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ORIGINAL ARTICLE

Role of Progranulin and its Implication in Knee Osteoarthritis among Iraqi Patients

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ABSTRACT

Background: Osteoarthritis is a complicated, chronic disorder of cartilage and bone, associated with homeostasis of bio-elements. The current study aims to assess the role of serum progranulin levels among Iraqi patients with knee osteoarthritis.

Patients and Methods: The study encompassed 50 patients aged 52.50 ± 3.12 years (25 males and 25 females), admitted to the at the Baghdad Medical City through the period from November 2021 to March 2022. All individuals were identified by physicians in a Rheumatology and Rehabilitation Outpatient Clinic and the clinical data was collected along with the assessment of biochemical parameters. Fasting serum glucose, lipid profile, calcium, magnesium, alkaline phosphatase, vitamin D3, and progranulin levels were determined.

Results: All patients in this study had higher levels of serum glucose and dyslipidemia with significant increases ($p < 0.05$) in alkaline phosphatase and progranulin levels and considerable reduced ($p < 0.05$) in serum calcium, magnesium, and vitamin D3 as compared to healthy group. Additionally, hypovitaminosis D were appeared in all patients and progranulin levels were (56.30±8.50 ng/mL) vs. (40.45 ± 8.90 ng/mL) in control group with OR and CI= 1.54 (0.97-2.58).

Conclusions: Inflammation has an integral role in the pathogenesis of osteoarthritis. Serum vitamin D_3 level is considerably low in patients with knee osteoarthritis paralleled to healthy individuals. However, this study demonstrates increased progranulin levels in knee osteoarthritis patients and associates with vitamin D level, disease activity, and inflammation among those patients.

Keywords: Knee osteoarthritis, Calcium, Magnesium, Alkaline phosphatase, Vitamin D3, Progranulin.

INTRODUCTION

Osteoarthritis (OA) is the most common type of arthritis comprising destruction of cartilage matrix. It most frequently occurs in the knees, followed by that in the hand and hip (1). The incidence of knee osteoarthritis (KOA) increases with age, and the incidence reaches 25% of the population over the age of 55 years (2).

The knee is the most commonly injured site in OA. Patients KOA experience severe pain and restricted movements. Knee OA is known to occur due to cartilage wear. This damage leads to chronic low-grade inflammation. A prolonged and irregular degree of inflammation leads to tissue destruction. The healing process is not effective because the joint is subject to wear and tear due to continuous use (3).

Calcium (Ca^{+2}) and magnesium (Mg^{+2}) are minerals have a significant roles in the body, particularly in the muscular and skeletal system. Calcium has consequences in glycogen metabolism, protein secretion, cell divisions and muscle contractions (4) . The Ca^{+2} homeostasis in the body depends on parathyroid hormone, vitamin D, calcitonin, and kidney function. Furthermore, Ca^{+2} and Mg^{+2} absorption is achieved in the intestine (5), and is stimulated by the active form of vitamin D with the commendation of a daily intake of 400 IU in adults. Similarly, Mg^{+2} helps to convert vitamin D into its dynamic form so that they are combined in the usage of different resistant forms of rickets (6). As a consequence, Ca^{+2} and Mg^{+2} insufficiency has been related with a greater risk of cardiovascular diseases (CVD), diabetes mellitus (DM), cancer, and even OA (7). Though, emerging indication recommends that serum ALP concentration is deliberated an inflammatory mediator related with cardiometabolic disorders (8).

Vitamin D is a steroid hormone that has many diverse biological actions in a number of target tissues. The main functions of vitamin D are Ca^{+2} balance and regulation of bone metabolism. Despite this, the full level of biological action of vitamin D remains to be determined with the variety of effects on different types of cells and tissues being reported. Vitamin D acts via the vitamin D receptor (VDR), and vitamin D controls circulating Ca^{+2} and phosphate through variability of renal reabsorption and intestinal absorption (9).

