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A Comparative Study of Vitamin D Level and Lactate Dehydrogenase Activity in Relation to Oxidative Stress in Women with Osteoporosis

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Abstract

Background: Osteoporosis (OP) is a silent disease that affects the microarchitecture of the skeleton bones, leading to a drop in mineral levels and an increase in fracture rates. This condition is more common in women than men, especially postmenopausal women, due to decreased production of estrogen, a hormone that regulates bone formation and bone resorption. Oxidative stress is a known factor in the development of OP, resulting from an imbalance in the production of oxidants and antioxidants. Hip and arm fractures are particularly common in women with OP.

Objective: The study aimed to evaluate the activity of Lactate Dehydrogenase (LDH) and oxidative stress (OS) levels in postmenopausal women with osteoporosis and varying levels of vitamin D, and to compare these groups based on the measured parameters.

Subjects and Methods: This study was conducted at the Baquba Teaching Hospital in Diyala, Iraq, from October 2022 to January 2023. The study involved 100 women whom were divided into two groups, 50 with normal vitamin D levels and 50 with abnormal vitamin D levels. The malondialdehyde (MDA) level was evaluated using the Satoh-modified approach.

Results: According to the statistical analysis conducted with the SPSS program, there was a significant difference ($P<0.001$) in Vitamin D levels between the groups ($OP_{n.\ D}$) and ($OP_{ab.D}$). Additionally, significant increases were observed in LDH activity ($P<0.001$) and MDA levels ($P<0.05$) for the group ($OP_{ab.D}$) in comparison to ($OP_{n.\ D}$).

Conclusion: The current study has indicated that women suffering from osteoporosis (OP) have higher levels of LDH and MDA. Additionally, there is an inverse correlation between vitamin D and these two variables; this correlation suggests that vitamin D may possess antioxidant properties that reduce the effects of oxidative stress (OS) in the body. Therefore, vitamin D may help in reducing LDH levels.

Keywords: Lactate dehydrogenase; Malondialdehyde; Osteoporosis; Oxidative stress; Vitamin D.

Introduction

Osteoporosis (OPs) is a serious health problem that results from an imbalance of bone turnover between resorption and production. It is marked that losing of bone mass leads to decreasing bone mineral density (BMD), and abnormal bone microarchitecture. These changes compromise the physical strength of the bone and make the skeleton more brittle, putting patients at an increased risk of fractures (mostly wrist, hip, and spine fractures) from minor trauma [1- 4]. Osteoporotic fracture is a serious medical issue that adversely affects the quality of life of those who suffer from it. However, OPs result in more than 8.9 million fractures annually, which implies that one is produced by this condition every three seconds. A hip fracture affects about 33% of patients, and up to 20% of those patients go on to die within a year after the fracture, mostly as a result of previous diseases [5]. In their lifetime: 1 in 3 women and 1 in 5 men over the age of 50 will sustain an osteoporotic fracture, according to the International Osteoporosis Foundation (IOF). Estrogen deficiency following the aging process is the primary cause of hormone-related OPs in postmenopausal women; it affects primarily women. According to estimates, 200 million men and women worldwide, mostly those over 60, suffer from OPs, a condition that is quite common [6]. Usually, the growth of healthy bones and the maintenance of appropriate calcium levels depend heavily on vitamin D (calciferol), a fat-soluble vitamin. Cholecalciferol, often known as vitamin D3, and ergocalciferol, sometimes known as vitamin D2, are the two most significant vitamin D components. In addition to playing a significant part in ensuring that calcium and phosphate concentrations are kept at an appropriate level, vitamin D is also important for the immune system and cell proliferation and differentiation [7]. Low vitamin D levels may contribute to bone loss and low BMD which is a key indicator of osteoporotic fractures. Where BMD decreases mainly in the arm, rib, and thoracic spines, total bone mineral contents are much lower, impacting on the quality of life due to fractures. According to estimates, Asia will account for more than half of all hip fractures

