C methyl group. Homocysteine can be recycled into methionine or converted into cysteine. Deficiencies of the vitamins B6, B9 and B12 impair such conversion and can lead to high serum homocysteine concentration [1,2]. The serum homocysteine level (S-Hcy) is inversely correlated

with serum folate concentration in children and adults [1]. High S-homocysteine has been linked to cardiovascular and neurodegenerative disease, diabetes, thrombosis [2,3]. Hyperhomocysteinemia is associated with alterations in vascular morphology, loss of endothelial anti-thrombotic function, and induction of a procoagulant environment [2]. High serum homocysteine (S-Hcy) levels are also associated with low bone mineral density (BMD) and increased fracture risk in postmenopausal women and also in experimental animals [4–15]. So far there are no complex data on S-Hcy and bone health in children and adolescents. Our aim was to evaluate S-Hcy levels in children with low BMD and/or prevalent low-energy trauma fractures in a retrospective pilot study.

Patients, Materials, Methods

Patients:

The charts of 19 children and adolescents (12 boys and 7 girls; mean age 14.9 ± 3.3 years) attending our Pediatric Bone Clinic were retrospectively evaluated. The inclusion criteria for this pilot study were at least two prevalent low-energy trauma fractures in personal history and/or low spinal L1-L4 BMD (below –2 SD Z-score). The mean number of prevalent fractures was 4.0 ± 2.6 (SD) per patient. The diagnostic distribution of the patients was: osteogenesis imperfecta (n = 3) and inadequately low bone density/osteoporosis of unknown origin (n = 16). At the time of the assessment, the patients were not taking any drugs known to influence bone metabolism. All subjects were on a standard central European diet, consisting mostly of meat and carbohydrates. None was on a diet poor in vitamin B, nor was receiving doses of vitamin B exceeding recommended daily allowances.

Materials:

The following biochemical parameters were assessed from a single morning blood draw in fasting patients: Serum levels of homocysteine, calcium, phosphate, blood urea nitrogen, creatinin, CrossLaps, serum activity of alkaline phosphatase.

Methods:

BMD was measured at spine (L1-L4) with dual energy X-ray absorptiometry (DXA) Lunar GE at the day of the blood draw. Measurement precision, expressed as coefficient of variation, was 1.0 %.

S-homocysteine level was evaluated by chemiluminescence (Immulite 2500 immunoassay system, Siemens Healthcare Diagnostics, Germany) and expressed in mol/L. The interassay variation was 2.06 % in samples with S-homocysteine concentration 7.43 μmol/L; 1.99 % with S-homocysteine 10.31 μmol/L, and 1.72 % with S-homocysteine 22.25 μmol/L, respectively.

Serum calcium (S-Ca) and serum phosphate (S-P) were assessed by colorimetric assay and expressed in mmol/L. Serum alkaline phosphatase activity (S-ALP) was measured by colorimetric assay and expressed in μkat/L. Serum CrossLaps (S-CTx) were assessed by means of electrochemiluminescence immunoassay – ECLIA on Elecsys-Cobas analyzers and expressed in ng/L.

Body height was measured on the day of the relevant blood draw to the nearest ±0.5 cm on a calibrated stadiometer.

Figure 1
S-Homocysteine (Z-Score) VS BMD (Z-Score)
R= –0.66; P = 0.01
S-Homocysteine, Y Axis
BMD, X Axis

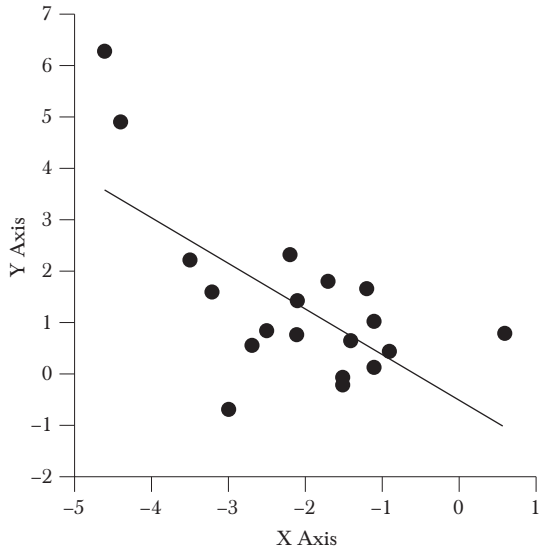
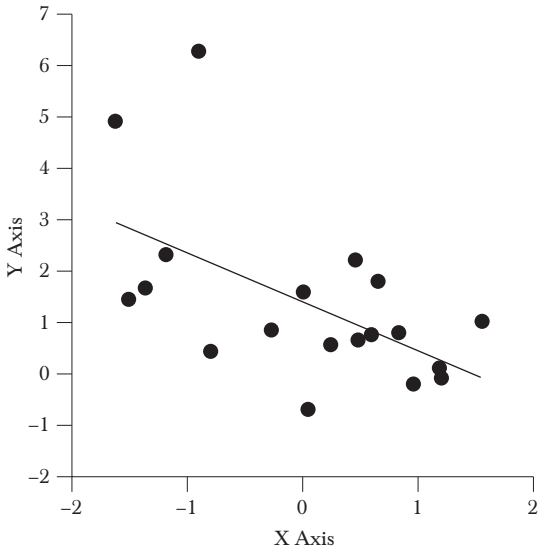


