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Osteoarthritis in Diabetic Patients: Correlation with Vitamin D Deficiency and Oxidative Stress Markers

Kamilia Ahmed Zaki [1], Ahmed El Sayed Abdullah El Tawil [2], Walid Arafat El Tohamy [3], Dalia Ali Jaber [1] and Nada Gamal [4]*

1 Biochemistry and Molecular Biology Department, 2 Clinical Pathology Department, 3 Orthopedic Surgery Department, 4 Medical Biochemistry and Desert Biology Department, Faculty of Medicine, Misr University of Science and Technology, 6th of October City, Giza, Egypt.

***Corresponding author:** Nada Gamal, **Email:** Nadagamalhamed@gmail.com, **Phone:** (+20) 1064351888

ABSTRACT

Background: Established risk factors for knee osteoarthritis (KOA) encompass prior joint trauma, advancing age, ethnic background, sex, and the structural configuration of the joint. Oxidative stress and inflammatory processes play pivotal roles in the pathogenesis of the condition. Vitamin D plays a crucial role in upholding skeletal integrity and facilitating the proper metabolism of bone and cartilage.

Objective: This study aimed to delve into the nexus between diabetes, oxidative stress, and vitamin D deficiency in individuals afflicted with osteoarthritis.

Methods: This study adopted a prospective case-control design involving 90 participants categorized into three equal cohorts: Group 1 comprising OA patients, Group 2 encompassing diabetic patients with osteoarthritis, and Group 3 constituting healthy volunteers. Mild to moderate osteoarthritis Diagnosis adhered to the criteria set forth by the American College of Rheumatology, with participants selected from Outpatient Orthopedic and Rheumatologic Clinics. Serum assessments were conducted across all participants to gauge levels of 25-hydroxyvitamin D (25(OH)D), malondialdehyde (MDA), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and total antioxidant capacity (TAC).

Results: Significantly disparate findings emerged among the groups in terms of 25(OH)D levels, MDA, TOS, SOD, GPX, CAT, and TAC. Noteworthy associations were delineated between diabetes and both the severity of vitamin D insufficiency and the extent of oxidative stress in osteoarthritis patients.

Conclusion: The study discerned an inverse relationship between vitamin D sufficiency and serum concentrations of oxidative stress markers, including MDA, TOS, SOD, and OSI. Furthermore, a positive correlation was unveiled between TAC and serum vitamin D levels.

Keywords: Osteoarthritis, Oxidative stress, Vitamin D.

INTRODUCTION

Osteoarthritis (OA) stands out as the prevailing type of arthritis encountered in clinical practice, recognized as a progressive ailment detrimentally impacting the musculoskeletal framework [1]. Particularly, the knee joint emerges as a frequent site affected by OA, ranking as the 11th primary contributor to global disability [2]. The onset of knee osteoarthritis (KOA) is shaped by oxidative stress and inflammation, alongside recognized predisposing factors including age, race, sex, prior joint trauma, and the anatomical structure of the joint [3].

Vitamin D plays a pivotal role in sustaining skeletal integrity and facilitating the proper functionality of bone and cartilage. It is proposed that vitamin D counters oxidative stress by activating a protective antioxidant mechanism termed the nuclear factor erythroid 2-related factor 2-Kelch-like ECH-associated protein 1 (Nrf2-KEAP1) pathway. Nevertheless, the relevance of these findings to individuals with KOA remains uncertain [4]. Vitamin D is believed to modulate inflammation and uphold immune function. Observational studies have correlated vitamin D insufficiency with increased inflammation in chronic conditions like osteoarthritis, complications during pregnancy due to elevated blood pressure, and infections in diabetic foot ulcers [3].

Epidemiological investigations have established associations between deficient levels of vitamin D and heightened knee discomfort, a greater incidence of osteoarthritis, and the onset and advancement of knee osteoarthritis. Vitamin D insufficiency is prevalent, with studies indicating a connection between inadequate vitamin D levels and increased knee pain. Moreover, prior research has firmly established a notable correlation between oxidative stress, reactive oxygen species (ROS), and the progression of osteoarthritis [1].

This study aimed to explore the relationship between diabetes, vitamin D deficiency severity, and the level of oxidative stress in individuals suffering from osteoarthritis.

PATIENTS AND METHODS

This research was structured as a prospective case-control investigation spanning one year, from June to December 2023, involving a total of 90 individuals categorized as follows: Group 1 comprised 30 individuals identified with osteoarthritis, group 2 included 30

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individuals with diabetes who were also diagnosed with osteoarthritis and group 3 consisted of 30 individuals without any health issues, serving as the control group.

The diagnosis of osteoarthritis in patients ranged from mild to moderate severity, adhering to the criteria set forth by the American College of Rheumatology. These patients were selected from those attending the Outpatient Departments of Orthopedics and Rheumatology at Misr University for Science & Technology Hospital.