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worldwide by 2050 [8-10]. Additionally, the many functions of vitamin D help explain why a decline in its level has been linked to a number of chronic disorders. In reality, a deficiency of vitamin D increases the risk of OPs and various other diseases and complications that affect how bones are metabolized, like autoimmune diseases, inflammatory bowel diseases (IBD), allergies, endocrinological disorders, bone marrow transplantation, and hematological malignancies [11]. Lactate dehydrogenase (LDH, EC 1.1.1.27), is a widely distributed enzyme that participates in carbohydrate metabolism by using the Nicotinamide adenine dinucleotide (NADH) coenzyme system to catalyze the interconversion of lactate and pyruvate. The body has large amounts of LDH, with high concentrations in the heart, liver, skeletal muscle, erythrocytes, and kidney and low concentrations in the lung, brain, and smooth muscle [12,13]. It has two isoforms, LDHA and LDHB [14]. The LDH enzymes are tetrameric enzymes that are made up of two distinct subunits, M and H. These two subunits can be combined to form five isozymes: LDH1 (4H), LDH 2 (3H, 1M), LDH 3 (2H, 2M), LDH 4 (1H, 3M), and LDH 5 (4M) [15]. Usually, LDH values in different tissues are typically 5000–15,000 times higher than normal serum concentrations [16]. High levels of the enzyme LDH usually indicate tissue damage [17]. Infections that cause tissue damage cause the enzyme LDH to migrate from the cytoplasm of cells to the bloodstream. Exercise, especially vigorous exercise, can enhance lipid peroxidation, inflammation, and OSs, which in turn cause tissue damage and raise LDH levels. Therefore, vitamin D reduces tissue damage by acting as an antioxidant [18]. Wiseman initially established that vitamin D is an antioxidant in 1993. On the other hand, LDH can play a significant role in controlling the redox status of cells. In cancer cells, LDH can show antioxidative and pro-oxidative activities simultaneously [19]. According to the definition of oxidative stress (OS; also known as molecular damage)" an increase in the oxidants (creation of derivatives of molecular oxygen and nitrogen: reactive oxygen species (ROS), and reactive nitrogen species (RNS), which lead to a disruption of redox signaling and control and/or molecular damage" [20]. In various cells and cellular compartments, ROS production is strictly controlled under normal physiological conditions. SO, OSs result from any disruption in the equilibrium between cellular production of ROS and antioxidant balance [21]. The mitochondrial activity of skeletal muscle is impacted by OSs, which is linked to vitamin D deficiency. In particular, vitamin D deficiency results in decreased oxygen consumption and impairment of mitochondrial activity. It's possible that the activation of the vitamin D receptor (VDR) is responsible for these harmful effects on muscle [22, 23]. Vitamin D3, its active metabolite 1,25-dihydroxycholecalciferol, vitamin D2, and pro-vitamin D3 (7-dehydrocholesterol) all suppressed the process of iron-dependent liposomal lipid peroxidation [20]. Vitamin D also acts as an antioxidant by inhibiting free radicals and the consequent oxidative modification of vital molecules [24, 25].

The study aimed to evaluate Lactate Dehydrogenase (LDH) activity and oxidative stress (OS) status in postmenopausal osteoporotic women with normal and abnormal vitamin D levels and to compare the two groups in terms of the relevant measured parameters.

Subjects and methods

Postmenopausal Iraqi women who attended the Baquba Teaching Hospital in Diyala, Iraq, were included in this study; which was conducted between October 2022 and January 2023. The study included 100 OP postmenopausal women who were grouped into two groups according to the T-score values obtained from the dual energy X-ray absorption spectroscopy; DEXA scan. Fifty OPs patients with normal vitamin D levels ($OP_{n.\ D}$) and 50 OPs patients with abnormal vitamin D ($OP_{ab.D}$); were included in the study. The body mass index (BMI; Kg/m^2) was calculated through weight in kg and length in (m^2). Interviews with the cases were conducted using a questionnaire form to collect the necessary information. Patients who were being treated with steroid medications for hypothyroidism, hyperthyroidism, rheumatoid arthritis, or diabetes mellitus were excluded from the samples. The ethics committee of the College of Science / University of Baghdad authorized the protocol.

