MCBU SBED
MANİSA CELAL BAYAR ÜNİVERSİTESİ SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ
MANISA CELAL BAYAR UNIVERSITY JOURNAL OF INSTITUTE OF HEALTH SCIENCE
ISSN: 2147-9607

ARAŞTIRMA MAKALESİ
RESEARCH ARTICLE
CBU-SBED, 2018, 5(3):107-111

The Possible Relationship Between Plasma Homocysteine, Folate and Vitamin B12 Levels and Limbus Vertebra

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Gönderim Tarihi / Received: 07.08.2018
Kabul Tarihi / Accepted: 24.09.2018

Öz

Amaç: Limbus vertebra (LV) gelişiminde rol alan birkaç mekanizma önerilmiş olmasına rağmen, LV'nin mekanizması hala belirsizdir. Bu çalışmanın amacı, LV'lı bir grupta Hcy, folat ve vitamin B12'nin plazma düzeylerini belirlemek ve yaş ve beden kitle indeksi eşleştirilmiş kadınlarla karşılaştırmak ve LV patofizyolojisine olası katkılarını araştırmaktır.

Gereç ve Yöntem: Bu çalışma, LV tanısı konan 171 premenopozal kadın ile LV'siz 190 yaş ve vücut kitle indeksi eşleştirilmiş premenopozal kadını içermektedir. Demografik, dual enerji X-ışını absorpsiyometri sonuçları ve plazma homosistein, folat ve vitamin B12 düzeyleri değerlendirilmiştir.

Bulgular: Antropometrik, klinik, dansitometrik ve biyokimyasal özellikler, Hcy düzeyleri dışında iki grup arasında istatistiksel olarak farklı değildi (p <0.001). Hcy düzeyleri LV grubunda yaşla birlikte anlamlı derecede negatif iken (r = -0.162, p = 0.034); kontrol grubunda böyle bir ilişki yoktu (r = 0.026, p = 0.721).

Tartışma: LV'li premenopozal genç kadınlar, yaş ve beden kitle indeksi eşleştirilmiş premenopozal kadınlara göre daha yüksek Hcy seviyelerine sahiptir. Yüksek Hcy seviyeleri, LV oluşumunun patogenezinde rol oynayabilir.

Anahtar Kelimeler: Folat, Homosistein, Limbus Vertebra, B12 Vitamini

Abstract

Aim: In spite of the fact that a few mechanisms have been proposed to take part in the development of the limbus vertebra, the mechanism of LV is still vague. The aim of this study was to determine the plasma levels of Hcy, folate and vitamin B12 in a group of women with LV and to compare with those of age-and body mass index-matched women in order to explore their possible contribution to the pathophysiology of LV.

Methods: The current study was based on a consecutive series of 171 premenopausal women who were diagnosed with LV and 190 age- and body mass index-matched premenopausal women without LV. Demographics, dual-energy X-ray absorptiometry results, and plasma homocysteine, folate, and vitamin B12 levels were assessed.

Results: The anthropometric, clinical, densitometric and biochemical features were not statistically different between two groups, except for Hcy levels ($p < 0.001$). While Hcy levels were significantly negatively correlated with age ($r = -0.162$, $p = 0.034$) in the LV group; there was no such relation in the control group ($r = 0.026$, $p = 0.721$).

Conclusions: Premenopausal young women with LV have higher Hcy levels compared to age- and body mass index-matched premenopausal women. High Hcy levels may play role in the pathogenesis of LV formation.

Key Words: Folate, Homocysteine, Limbus Vertebra, Vitamin B12.

1. Introduction

Limbus vertebra (LV) is a bone deformity caused by intrabody herniation of the nucleus pulposus through a defect through the vertebral endplate and results in the separation of a smooth triangular bone fragment [1,2]. Although it has been proposed that genetics and cumulative mechanical stress on an immature skeleton play a role in the development of the LV, the mechanism of LV formation is still unclear.