Figure 2
Homocysteine (Z-Score) VS S-ALP (Z-Score)
R= –0.56; P = 0.03
S-Homocysteine, Y Axis
S-ALP, X Axis



To eliminate the influence of age, the obtained results of body height, S-Hcy, S-ALP, S-CTx were calculated as standard deviation scores (SDS) or Z-scores by the equation SDS = (actual individual value – mean value for age and sex)/standard deviation for age and sex. The BMD reference data (concerning European paediatric population) were supplied by the manufacturer within the DXA software package. The following, previously published values, served as reference data: Czech anthropometric parameters from a 2001 survey [16], previously obtained Hcy values of healthy Czech paediatric population [17], S-ALP values of Czech children [18] and S-CTx levels of healthy British population [19]. For statistical analysis, Sigmaplot 2.0 and Systat programme was used. The statistical analysis was performed by unpaired t-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, $p < 0.05$ was required for statistical significance.

Results:

The mean S-HCy was 10.9 mol/L ± 3.4 (SD). The mean L1-L4 BMD was 0.818 g/cm^2 ± 0.138 (SD). The mean S-ALP value was 3.6 μkat/L ± 2.1 (SD) and mean S-Crosslaps value was 925 ng/L ± 522 (SD). When converted to Z-scores and compared with reference values, the S-Hcy Z-score was significantly higher and L1-L4 BMD Z-score was significantly lower, respectively, in comparison with reference values (Table 1). S-ALP Z-score did not differ from reference values, while S-CrossLaps were higher (Table 1). S-Ca and S-P levels were within normal reference range (mean ± SD: 2.36 ± 0.09 mmol/L; range 2.18–2.54; and 1.52 ± 0.19 mmol/L; range 1.16–1.99, respectively). Mean body height Z-score was –0.06 ± 1.04 which did not differ from reference values (Table 1). There was no correlation between body height and BMD Z-scores (r = –0.004). S-Hcy was inversely correlated to L1-L4 BMD (r = –0.66; p = 0.01) (Figure 1) and S-ALP (Figure 2) (r = –0.56; p = 0.03) and not related to number of prevalent fractures (r = 0.11) or S-CrossLaps (r = –0.14).

Table 1: Patient data expressed as Z-scores ± SD

Parameter	Mean	SD	p†
S-homocysteine	1.4	1.8	**0.001**
L1-L4 BMD	–2.1	1.3	**0.0001**
S-ALP	0.03	1.0	0.91
S-CrossLaps	1.5	1.9	**0.001**
Height	–0.06	1.0	0.27

BMD – bone mineral density; S-ALP – serum alkaline phosphatase aktivity
†Compared to reference data

Discussion:

Our results suggest negative influence of elevated S-Hcy on bone formation and BMD in children with fractures, while bone resorption was increased in this group of pediatric patients. The low BMD in our patients was not influenced by body height, as the body height did not differ from reference values and was there was no correlation between BMD and body height.

In adult patients, a negative relationship between S-Hcy and BMD has been reported by several authors [4,5]. Another study revealed high S-Hcy in osteoporotic postmenopausal women with no relationship to BMD values [8]. Yet another study indicated that high homocysteine levels are associated with an increased risk of hip fracture, which could be accounted for by poor renal function [12]. In addition, hyperhomocysteinemia was found to be associated with impaired fracture healing in mice [13].

Osteoporosis and higher fracture risk in patients with high S-Hcy is currently explained by accumulation of homocysteine in bone resulting in a distinct reduction of cancellous bone and a drop in bone strength. Furthermore, homocysteine stimulates osteoclast activity [15]. A reduced methylation capacity of bone cells might be another relevant pathomechanism [14].

We did not find any relationship between number of prevalent fractures and S-Hcy in our patient group. We may hypothesize that due to low number of patients, the study was not powered enough to detect significant relationship between these two parameters, and a more extensive research should be performed. We are aware of other shortcomings, including the fact that methylenetetrahydrofolate reductase levels and vitamin B status were not evaluated in our patients.

Conclusion

Our results suggest that elevated S-Hcy could be a risk factor of impaired bone health in children and adolescents. Further studies are necessary for more detailed clarification of these issues.

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