A volume of 5 mL of venous blood from the collected samples was put into the tube at about 25ºC and centrifuged for 5 minutes at 4,000 rpm; the serum was examined right away after being obtained. The LDH activity was determined by using Cobas c 111 Analyzer automatically after calibration (Roche/Hitachi Cobas c systems) technique. The MDA evaluation was done by using the Satoh-modified method [16]. The vitamin D test was done using VIDAS® 25 OH Vitamin D Total - bioMerieux - France. The Statistical Package for Social Sciences (SPSS) version 26.0 was employed to analyze the data.

Results

The women of (the $OP_{n.\ D}$) group were taking (1000 UI/day) of a vitamin D supplement but had stopped taking it a month before the test, while ($OP_{ab.\ D}$) Women never took a vitamin D supplement.

Table 1 show the mean ± SD values for age, BMI, and T-score; BMD value for the two groups Only BMD seemed to be significantly different between the two groups.

Table 1: Mean ± SD values for selected variables in the two study groups

Variables	Study groups	Mean	±SD	Student t-test results	
				p-value	Sig.
Age (years)	$OP_{n.\ D}$	58.4	6.34	p>0.05	N. S
	$OP_{ab.\ D}$	56.1	6.27		
BMI (kg/cm^2)	$OP_{n.\ D}$	30.1	4.14	p>0.05	N. S
	$OP_{ab.\ D}$	29.1	3.62		
T-score	$OP_{n.\ D}$	-3.2	0.57	p>0.05	N. S
	$OP_{ab.\ D}$	-2.9	1.26		
BMD (g/cm^2)	$OP_{n.\ D}$	0.72	0.06	p<0.05	Sig.
	$OP_{ab.\ D}$	0.71	0.11		

Table 2 shows highly significant differences in vitamin D and LDH activity between ($OP_{n.\ D}$) and ($OP_{ab.\ D}$), while MDA level was significantly different between the two groups.

Table 2: Mean ± SD values for selected lab tests in the two study groups

Variables	Study groups	Mean	±SD	Student t-test results	
				p-value	Sig.
Vitamin D (ng/ml)	$OP_{n.\ D}$	30.0	6.55	p<0.001	H. S
	$OP_{ab.\ D}$	7.4	2.71		
LDH activity (U/L)	$OP_{n.\ D}$	171.6	46.76	p<0.001	H. S
	$OP_{ab.\ D}$	212.3	24.68		
MDA (mmol/L)	$OP_{n.\ D}$	15.1	6.84	p<0.05	Sig.
	$OP_{ab.\ D}$	20.9	9.95		

The correlation between LDH with vitamin D and MDA is shown in Figures 1 and 2 respectively).

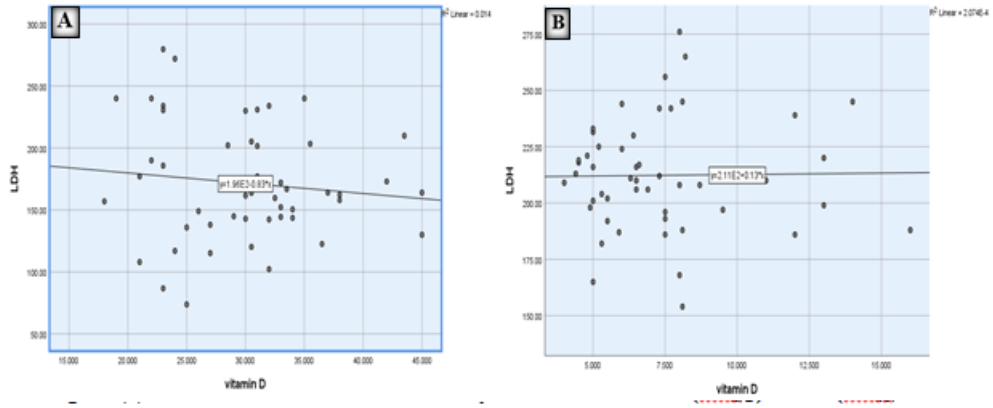

Figure (1): Correlation between LDH activity and vitamin D in A ($OP_{n.\ D}$) and B ($OP_{ab.\ D}$)

