Case study
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Estimation of Bone Metabolic Biomarkers (MDA, BSAP, Calcium, and Vitamin D3) in Elderly Individuals Aged 50 to 70 Years of Both Sexes

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تقدير المؤشرات الحيوية الاستقلابية للعظام(MDA ، وBSAP، والكالسيوم، وفيتامين D3) لدى كبار السن الذين تتراوح أعمارهم بين 50 و70 عامًا من كلا الجنسين

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ABSTRACT

Background: Osteoporosis (OP) is a long-term skeletal disturbance marked by loss of bone mass and bone tissue degradation, which increases fragility and the risk of fractures. Because of hormonal changes that speed up bone resorption, postmenopausal women are most affected by this disorder. Oxidative stress indicators are among the many biomarkers that are essential for understanding the metabolic mechanisms that underlie osteoporosis.

Materials and Methods A case-control study of 120 participants (80 patients, 40 controls) was conducted. Serum levels of MDA, BSAP, D3 and Ca+ were measured using ELISA kits, Statistical analysis was performed using SPSS, with a significance level set at $P < 0.05$.

Results: A statistical analysis using the SPSS program demonstrated a significant reduction in vitamin D levels among patients suffering from osteoporosis and a healthy control group ($P<0.005$). Furthermore, individuals with osteoporosis showed elevated of BSAP, and malondialdehyde (MDA) levels, indicating heightened oxidative stress.

Conclusion: This study highlights the association between oxidative stress and osteoporosis severity. The results suggest that vitamin D may play a crucial role in counteracting oxidative damage and preserving bone integrity.

Key words: Malondialdehyde; Oxidative stress; Osteoporosis; BSAP; Vitamin D.

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INTRODUCTION

The World Health Organization (WHO) is asserts that osteoporosis is characterized by a bone mineral density (BMD) that falls more than -2.5 standard deviations below the average normal value; in contrast, osteopenia is defined as a BMD ranging between -1 and -2.5 standard deviations. moreover, postmenopausal women account for approximately 80% of individuals affected by osteoporosis [1].

Numerous elements, including oxygen supply, nutrients, hormones, cytokines, growth factors, and oxidative stress, can have a typical impact on bone tissue. According to a recent research, oxidative stress is a key factor in the pathophysiology of osteoporosis, bone tissue will be destroyed by excessive levels of free radicals in the blood when there is oxidative stress; therefore, osteoblasts will produce antioxidants in order to protect bone cells from injury caused by free radicals[2] .

Lipid peroxidation causes damage to cells in the body, such as mitochondria, DNA, lipids, and proteins, which then leads to the production of some reactive aldehydes.[3]

Malondialdehyde (MDA) has been demonstrated to play a significant pathogenic role in degenerative disorders, such as osteoporosis, which is marked by increased oxidative stress, this is due to the ability of MDA to form connections with proteins, such as those involved in cell death. These connections can cause functional damage and accelerate molecular turnover [4]. MDA, a byproduct of lipid peroxidation, is frequently utilized as a biomarker of oxidative stress in a variety of health concerns, including osteoporosis, cancer, asthma, and cardiovascular disease, the assessment of MDA levels can be performed in various biological specimens, such as blood, plasma, and tissue, through a range of analytical methods [5].

Exercise has been reported to improve bone health due to the mechanical load sensitivity of bones; weight-bearing exercises, including hopping and jogging, in conjunction with progressive resistance training, whether in isolation or in combination, have been demonstrated to be effective, are beneficial in stimulating and promoting osteogenic responses in bone [6].

Resistance training involves different muscle contractions (isotonic, isokinetic, and isometric). Bone turnover markers (BTMs) are indicators of bone metabolism and the remodeling process[7]. It has been determined that certain blood and urine molecules can be utilized as biological markers (BTMs) in the assessment of bone turnover dynamics; these molecules offer a highly effective approach to evaluating the metabolic status of the bone, exhibiting sensitivity to both short-term and long-term therapeutic intervention periods.[8].

BTMs fall in the categories of bone formation and bone resorption; the former are the result of the direct or indirect actions of active osteoblasts, secreted by osteoblasts at different stages with different characteristics of osteoblast function and bone growth. Researchers measure all bone formation markers using blood serum. The majority of the bone resorption markers are the product of bone collagen degradation, and their levels are detected in blood serum and urine [9].