Many factors contribute to bone health. Association studies have identified vitamins and vitamin-related factors that are related to fractures or bone mineral density (BMD). Homocysteine (Hcy) is a non-protein amino acid that is biosynthesized from methionine and either recycled into methionine or converted into cysteine with the aid of the B-group vitamins. Results from previous literature have reported an association between plasma concentrations of Hcy and altered bone mechanism through proposed mechanisms of a) induction of apoptosis in human bone marrow stromal cells [3], b) stimulation of osteoclastogenesis and

osteoclast activity [4,5], c) reduction in bone blood flow and consequently changes of the biomechanical properties of bone [6], d) reduction of bone strength by interfering with collagen crosslinks [7, 8], and e) altered bone matrix formation [9]. Serum homocysteine is regulated by vitamin B12 and folic acid, and several studies have analyzed the relationship between B-group vitamin levels and altered bone mechanism [5,10,12].

The aim of this study was to determine the plasma levels of Hcy, folate and vitamin B12 in a group of women with LV and to compare with those of age-and body mass index-matched women in order to explore their possible contribution to the pathophysiology of LV.

2. Material and Methods

2.1. Study subjects

The current study was based on a consecutive series of 171 premenopausal women who took a medical examination at the neurosurgery outpatient clinic from January 2016 to January 2017, and who were diagnosed with LV by X-ray, CT and MR imaging and 190 age- and body mass index-matched premenopausal women without LV. Patients with following conditions were excluded: (a) patients who were on medications affecting the levels of Hcy, folate, vitamin B12 or bone metabolism, including vitamin B12 and/or folic acid supplements, anticonvulsants, glucocorticoids, diuretics, vitamin D supplements, and bisphosphonate hormone replacement therapy for at least 6 months before the evaluation, (b) patients with medical conditions affecting bone metabolism, including malignancy, chronic infectious or inflammatory diseases, systemic gastrointestinal, renal, respiratory diseases, and thyroid disorders, (c) patients with diagnosed osteopenia/osteoporosis, (d) patients with severe physical inactivity, (e) post-menopausal patients, (f) ages less than 18 and more than 35, (g) cigarette smoking for more than 10/day, (h) alcohol consumption over two glasses/day, and (i) radiographic contrast material (barium) use within the past 7 days or nuclear medicine studies within the past 3 days of dual-energy X-ray absorptiometry.

2.2 Data Collection

The data was collected from the patients' medical records, regarding (a) age, (b) weight, (c) height, (d) BMI, (e) smoking status, (f) alcohol consumption, (g) regular exercise (2–3 days/week), (i) DXA results, (k) homocysteine levels, (l) vitamin B12 levels, and (m) folate levels

2.3.DXA Scans

Lumbar spine BMD values were obtained from the firstto the fourth lumbar vertebral body by DXA using Discovery Wi DXA scanner (Hologic®, Bedford, MA, USA). The results were reported as T scores (standard deviation above or below values for young healthy population) and osteoporosis is defined as any T-score lower than -2.5, osteopenia as any T-score between -2.5 and -1.0, and normal bone density as any T-scores equal to or greater than -1.0.

2.4. Laboratory examination

The standard application of our institution for laboratory examinations of plasma homocysteine, folate, and vitamin B12 levels includes: sampling from an antecubital vein between 8 AM and 9 AM, in the fasting state (overnight), placing on ice and centrifuging within 1 h, and immediately storing the separated plasma in two different tubes at -25°C until assayed.

Homocysteine: The reference values are 5-12 μmol/L, with a sensitivity of 0.4 μmol/L, and within-run and between-run coefficient of variation (CVs) of 4.57% and 6% respectively.

Vitamin B12: The reference values are 126,5-505 pg/ml, with a sensitivity of 70.13 pg/ml, and within-run and between-run coefficient of variation (CVs) of 3.1% and 4.8%, respectively.

Folic acid: The reference values are 3,1-19,9 ng/ml, with a sensitivity of 0.06 ng/ml, and within-run and between-run coefficient of variation (CVs) of ≤ 8% and ≤ 12%, respectively.

2.5 Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences 21.0 (SPSS Inc., Chicago, IL, USA). Results of continuous variables are reported as means with standard deviations or frequencies. Categorical data were compared using Chi-square or Fisher's exact test, and continuous variables were analyzed with one-way ANOVA with post-hoc test or Kruskal-Wallis test, where appropriate. Simple regression models were used to detect linear relationships between variables. A two-tailed p-value less than 0.05 was considered significant.

3. Results

A total of 361 patients were included in this study. The basic characteristics of the study population are given in Table 1. The anthropometric, clinical, densitometric and biochemical features were not statistically different between two groups, except for Hcy levels ($p < 0.001$). While Hcy levels were significantly negatively correlated with age ($r = -0.162$, $p = 0.034$) in the LV group, there was no such relation in the control group ($r = 0.026$, $p = 0.721$).