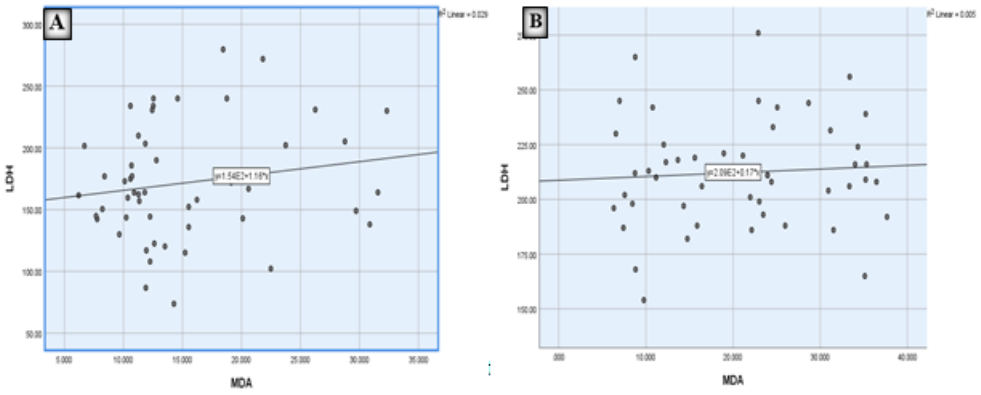

Figure (2): Correlation between LDH activity and MDA in A ($OP_{n.D}$) and B ($OP_{ab.D}$)

Discussion

All the women in group ($OP_{n.D}$) were taking vitamin D prior to the commencement of the study, which explains the finding that their vitamin D levels were significantly higher than those in the ($OP_{ab.D}$) group. Taking this daily supplement improved the level of BMD, as was shown in Table 2. Vitamin D supplementation has a beneficial effect on enhancing BMD in women with OPs [23], which is consistent with our findings (the differences between the two

groups were significant for both the vitamin and BMD).

The OSs are affected by many diseases [28-30], its common biomarker is MDA (an end-product of lipid peroxidation induced by ROS and is considered an OS indicator) [13,31]. According to certain studies, the MDA levels were markedly elevated in OPs, this fact is in agreement with previous studies that showed increasing MDA levels in OPs patients [30,32,33], and is consistent with our findings (Table 2). The study also showed that MDA levels fell as vitamin D levels rise and vice versa [34]. The current study found a significant increase in serum LDH activity in ($OP_{ab.\ D}$) compared to ($OP_{n.D}$) (Table 2).

Wiseman initially established that vitamin D is an antioxidant in 1993. On the other hand, LDH can play a significant role in controlling the redox status of cells. In cancer cells, LDH can show antioxidative and pro-oxidative activities simultaneously [38]. Also, according to Abdlkarem and Zainulabdeen LDH can play as an indicator for increasing OSs [30]. The results in Figures 1 and 2 prove this information.

According to the findings of the current study for groups ($OP_{n.D,}$ and $OP_{ab.D}$), vitamin D appeared to be inversely related to the enzyme, as the blood level of the enzyme decreased when vitamin D levels increased. The data reported for the same groups, Figure (2) also demonstrates a linear relationship between OSs products (MDA) and the LDH activity. The correlation of LDH with MDA, and vitamin D has been shown in (Figures 1 and 2, respectively) by comparing patients who had vitamin D deficiency with patients who had adequate vitamin D concentration. Overall, the correlation coefficient was not significant and revealed a weak correlation value. For ($OP_{ab.\ D}$) group, LDH activity increased acting as a pro-oxidant causing an increase in OS status (by MDA level). On the other hand, in the ($OP_{n.\ D}$) group, the vitamin intake as a supplement affected the results, because of the antioxidant action of this vitamin via reducing OSs, as a result of decreasing: LDH levels in the blood, and the levels of MDA, respectively, which help to, at the very least, stop the progression of the disease. The results were expected to be more pronounced, but women may need adequate vitamin intake for long periods (the vitamin values were normal at the lowest level (30.0 ± 6.55; lower limit) for the ($OP_{n.\ D}$) range of expected values (30-100 ng/ml) for healthy individuals. Another reason for these results is the comparison between a different (unpaired) person; a follow-up study may give clearer results.