The potential variation in the concentration of BTMs may serve as an indicator of the status of bone metabolism; consequently, there has been a continuous increase in the utilization of these substances. While there is a growing body of literature attributing the beneficial effects of

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resistance training to bone health, there is paucity of research addressing the impact of resistance training on the microcirculation of bone tissue and bone turnover markers [10].

Indeed, as posited by certain authors, the efficacy of exercises in regard to bone tissue mineralization (BTM) has been a subject of debate. This is due to the fact that, under typical circumstances, any increase in bone formation is accompanied by an increase in bone loss. Monitoring BTMs in clinical practice may facilitate the elucidation of the physiological mechanisms responsible for the osteogenic effect of therapeutic intervention [11].

MATERIALS AND METHODS

This study included a total of 120 participant (80 osteoporosis patient ,40 healthy control) of different ages undergoing at osteoporosis at the Merjan Medical City from November 1, 2024 to January 12, 2025 classified based on gender (40 man40 woman among patient-20 men ,20 women among control) and according to age group (50-59,60-69,70-79) , after recording information about patients BMI was also calculated using the formula weight (kg)/height² (m^2), and participants were categorized into normal weight, overweight, and obese groups, taken 5ml of blood were collected for biochemical study the study contributed by utilizing the ELISA the measurement should be taken in accordance with the manufacturer's instructions, MDA ; BSAP ,D3,Ca+ concentration of both affected and healthy, and they were measured using (ELISA kits from BT Lab- China) [12]. Data was statically analyzed by spss.

RESULTS AND DISCUSSION

The patients and controls were divided and analyzed based on gender, age categories (50–59, 60–69, 70–79 years), and BMI (normal weight, overweight, obese). BMI was calculated using standard anthropometric methods. In The table (1) shows the distribution of gender and Body Mass Index (BMI) between the two groups. It is noticeable that there is a higher percentage of overweight and obesity among osteoporosis patients compared to the control group, which may indicate a link between the study groups.

Table (1) General data of study group (osteoporosis patients and healthy control)

Parameter	Healthy control (N=40)	osteoporosis patient (N=80)
Gender		
Male	20	40
Female	20	40
BMI		
Healthy weigh	12	31
Over weight	16	21
Obesity	12	28

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The table indicate a significant increase in BSAP levels (P=0.027) and MDA levels (P=0.000) in osteoporosis patients compared to the control group. The significant difference at P<0.01 indicates biological changes associated with osteoporosis. These results support the hypothesis of increased oxidative stress (MDA) and bone formation activity (BSAP) in osteoporosis cases.

Table (2): The level of parameter (BSAP(U/L) , MDA (nmol/ml)) of osteoporosis patients and normal subjects.

	osteoporosis patients (n=80)	**Controls (n=40)**	**P. Value of T-Test**
BSAP(U/L)	131.95±12.29	91.19±25.92	0.027*
MDA (nmol/ml)	149.49±34.75	61.34±15.43	0.000**

T-test , values (mean ± S.D)

In table (3) there is a significant decrease in calcium levels (P=0.002) and vitamin D3 levels (P=0.000) in patients compared to the control group. This finding reinforces the hypothesis that vitamin D and calcium deficiency is a major factor in osteoporosis development.

Table (3): level of biochemical (S. Ca mg/dl, D3 ng/ml) in osteoporosis patient and control subjects.

	osteoporosis patients (n=80)	**Controls (n=40)**	**P. Value of T-Test**
S. Ca mg/dl	8.63±0.98	9.18±0.59	0.002*
D3 ng/ml	18.86±2.84	28.34±4.56	0.000**

T-test , values (mean ± S.D)

There are no significant differences were found between males and females in the levels of calcium, vitamin D3, BSAP, and MDA (P>0.05), suggesting that gender is not a clear influencing factor for these variables within the study sample as showed in table 4.

Table (4): Level of some biomarkers (BSAP(U/L), MDA (nmol/ml), Ca mg/dl, D3 ng/ml) in osteoporotic patients according to sexes

Parameter	Male (N0 . 40)	Female (No. 40)	P.vale
	Mean ± SD	**Mean ±SD**	**P. value**
Calcium(mg/dl)	8.72±0.90	8.54± 1.07	0.484
D3 (ng/ml)	19.23±6.08	18.50±5.67	0.636
BSAP (U/L)	135.58 ± 46 .72	128.32 ±30.50	0.805
MDA(nmol/ml)	145.97 ±33.33	153.00±36.33	0.483

ANOVA test, values (mean ± S.D)

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The tables (5-6) show slight differences in BSAP and MDA levels among different age groups, with a tendency for these levels to increase with age, though without strong statistical significance (P>0.05). This result may indicate that the age-related impact on osteoporosis is gradual rather than abrupt.