4. Discussion

In our retrospective case-control study on the possible contribution of Hcy and related factors to the pathophysiology of LV, plasma Hcy levels were found to be significantly higher in the LV group compared to controls and higher levels of Hcy were detected in the young age.

Although the mechanism of LV formation is still unclear, it has been proposed that genetics and cumulative mechanical stress on an immature skeleton

play a role in the development of the LV [13, 14]. The maturation process of collagen requires at least 10 different enzymes and the process of cross-linking involves both enzymatic catalysis and a series of condensation reactions to form intra- and intermolecular cross-links [15-17].

Table 1. Basic characteristics of the study population

	Total subjects (n=361)	Patients with LV (n=171)	Healthy controls (n=190)	p
Age, years	30.3±2.82	30.2±2.86	30.4±2.78	0.485
Height, cm	166.4±6.03	166.1±6.44	166.7±5.63	0.314
Weight, kg	63.5±11.37	63.4±11.01	63.5±11.72	0.943
Body mass index, kg/m^2	23.0±4.08	23.0±4.07	22.9±4.10	0.677
BMD- Lumbar Total, g/cm^2	1.095±0.124	1.088±0.125	1.101±0.123	0.153
T-score Total	0.43±1.113	0.37±1.115	0.49±1.112	0.180
Homocysteine, µmol/L	9.52±3.321	10.58±4.032	8.57±2.113	**<0.001**
Vitamin B12, pg/ml	302.9±109.67	294.4±110.23	310.5±108.89	0.147
Folic acid, ng/ml	9.06±3.609	8.93±3.764	9.18±3.470	0.268
Current smoking, %	22.4	20.5	24.2	0.449
Alcohol consumption, %	9.7	7.6	11.6	0.217
Regularexercie, %	23.8	23.4	24.2	0.902

LV: Limbus vertebra; BMD: bone mineral density

It might be expected that alterations in the activity of these enzymes with lathyrogens such as homocysteine would result in changes in collagen maturation and cross-linking, and thus in bone mechanical properties determined by collagen structure [7]. Homocysteine concentrations were shown to be increased across age groups, higher among the oldest than among the middle-aged group [18, 19]. Although not significant ($p = 0.721$), such a tendency was present in the control group in our study, but on the other hand, we found significant negative correlation between levels of Hcy and age ($r = -0.162$, $p = 0.034$) in the LV group, which may demonstrate increase in Hcy levels during the early adulthood, thus interfering with the collagen maturation.

Plasma Hcy, folate, and vitamin B12 levels have been reported to be involved in lower BMD values [4, 18, 20,23]. The association between Hcy and BMD has been a topic of particular interest, but the results from individual research have been inconsistent. Bozkurt et al. [24] found that plasma Hcy levels were associated with osteoporosis in a sample of Turkish postmenopausal women. Moreover, Baines [20] and Gjesdal [18] showed a significant association of Hcy with BMD as well as significantly higher Hcy levels in the osteoporosis group. In the same way, Ouzzif et al. [25] reported that Hcy was significantly higher in the osteoporotic group, suggesting the level of Hcy to be inversely related to BMD at the lumbar spine in Moroccan postmenopausal women. However, there are also contrasting reports on the relationship between Hcy and low BMD [26-29]. It was concluded that the proposed mechanistic link between Hcy and osteoporosis was much more complicated and could not be explained only through BMD. The inconsistencies in these results may be related to different socio-demographic factors, dietary habits, the age of participants and BMD measurement sites. Our result in the LV group, although not statistically significant ($r = -0.051$, $p = 0.331$), was in accordance with previous studies that concluded that an elevated Hcy level was related to low BMD. The focus of several studies has been also on vitamin B12 and folic acid levels as far as they are related to the metabolism of Hcy [20, 21, 27, 30]. A statistically non-significant difference in serum vitamin B12 and folic acid concentrations were found between LV and control groups in the current study (294.4 ± 110.23 vs. 310.5 ± 108.89, $p = 0.147$ and 8.93 ± 3.764 vs. 9.18 ± 3.470, $p = 0.268$, respectively). These findings are consistent with the results of previous studies suggesting a lack of relationship between low levels of vitamin B12 and folic acid and BMD [26,28, 31].