Inclusion criteria: Individuals aged between 20 to 70 years, of any gender, who consented to partake in the study.

Exclusion criteria: Individuals with type 1 diabetes, liver failure, chronic kidney diseases, inflammatory conditions, cancer, hyperparathyroidism, and those who had recently taken vitamin D supplements.
All participants underwent assessments of their body metrics and physical activity levels.

Collection and processing of blood samples:

On the day of the procedure, after a 10-hour overnight fast, a 10-milliliter blood sample was drawn from each participant's antecubital vein. These samples were then centrifuged at 3,000 revolutions per minute for ten minutes at 4 degrees Celsius to separate the serum, which was subsequently stored at -80 ◦C until further analysis.

Analytical Methods:

The level of serum 25-hydroxyvitamin D (25(OH)D) was determined using an electro-chemiluminescence immunoassay (ECLIA) on a Roche Elecsys system in Germany, assessing both 25(OH)D3 and 25(OH)D2 forms. The precision and accuracy of the assay were ensured by employing MassCheck control specimens. To measure the activity of erythrocyte superoxide dismutase (SOD), we utilized an ELISA kit from Elabscience (Cat. No. E-BC-K022-S), based in Houston, Texas, USA, employing the competitive-ELISA technique. The concentration of malondialdehyde (MDA) in the serum was quantified using a specific kit (Cat. No. E-El-0060) from Elabscience, following a protocol based on the thiobarbituric acid reactive substances method. For the assessment of total antioxidant capacity (TAC), the ferric-reducing antioxidant power method was applied, utilizing a kit from Elabscience (Cat. No; E-BC-K219-M). The total oxidant status (TOS) of the serum was evaluated using Erel's method, which involves the oxidation of the ferrous ion-o-dianisidine complex to ferric ions by the oxidants present in the sample.

Calculation of the oxidative stress index (OSI):

The OSI was computed by taking the ratio of TOS to TAC, with TOS measured in mol H_2O_2 equivalents per liter and TAC in mmol Trolox equivalents per liter, resulting in the formula: OSI (arbitrary unit) = TOS/TAC.

Ethical considerations: The study was conducted following approval from The Research Ethics Committee at Misr University for Science & Technology, Egypt. Written informed consents were obtained from all participants after they were briefed on the study's requirements, objectives, and potential risks. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring the protection of their confidentiality and privacy. This work adheres to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving human subjects.

Statistical analysis

Statistical analysis was conducted using SPSS version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). The collected data were tabulated and subjected to statistical analysis. The Chi-square (X^2) test was employed to compare proportions among qualitative parameters, while the independent T-test was utilized to compare two independent groups with parametric quantitative data. All statistical comparisons were two-tailed, with a significance level set at a P-value of ≤ 0.05 indicating significance, a P-value of ≤ 0.001 indicating highly significant difference, and a P-value of > 0.05 indicating a non-significant difference.

RESULTS

There were no significant differences between the two groups regarding age and sex. Similarly, there were no significant differences between the two groups in terms of weight or BMI. Similarly, no significant differences were observed in terms of cigarette smoking, physical activity, and duration of disease **(**Tables 1 and 2).

Table (1): Comparison between the three groups as regards demographic data

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P value
Age, years				>0.05
Mean ± SD	47.4 ± 4.4	47.3 ± 4.5	46.8 ± 4.1	
Median (Minimum - Maximum)	50 (40 - 60)	50 (40 - 60)	50 (40 - 60)	
Sex				>0.05
Male	14 (46.7%)	13 (43.3%)	15 (50%)	
Female	16 (53.3%)	17 (56.7%)	15 (50%)	

X^2: Chi Square, ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant.

Table (2): Comparison between three groups as regard anthropometric data

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P value
Weight (kg)				>0.05
Mean ± SD	82.5 ± 8.4	83.5 ± 8.1	83.1 ± 8.5	
Median (Minimum - Maximum)	85 (70 - 95)	85 (70 - 95)	85 (70 - 95)	
BMI (kg/m^2)				>0.05
Mean ± SD	28.2 ± 3.1	27.9 ± 3.05	28.3 ± 2.95	
Median (Minimum - Maximum)	30 (25 - 32)	30 (25 - 32)	30 (25 - 32)	

X2: Chi Square, ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant.

However, significant differences were noted between the two groups concerning levels of 25-hydroxyvitamin D (25(OH)D), malondialdehyde (MDA) (nmol/mL), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPX) (U/gHb), catalase (CAT) (K/gHb), and total antioxidant capacity (TAC) (P<0.001) (Table 3).