Table (5): level of the biomarkers (BSAP(U/L), MDA (nmol/ml)) in patient with osteoporosis and normal one in relation to sex, age.

Parameter		Male (No. 40)		Female (N0.40)		
	Age Groups	**No.**	**Mean ± S D**	**No**	**Mean ±S D**	**P. value**
BSAP (U/L)	50 - 59 years	15	110.24±24.38	15	108.59±20.75	0.847
	60 - 69 years	15	129.36± 27.58	15	160.06±53.61	
	70 - 79 years	10	159.24± 21.43	10	117.79±43.81	
MDA (nmol/ml)	50 - 59 years	15	131.33 ± 20.84	15	165.89±27.24	0.440
	60 - 69 years	15	146.77± 21.06	15	154.45±25.63	
	70 - 79 years	10	156.35± 33.18	10	145.63± 36.89	

the result was mean ± S.D

Table (6): level of biochemical parameter vitamin D3 (ng/ml) and calcium(mg/dl)) in osteoporotic patients and healthy subjects of both sexes.

Parameter		Male (No. 40)		Female (N0.40)		
	Age Groups	**No.**	**Mean ± S D**	**No**	**Mean ±S D**	**P . value**
S, Ca (mg/dl)	50 - 59 years	15	8.53 ± 0.87	15	8.44±1.15	0.951
	60 - 69 years	15	8.86 ± 0.82	15	8.49±0.75	
	70 - 79 years	10	8.76± 1.02	10	8.62±1,25	
D3 (ng/ml)	50 - 59 years	15	19.56 ± 6.09	15	20.39±6.60	0.637
	60 - 69 years	15	19.99± 5.68	15	15.82±4.52	
	70 - 79 years	10	18.41±6.77	10	19.29±5.61	

ANOVA test, values (mean ± S.D)

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The Table (7) demonstrates the efficiency of biochemical markers (BSAP, MDA, Ca, D3) in diagnosing osteoporosis using sensitivity, specificity, and area under the curve (AUC) values. BSAP shows the highest sensitivity (0.983), while MDA has the highest AUC (0.954), indicating it as a strong marker for identifying osteoporosis. These statistical values strengthen the reliability of using these parameters in clinical practice.

Table (7): sensitivity and specificity of study parameters (BSAP(U/L), MDA (nmol/ml), Ca mg/dl, D3 ng/ml) .

Parameters	Sensitivity	Specificity	AUC	Cut off	P. Value	Asymptotic 95% Confidence Interval	
						Lower	Upper
Ca (mg/dl)	0.650	0.975	0.333	8.15	0.005	0.229	0.437
D3 (ng/ml)	0.850	0.950	0.219	13.85	0.000	0.120	0.318
BSAP(U/L)	0.983	0.875	0.682	65.53	0.002	0.576	0.788
MDA(nmol/ml)	0.967	0.250	0.954	87.06	0.000	0.918	0.989