The strength of this study is that all the measurements were performed with a single DXA scanner and by a single biochemistry lab. However, limitations such as small sample size, lack of data on biochemical markers of bone turnover and vitamin D should be considered. Studies with large sample size are needed to provide possible contribution of Hcy in the pathogenesis of LV.

5. Conclusion

In conclusion, results of this study show that Hcy levels are higher in young women with LV. Nevertheless, it seems necessary to design large longitudinal prospective studies in order to definitively characterize the bone loss and high Hcy levels accounting in LV patients.

6. Kaynaklar

1. Ghelman B, Freiberger RH. The limbus vertebra: an anterior disc herniation demonstrated by discography. AJR American journal of roentgenology. 1976;127(5):854-5.
2. Hellstrom M, Jacobsson B, Sward L, Peterson L. Radiologic abnormalities of the thoraco-lumbar spine in athletes. Acta radiologica. 1990;31(2):127-32.
3. Kim DJ, Koh JM, Lee O, Kim NJ, Lee YS, Kim YS, Park JY, Lee KU, Kim GS. Homocysteine enhances apoptosis in human bone marrow stromal cells. Bone. 2006;39(3):582-90.
4. Herrmann M, Widmann T, Colaianni G, Colucci S, Zallone A, Herrmann W. Increased osteoclast activity in the presence of increased homocysteine concentrations. Clinical chemistry. 2005;51(12):2348-53.
5. Vaes BL, Lute C, Blom HJ, Bravenboer N, de Vries TJ, Everts V, Dhonukshe-Rutten RA, Muller M, de Groot LC, Steegenga WT. Vitamin B(12) deficiency stimulates osteoclastogenesis via increased homocysteine and methylmalonic acid. Calcified tissue international. 2009;84(5):413-22.
6. Tyagi N, Kandel M, Munjal C, Qipshidze N, Vacek JC, Pushpakumar SB, Metreveli N, Tyagi SC. Homocysteine mediated decrease in bone blood flow and remodeling: role of folic acid. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2011;29(10):1511-6.
7. Blouin S, Thaler HW, Korninger C, Schmid R, Hofstaetter JG, Zoehrer R, Phipps R, Klaushofer K, Roschger P, Paschalis EP. Bone matrix quality and plasma homocysteine levels. Bone. 2009;44(5):959-64.
8. Herrmann M, Tami A, Wildemann B, Wolny M, Wagner A, Schorr H, Taban-Shomal O, Umanskaya N, Ross S, Garcia P, Hubner U, Herrmann W. Hyperhomocysteinemia induces a tissue specific accumulation of homocysteine in bone by collagen binding and adversely affects bone. Bone. 2009;44(3):467-75.
9. Thaler R, Agsten M, Spitzer S, Paschalis EP, Karlic H, Klaushofer K, Varga F. Homocysteine suppresses the expression of the collagen cross-linker lysyl oxidase involving IL-6, Fli1, and epigenetic DNA methylation. The Journal of biological chemistry. 2011;286(7):5578-88.
10. Kim GS, Kim CH, Park JY, Lee KU, Park CS. Effects of vitamin B12 on cell proliferation and cellular alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells and UMR106 osteoblastic cells. Metabolism: clinical and experimental. 1996;45(12):1443-6.
11. Roman-Garcia P, Quiros-Gonzalez I, Mottram L, Lieben L, Sharan K, Wangwiwatsin A, Tubio J, Lewis K, Wilkinson D, Santhanam B, Sarper N, Clare S, Vassiliou GS, Velagapudi VR, Dougan G, Yadav VK. Vitamin B(1)(2)-dependent taurine synthesis regulates growth and bone mass. The Journal of clinical investigation. 2014;124(7):2988-3002.
12. Holstein JH, Herrmann M, Splett C, Herrmann W, Garcia P, Histing T, Graeber S, Ong MF, Kurz K, Siebel T, Menger MD, Pohlemann T. Low serum folate and vitamin B-6 are associated with an altered cancellous bone structure in humans. The American journal of clinical nutrition. 2009;90(5):1440-5.
13. Koyama K, Nakazato K, Min S, Gushiken K, Hatakeda Y, Seo K, Hiranuma K. COL11A1 gene is associated with limbus vertebra in gymnasts. International journal of sports medicine. 2012;33(7):586-90.