There are several proteins that are complicated in the low-grade inflammation, tissue destruction, and in the healing of the cartilage in the KOA. Consequently, these proteins may have either pro-inflammatory or anti-inflammatory properties and frequently work to maintain immune homeostasis (10). These are interleukins, tumor necrosis factor-alpha (TNF-α), osteocalcin, progranulin (PGRN), and other cytokines.

Progranulin (PGRN) is a growth factor for glycoprotein excreted by neuron cells, glia cells, leucocytes, chondrocytes, and epithelial cells. It is also excreted by adipose tissue. It has been proposed that high-fat diets increase the PGRN synthesis in adipose tissues. The PGRN is the endogenous ligand of TNF-α receptors. It provokes the possessions of TNF-α receptors by binding to TNF-α receptors and has anti-inflammatory influences other than its anti-inflammatory influences, it is active in cases of wound healing, neuronal recovery, and tumorgenesis (11). It has been investigated that PGRN has a role in immune-mediated inflammatory diseases due to its anti-inflammatory possessions (12). The PGRN has been also revealed to be a novel indicator for chronic inflammation, obesity, type 2 DM, and CVD (13). It has been proposed that PGRN may be overexpressed in patients with OA. However, these patients tend to be obese or overweight and obesity is one of the factors that may raise PGRN levels (14).

The aim of the present study is to assess the role of serum PGRN levels among Iraqi patients with knee osteoarthritis

PATIENTS AND METHODS

The present study was performed through November 2021 to March 2022 at Baghdad Teaching Hospital/ Baghdad Medical City, with a total of 50 KOA patients; their ages ranged from 40-55 years. They were equated with 40 healthy subjects as control group. All patients were diagnosed by physicians in a Rheumatology and Rehabilitation Outpatient Clinic. Informed consent was taken from all cases prior to their enrollment in this study. Waist circumference (WC), systolic-, and diastolic blood pressure (SBP, DBP) were measured and body mass index (BMI) was obtained from the patient's measured weight and height calculated as weight (Kg)/height(M^2) and categorized according to WHO standard cut offs (15).

Exclusion Criteria: Patients with diabetes, rheumatic disease, malignancy, liver, renal, or thyroid disorders were excepted. Also, individuals on treatments that might disturb bone metabolism as diuretics, β-blockers, bisphosphonates, steroids, vitamin D or any minerals were not included in this study.

Biochemical Measurements: Laboratory assessments were down, which encompassed fasting serum glucose (FSG), total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C),

very low density lipoprotein cholesterol (VLDL), serum Ca^{+2}, and alkaline phosphatase (ALP). All these tests were measured using a chemical analyzer (Abbott C4000). Serum vitamin D3 was estimated using mini VIDAS, BioMerieux kit, France. Hypovitaminosis is defined by most experts as a serum vitamin D level less than 20 ng/mL, while a level of greater than 30 ng/mL is deliberated to be normal and a level of 20–30 ng/mL defines vitamin D insufficiency (16). The PGRN level was assessed using enzyme-linked immunosorbent assay (ELISA) kit (Catalogue No. RDEEH4816) with sensitivity: 0.188 ng/mL.

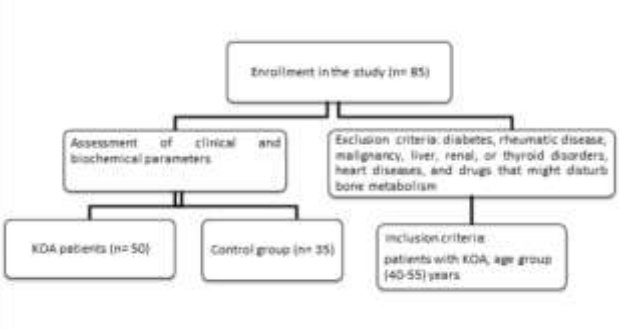

Figure 1: Flow diagram of the current study

Statistical Analysis: All statistics are shown as means ± SD for all parameters. The t-test was used to equate experimental groups. Also, Odd ratio (OR), confidence interval (CI), for PGRN and correlation coefficient (r) between variables were done in this study. A p-value < 0.05 was deliberated statistically significant.