the result was mean ± S.D

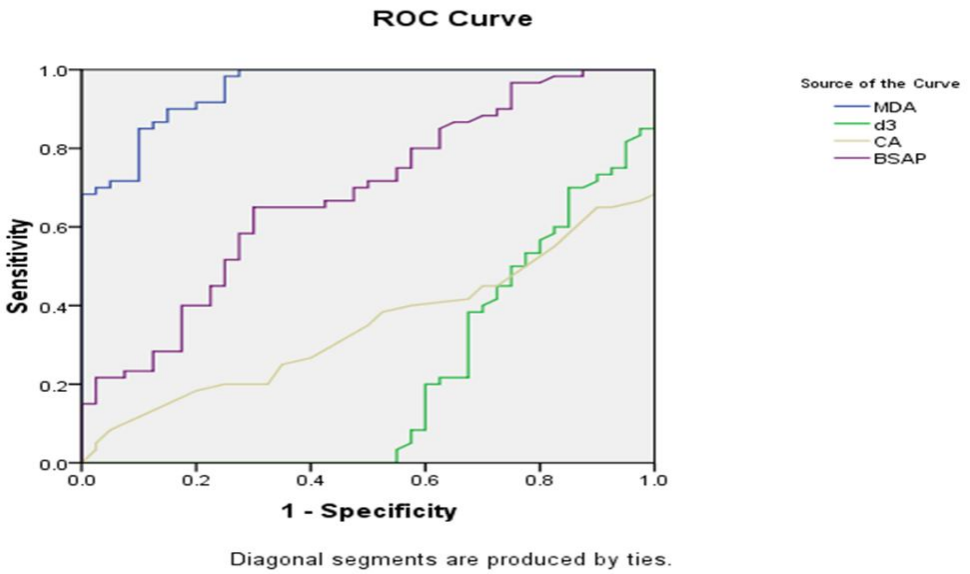

Figure (5): R O C curve to predict disease activity by parameter

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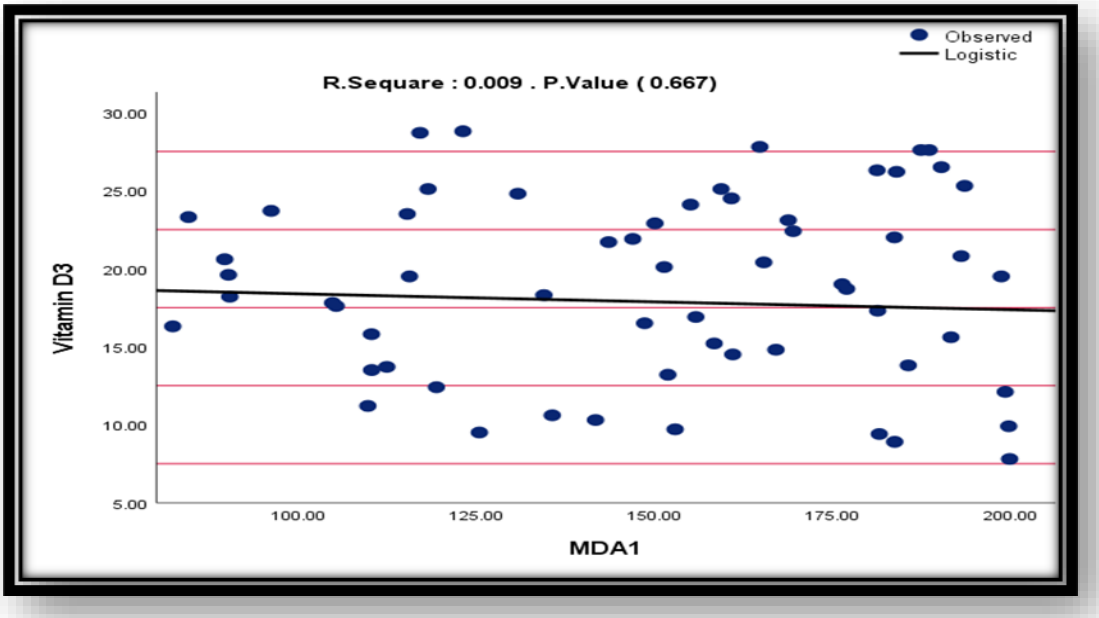