Table (3): Comparison of 25(OH)D levels, malondialdehyde (MDA) (nmol/mL), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPX) (U/gHb), catalase (CAT) (K/gHb) and total antioxidant capacity (TAC) between the three groups

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P value
25(OH)D levels (ng/mL)				<0.001
Mean ± SD	19.7 ± 4.9	22.2 ± 5.4	42.5 ± 8.1	
Malondialdehyde (MDA) (nmol/mL)				<0.001
Mean ± SD	2.22 ± 0.45	2.3 ± 0.41	1.72 ± 0.25	
Total oxidant status (TOS) (µmol H2O2 Equiv./L)				<0.001
Mean ± SD	12.7 ± 2.8	12.9 ± 2.5	9 ± 1.4	
Superoxide dismutase (SOD) (U/gHb)				<0.001
Mean ± SD	1251 ± 220	1245 ± 180	1181 ± 108	
Catalase (CAT) (K/gHb)				<0.001
Mean ± SD	230 ± 40	228 ± 35	220 ± 25	
Total antioxidant capacity (TAC) (mmol/L)				<0.001
Median (Minimum - Maximum)	1.7 (1 - 3)	1.7 (1 - 3)	2.4 (1.5 - 4)	
Glutathione peroxidase (GPX) (U/gHb)				<0.001
Mean ± SD	45.4 ± 12.8	44.7 ± 10.8	42.8 ± 10.4	

X2: Chi Square, ANOVA Test, P value < 0.05: Statistically significant difference.

Strong significant correlations were identified between diabetes and both the severity of vitamin D deficiency and the extent of oxidative stress in patients with osteoarthritis (Table 4).

Table (4): Correlations between diabetes with the severity of vitamin D deficiency, extent of oxidative stress in patients with osteoarthritis

Correlations		
		Diabetes
Severity of vitamin D deficiency	r	-.495-**
	P	**<0.0001**
MDA	r	.498**
	P	**<0.0001**
TOS	r	.560**
	P	**<0.0001**
SOD	r	.720**
	P	**<0.0001**
GPX	r	.515**
	P	**<0.0001**
CAT	r	0.38
	P	**<0.0001**
TAC	r	.48**
	P	**<0.0001**

P value< 0.05 is significant, P value < 0.05: Statistically significant difference.

In the univariate correlation analysis, significant correlations emerged between diabetes and the severity of vitamin D deficiency, as well as the extent of oxidative stress in patients with osteoarthritis (Table 5).

Table (5): Univariate Correlations between diabetes with the severity of vitamin D deficiency, extent of oxidative stress in patients with osteoarthritis

		Iron deficiency anemia
Severity of vitamin D deficiency	**Correlation**	0.348
	Significance	**<0.0001**
MDA	**Correlation**	0.471
	Significance	**<0.0001**
TOS	**Correlation**	0.412
	Significance	**<0.0001**
SOD	**Correlation**	0.357
	Significance	**<0.0001**
GPX	**Correlation**	0.462
	Significance	**<0.0001**
CAT	**Correlation**	0.422
	Significance	**<0.0001**
TAC	**Correlation**	0.35
	Significance	**<0.0001**

P value < 0.05: Statistically significant difference.

The multivariate correlation analysis also revealed significant correlations between diabetes and the severity of vitamin D deficiency, along with the extent of oxidative stress in patients with osteoarthritis (Table 6).

Table (6): Multivariate Correlations between diabetes with the severity of vitamin D deficiency, extent of oxidative stress in patients with osteoarthritis

Variable		Value
Severity of vitamin D deficiency	**Correlation**	**71.305**
	Significance	**<0.0001**
MDA	**Correlation**	**20.495**
	Significance	**<0.0001**
TOS	**Correlation**	**25.595**
	Significance	**<0.0001**
SOD	**Correlation**	**72.35**
	Significance	**<0.0001**
GPX	**Correlation**	**21.49**
	Significance	**<0.0001**
CAT	**Correlation**	**25.92**
	Significance	**<0.0001**
TAC	**Correlation**	**25.56**
	Significance	**<0.0001**

Correlation regression: ANOVA, P value < 0.05: Statistically significant difference.

The sensitivities of malondialdehyde (MDA), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and total antioxidant capacity (TAC) were 88%, 85%, 87%, 85%, 84%, and 85% respectively. The specificities for the same biomarkers were 89%, 88%, 87%, 88%, 89%, and 88% respectively (Figure 1).