Conclusion

The current study shows higher levels of LDH and MDA in women with OP, in addition to an inverse relationship between these two variables and vitamin D which may suggest an antioxidant effect for vitamin D that reduces the effects of OS, and thus reducing LDH levels.

Authors' declaration:

Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in the College of Science, University of Baghdad, according to the code number (CSEC/1123/0112) on (10/ 11/ 2023).

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Author Contributions:

Study conception & design: (Jwan A. Zainulabdeen). Literature search: (Husham A. Abdlkarem). Data acquisition: (Husham A. Abdlkarem). Data analysis & interpretation: (Husham A. Abdlkarem). Manuscript preparation: (Husham A. Abdlkarem). Manuscript editing & review: (Jwan A. Zainulabdeen).

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دراسة مقارنة مستوى فيتامين د ونشاط اللاكتيت ديهايدروجينيز وعلاقته بالإجهاد التأكسدي لدى النساء المصابات بهشاشة العظام

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الخلاصة

خلفية البحث: هشاشة العظام (OP) هو مرض صامت يؤثر على البنية الدقيقة لعظام الهيكل العظمي، مما يؤدي إلى انخفاض مستويات المعادن وزيادة معدلات الكسور. هذه الحالة أكثر شيوعا لدى النساء من الرجال، وخاصة النساء بعد انقطاع الطمث، وذلك بسبب انخفاض إنتاج هرمون الاستروجين، وهو الهرمون الذي ينظم تكوين العظام وارتشاف العظام. يعد الإجهاد التأكسدي عاملاً معروفًا في تطور OP، الناتج عن خلل في إنتاج المواد المؤكسدة ومضادات الأكسدة. تعد كسور الورك والذراع شائعة بشكل خاص عند النساء المصابات بـ OP.

الهدف: هدفت الدراسة إلى تقييم نشاط مستويات هيدروجيناز اللاكتات (LDH) والإجهاد التأكسدي (OS) لدى النساء بعد انقطاع الطمث المصابات بهشاشة العظام ومستويات مختلفة من فيتامين د ومقارنة هذه المجموعات بناءً على المعلمات المقاسة.

المرضى وطرق البحث: أجريت هذه الدراسة في مستشفى بعقوبة التعليمي في ديالى، العراق، في الفترة من أكتوبر 2022 إلى يناير 2023. وشملت الدراسة 100 امرأة تم تقسيمهن إلى مجموعتين، 50 بمستويات طبيعية من فيتامين د و50 بمستويات غير طبيعية من فيتامين د. تم استخدام نتائج T-score من التحليل الطيفي لامتصاص الأشعة السينية مزدوج الطاقة (DEXA)، لتحديد النساء في كل مجموعة. تم قياس نشاط LDH باستخدام نهج محلل Cobas c 111 الآلي (أنظمة Roche / Hitachi Cobas c). تم تقييم مستوى MDA باستخدام النهج المعدل بواسطة Satoh. تم تحديد مستويات فيتامين د باستخدام اختبار VIDAS® 25 OH Vitamin D Total الذي أجرته شركة bioMerieux - فرنسا.

النتائج: ووفقاً للتحليل الإحصائي الذي أجري باستخدام برنامج SPSS، كان هناك فرق معنوي (P<0.001) في مستويات فيتامين د بين المجموعتين (OPn.D) و (OPab.D). بالإضافة إلى ذلك، لوحظت زيادات معنوية في نشاط LDH (P<0.001) ومستويات MDA (P<0.05) لمجموعة (OPab.D) مقارنة بـ (OPn.D).

الإستنتاج: أشارت الدراسة الحالية إلى أن النساء اللاتي يعانين من هشاشة العظام (OP) لديهن مستويات أعلى من LDH وMDA. بالإضافة إلى ذلك، هناك علاقة عكسية بين فيتامين د وهذان المتغيرين، ويشير هذا الارتباط إلى أن فيتامين د قد يمتلك خصائص مضادة للأكسدة تقلل من آثار الإجهاد التأكسدي (OS) في الجسم. لذلك، قد يساعد فيتامين د في تقليل مستويات LDH..

الكلمات المفتاحية: هشاشة العظام، فيتامين د، ديهايدروجينيز اللاكتيت ، الإجهاد التأكسدي.