Figure 6: Correlation coefficient between D3 and MDA

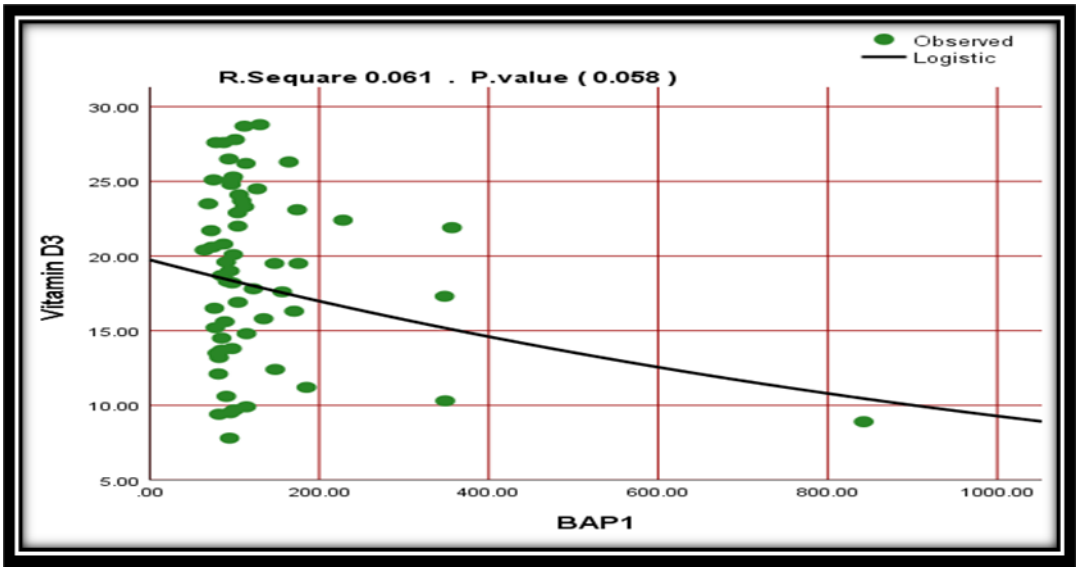

Figure 7: Correlation coefficient between D3 and BSAP

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The general characteristics of the study participants indicate a higher prevalence of osteoporosis in overweight and obese individuals compared to those with a healthy weight. Of the 80 individuals with osteoporosis, only 31% were considered to be at a healthy weight, while 49% were overweight. These results suggest that body weight significantly impacts bone metabolism, perhaps by increasing metabolic stress and converting bone work with greater weight. These results emphasize the importance of monitoring the body structure of osteoporotic patients, as weight management can be an important factor in improving bone health and reducing the fracture risk. In addition, the intervention of weight management and promoting a healthy lifestyle can be advantageous to reduce the effect of osteoporosis and increase the general skeletal integrity. Personal nutrition and implementation of exercise programs that suit individual needs can provide a comprehensive approach to support bone health while addressing weight problems in these patients[14].

In addition to the management of body mass, there is a previous study that showed the significance of nutrition in the context of bone health cannot be overstated. A balanced diet that is rich in essential nutrients such as calcium, vitamin D and omega-3 fatty acids is of crucial importance for the maintenance of optimal bone density and the prevention of further deterioration in patients suffering from osteoporosis[15] .

Reactive oxygen species (ROS) are molecules with a high degree of reactivity, which have the capacity to attack virtually all components of cells. This process can result in additional tissue damage. When the body is unable to neutralize the production of free radicals, oxidative stress occurs. The condition is defined by an imbalance between antioxidants and prooxidants, which is on the latter side and as a result of cell damage. In osteoporotic patients, oxidative stress is clarified by high levels of oxidative stress markers than healthy individuals. In particular, a well - diagnosed biomarker of oxidative stress, Serum Malondildehyde (MDA) was clearly increased in osteoporotic patients compared to the control group. These findings in the same way guide speculation that oxidative stress is an important element in the improvement of osteoporosis. Similar results were reported by [16], who also found significantly increased serum MDA levels in osteoporotic women compared to healthy controls.

Based on the presented proof, MDA may act as a dependable indicator of lipid peroxidation due to reactive oxygen species (ROS). The findings of this look at are supported by using a enormous inverse correlation among MDA ranges and both general and lumbar backbone bone mineral density (BMD) in osteoporotic woman.

However, in contrast to these findings, [17] investigated plasma MDA as a marker o f free radical-mediated lipid peroxidation and found no significant differences among the studied groups

Moreover, the results of the present study are in alignment with those of [18], who advanced the hypothesis that heightened osteoclastic activity in women with low BMD may have been accountable for the augmented production o f ROS in the form of superoxide. This hypothesis

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was substantiated by the elevated serum MDA levels observed in the study population compared with the control group[18].

The results demonstrate significantly higher levels of bone-specific alkaline phosphatase (BSAP) in osteoporotic patients compared to the healthy control group. The mean BSAP level in osteoporotic patients was significantly higher than in the control group. This indicates increased bone turnover in osteoporotic patients. Similarly, the comparison of biochemical parameters between osteoporotic and healthy subjects shows a significant reduction in serum calcium (S.Ca) and vitamin D (D3) levels in osteoporotic patients. The mean calcium level in osteoporotic patients was significantly lower than that in the control group. Similarly, vitamin D levels were markedly lower in osteoporotic patients compared to controls. This suggests that vitamin D deficiency is a major contributing factor to osteoporosis and highlights the need for vitamin D supplementation in at-risk populations.[19]

In Comparing biochemical markers between male and female osteoporotic patients indicates no significant differences in calcium, vitamin D3, BSAP, and MDA levels between the sexes. This suggests that osteoporosis affects both men and women similarly in terms of metabolic markers. However, the slight variation in MDA levels in female's vs males may indicate a slightly higher oxidative stress burden in women. When analyzing osteoporosis patients by age groups, the study found that bone turnover markers increased with age. In individuals aged 70-79, BSAP levels were higher compared to younger age groups, indicating increased bone degradation. Additionally, MDA levels showed variability, with the highest oxidative stress burden observed in the 50-59 age group among females. These findings suggest that bone metabolism deteriorates with age, and oxidative stress may contribute to early-stage osteoporosis.[20]

The study observed a gradual decline in serum calcium and vitamin D levels with increasing age. The lowest vitamin D levels were found in individuals aged 60-69 years, particularly in females. This further supports the role of vitamin D deficiency in osteoporosis and indicates that older individuals may require higher doses of vitamin D supplementation[21].