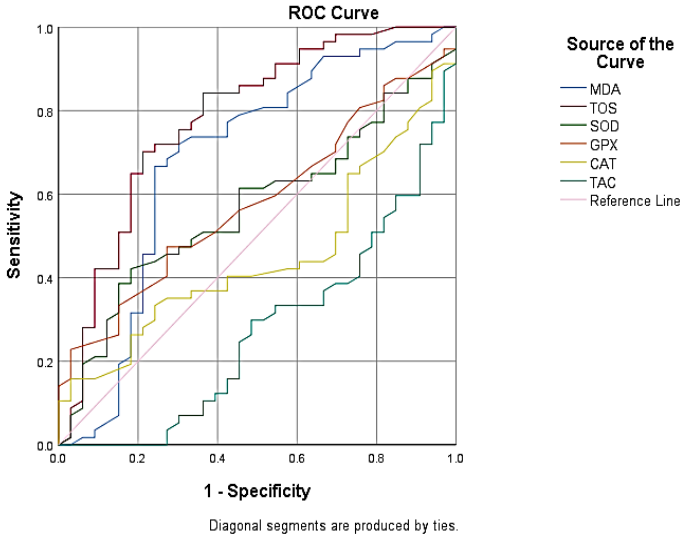

Figure (1): ROC curve analysis of oxidative stress in patients with osteoarthritis.

DISCUSSION

Vitamin D is renowned for its dual function in reducing inflammation and averting immunosuppression. Observational studies suggest that a deficiency in vitamin D is associated with increased inflammation in chronic ailments, such as osteoarthritis, pregnancy-related hypertension, and diabetic foot infections [1]. Additionally, research indicates that inadequate levels of

vitamin D contribute to heightened knee pain, a greater prevalence of osteoarthritis, and the initiation and progression of knee osteoarthritis [5]. Hence, our study aimed to compare the levels of vitamin D deficiency between osteoarthritis patients with and without diabetes, while also evaluating oxidative stress levels in both cohorts.

Our findings revealed no significant disparities in age or gender between the three groups. Similarly, there were no noteworthy distinctions in weight or BMI between them. These results align with those reported by **Amirkhizi *et al.*** [1] who likewise observed no significant variations in age, gender, or body composition parameters among their study cohorts. In contrast, **Asghari *et al.*** [6] observed that individuals with obesity exhibited higher measures of weight, BMI, waist circumference, fat mass, and visceral fat compared to those with normal weight, despite similar age, gender, physical activity, and smoking habits due to the study's design.

The study revealed significant differences between the three groups in terms of malondialdehyde (MDA), total oxidant status (TOS), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), and total antioxidant capacity (TAC), indicating notable variations in oxidative stress and antioxidant defense mechanisms. A comprehensive meta-analysis conducted by **Moslemi *et al.*** [7], which synthesized findings from 23 studies across diverse demographics and clinical conditions, demonstrated marked enhancements in MDA levels (Effect Size (ES) = -0.37; 95% Confidence Interval (CI): -0.48, -0.25, $p < 0.001$) and inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP) (ES = -0.42; 95% CI: -0.55, -0.29, $p < 0.001$) and tumor necrosis factor-alpha (TNF-α) (ES = -0.27; 95% CI: -0.42, -0.12; $p < 0.001$) subsequent to vitamin D supplementation. Supporting these outcomes, a randomized controlled trial by **Cojic *et al.*** [8], which encompassed patients with Type 2 diabetes mellitus (T2DM) implemented interventions involving the administration of at least 14,000 IU of vitamin D per week. Initially, the deficient group received 50,000 IU per week for three months, followed by a reduction to 14,000 IU per week for another three months. This intervention led to a decrease in reactive oxygen species (ROS) levels at a six-month follow-up and a reduction in the inflammatory marker CRP, although the latter change did not reach statistical significance.

The study uncovered a robust and notable association between diabetes, vitamin D insufficiency, and the degree of oxidative stress among individuals afflicted with osteoarthritis. This correlation remained consistently evident across both univariate and multivariate correlation analyses, underscoring the interconnection among diabetes, reduced vitamin D levels, and heightened oxidative stress in this patient population. In a study by **Wee *et al.*** [9], a significant relationship was identified between vitamin D deficiency and heightened levels of malondialdehyde (MDA) alongside diminished activity of superoxide dismutase (SOD) within the small subcutaneous arteries of diabetic individuals, indicating escalated oxidative stress within microvascular tissues. Furthermore, elevated levels of vascular tissue tumor necrosis factor-alpha (TNF-α) and angiotensin II (Ang II) were notably observed. Nevertheless, no correlation was found between vitamin D deficiency and glycemic indicators such as fasting blood glucose (FBG) and hemoglobin A1c (HbA1c) in these patients. To the best of our knowledge, these findings have not been previously documented.

CONCLUSION

An inverse correlation was found between the status of vitamin D and serum concentrations of oxidative stress indicators such as MDA, TOS, SOD, and OSI. There was a favorable correlation between TAC and serum levels of vitamin D. To provide conclusive evidence for these findings, additional research must be conducted using prospective research approaches and large sample sizes.

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Conflict of Interest: Nil.

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