14. Bennett DL, Nassar L, DeLano MC. Lumbar spine MRI in the elite-level female gymnast with low back pain. Skeletal radiology. 2006;35(7):503-9.
15. Yamauchi M. Collagen: The Major Matrix Molecule in Mineralized Tissues. In: Anderson JJB, Garner SC, editors. Calcium and Phosphorus Nutrition in Health and Disease. Florida: CRC Press; 1995. p. 127-47.
16. Bailey AJ, Paul RG, Knott L. Mechanisms of maturation and ageing of collagen. Mechanisms of ageing and development. 1998;106(1-2):1-56.
17. Knott L, Bailey AJ. Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance. Bone. 1998;22(3):181-7.
18. Gjesdal CG, Vollset SE, Ueland PM, Refsum H, Drevon CA, Gjessing HK, Tell GS. Plasma total homocysteine level and bone mineral density: the Hordaland Homocysteine Study. Archives of internal medicine. 2006;166(1):88-94.
19. Jacques PF, Rosenberg IH, Rogers G, Selhub J, Bowman BA, Gunter EW, Wright JD, Johnson CL. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. The American journal of clinical nutrition. 1999;69(3):482-9.
20. Baines M, Kredan MB, Usher J, Davison A, Higgins G, Taylor W, West C, Fraser WD, Ranganath LR. The association of homocysteine and its determinants MTHFR genotype, folate, vitamin B12 and vitamin B6 with bone mineral density in postmenopausal British women. Bone. 2007;40(3):730-6.
21. Swart KM, van Schoor NM, Lips P. Vitamin B12, folic acid, and bone. Current osteoporosis reports. 2013;11(3):213-8.
22. Bahtiri E, Islami H, Rexhepi S, Qorraj-Bytyqi H, Thaci K, Thaci S, Karakulak C, Hoxha R. Relationship of homocysteine levels with lumbar spine and femur neck BMD in postmenopausal women. Acta reumatologica portuguesa. 2015;40(4):355-62.
23. Zhang H, Tao X, Wu J. Association of homocysteine, vitamin B12, and folate with bone mineral density in postmenopausal women: a meta-analysis. Archives of gynecology and obstetrics. 2014;289(5):1003-9.
24. Bozkurt N, Erdem M, Yilmaz E, Erdem A, Biri A, Kubatova A, Bozkurt M. The relationship of homocyteine, B12 and folic acid with the bone mineral density of the femur and lumbar spine in Turkish postmenopausal women. Archives of gynecology and obstetrics. 2009;280(3):381-7.
25. Ouzzif Z, Oumghar K, Sbai K, Mounach A, Derouiche el M, El Maghraoui A. Relation of plasma total homocysteine, folate and vitamin B12 levels to bone mineral density in Moroccan healthy postmenopausal women. Rheumatology international. 2012;32(1):123-8.
26. Cagnacci A, Bagni B, Zini A, Cannoletta M, Generali M, Volpe A. Relation of folates, vitamin B12 and homocysteine to vertebral bone mineral density change in postmenopausal women. A five-year longitudinal evaluation. Bone. 2008;42(2):314-20.
27. Haliloglu B, Aksungar FB, Ilter E, Peker H, Akin FT, Mutlu N, Ozekici U. Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women. Archives of gynecology and obstetrics. 2010;281(4):663-8.
28. Rumbak I, Zizic V, Sokolic L, Cvijetic S, Kajfez R, Colic Baric I. Bone mineral density is not associated with homocysteine level, folate and vitamin B12 status. Archives of gynecology and obstetrics. 2012;285(4):991-1000.
29. van Meurs JB, Dhonukshe-Rutten RA, Pluijm SM, van der Klift M, de Jonge R, Lindemans J, de Groot LC, Hofman A, Witteman JC, van Leeuwen JP, Breteler MM, Lips P, Pols HA, Uitterlinden AG. Homocysteine levels and the risk of osteoporotic fracture. The New England journal of medicine. 2004;350(20):2033-41.
30. Cagnacci A, Baldassari F, Rivolta G, Arangino S, Volpe A. Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women. Bone. 2003;33(6):956-9.
31. Kakehasi AM, Carvalho AV, Maksud FA, Barbosa AJ. Serum levels of vitamin B12 are not related to low bone mineral density in postmenopausal Brazilian women. Revista brasileira de reumatologia. 2012;52(6):863-9.