The diagnostic performance of the studied biomarkers showed that BSAP and MDA had high sensitivity and specificity for osteoporosis detection. The cut-off value for These results suggest that oxidative stress markers could serve as reliable indicators for osteoporosis screening[22].

The statistical findings indicate no significant relationships between age, sex, BMI, and vitamin D3 levels, as well as notable differences between osteoporosis patients and healthy controls in terms of biochemical markers. These results align with existing literature on bone health and osteoporosis risk factors. The strong negative correlation between age and vitamin D3 levels suggests that as individuals age, their vitamin D3 levels decline. This trend is well-documented in medical research, where aging is associated with reduced skin synthesis of vitamin D, lower sun exposure, and decreased dietary intake [23].

A study by [24] found that older adults tend to have significantly lower vitamin D levels, increasing their risk of osteoporosis and fractures.

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Compston [25]found that postmenopausal women are more likely to develop osteoporosis due to hormonal changes affecting bone metabolism. While BMI itself is not a direct indicator of bone health, higher BMI is often associated with greater mechanical loading on bones, which may provide some protective effect against osteoporosis .Weaver highlighted that vitamin D deficiency is a major risk factor for osteoporosis, as it leads to impaired calcium absorption and increased bone resorption[14].

CONCLUSION

In this study show strongly indicate that osteoporosis is associated with increased oxidative stress, higher bone turnover, and deficiencies in essential nutrients such as calcium and vitamin D. The findings underscore the importance of early diagnosis and intervention strategies, including antioxidant therapy, calcium and vitamin D supplementation, and lifestyle modifications such as exercise. Future studies should explore the long-term effects of these interventions on bone health.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

المقدمة: هشاشة العظام (OP) هو اضطراب طويل الأمد في الهيكل العظمي يتميز بفقدان كتلة العظام وتدهور أنسجة العظام، مما يزيد من هشاشتها وخطر الإصابة بالكسور. وبسبب التغيرات الهرمونية التي تسرّع من ارتشاف العظام، فإن النساء بعد سن اليأس هن الأكثر تأثراً بهذا الاضطراب. تعد مؤشرات الإجهاد التأكسدي من بين العديد من المؤشرات الحيوية الضرورية لفهم آليات الأيض التي تكمن وراء هشاشة العظام.

طرق العمل: أُجريت دراسة حالة وشواهد على 120 مشاركًا (80 مريضًا و40 شخصًا من الضوابط). تم قياس مستويات المصل من MDA، BSAP، D3و +Caباستخدام مجموعاتELISA ، وتم إجراء التحليل الإحصائي باستخدامSPSS ، مع تحديد مستوى الدلالة عند $P < 0.05$.

النتائج: كشف تحليل إحصائي باستخدام برنامج SPSS عن انخفاض كبير في مستويات فيتامين (د) بين المرضى الذين يعانون من هشاشة العظام مقارنة بمجموعة التحكم الصحية ($P <0.005$). علاوة على ذلك، أظهر الأفراد الذين يعانون من هشاشة العظام مستويات مرتفعة من مالونديالدهيد (MDA) و (BSAP) . مما يشير إلى زيادة الإجهاد التأكسدي. وحددت الدراسة أيضًا وجود علاقة سلبية بين مستويات فيتامين (د) وعلامات الأكسدة

الاستنتاج: تسلط هذه الدراسة الضوء على العلاقة بين الإجهاد التأكسدي وشدة هشاشة العظام. وتشير النتائج إلى أن فيتامين (د) قد يلعب دورًا حاسمًا في مواجهة الضرر التأكسدي والحفاظ على سلامة العظام

الكلمات المفتاحية: مالونديالديهيد؛ الإجهاد التأكسدي؛ هشاشة العظام؛ الفوسفاتيز القلوي الخاص بالعظام؛ فيتامين د.