RESULTS

The distribution of OA stages in patients group are demonstrated in table 1. Grade II has the higher percent (50%) among patients group. While, the grade IV has the lower percent (10%).

Table 1: The distribution of OA stages in patients group

Grade	Patients n(%)
I	12 (24%)
II	25 (50%)
III	8 (16%)
IV	5 (10%)
Total	50 (100%)

Table 2: Clinical and biochemical characteristics of KOA patients and control

Parameters	KOA	Control	p-value
Number (Female/Male)	50 (25/25)	35 (20/15)	0.082
Age (Years)	52.50 ± 3.12	48.20 ± 3.82	0.060
WC (cm)	93.58 ± 4.16	74.33 ± 5.40	0.001
BMI (Kg/m^2)	32.15 ± 3.10	24.82 ± 1.12	0.040
SBP (mmHg)	136.40 ± 2.07	118.50 ± 1.65	0.001
DBP (mmHg)	100.05 ± 3.16	78.30 ± 1.10	0.001
FSG (mg/dL)	112.75 ± 8.45	92.87 ± 3.82	0.001
TC (mg/dL)	300.16 ± 10.74	130.75 ± 9.62	0.0001
TAG (mg/dL)	220.04 ± 5.78	105.70 ± 4.32	0.0001
HDL-C (mg/dL)	42.10 ± 5.40	57.62 ± 2.81	0.010
LDL-C (mg/dL)	214.04 ± 4.18	51.99 ± 5.96	0.0001
VLDL (mg/dL)	44.02 ± 1.16	21.14 ± 0.85	0.001
Duration of OA (Years)	3.50 ± 2.35	-	-

Clinical and biochemical characteristics of KOA patients and control are presented in table 2. There were considerable rises ($p < 0.05$) in WC, BMI, SBP, DBP, FSG, TC, TAG, LDL-C, and VLDL, whereas a considerable decrease ($p < 0.05$) was detected in serum HDL-C level in KOA patients as paralleled to the controls. Moreover, the duration of OA was 3.50 ± 2.35 years. Considerable reduces ($p < 0.05$) in serum Ca^{+2}, Mg^{+2}, ALP, and vitamin D3 were found in KOA patient as compared to the controls. While a considerable elevation ($p < 0.05$) was detected in serum ALP concentration in KOA patient as compared to the controls (Table 3).

Additionally, there was a considerable increase (p= 0.001) in serum PGRN concentration in KOA patient as compared to the controls (Table 4).

Data are expressed as mean±SD. $p < 0.05$: significant, WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FSG: fasting serum glucose, TC: total cholesterol, TAG: triacylglycerol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, VLDL: very low density lipoprotein.

Table 3: Biochemical characteristics of serum Ca, Mg, ALP, and vitamin D3 levels in patients and controls

Parameters	KOA (n= 50)	Control (n= 35)	p-value
Ca^{+2} (mg/dL)	4.38 ± 1.10	10.48 ± 1.45	0.04
Mg^{+2} (g/L)	0.50 ± 0.10	1.10 ± 0.40	0.03
ALP (U/L)	78.33 ± 10.15	57.33 ± 9.21	0.65
Vitamin D3 (ng/mL)	8.45 ± 4.32	48.82 ± 3.15	0.0001

Data are expressed as mean±SD. $p < 0.05$: significant, Ca^{+2}: calcium, Mg^{+2}: magnesium, ALP: alkaline phosphatase.

Table 4: State of PRGN levels in patients and controls

Parameters	KOA (n= 50)	Control (n= 35)	p-value	OR (CI)
PGRN (ng/mL)	56.30 ± 8.50	40.45 ± 8.90	0.01	1.54 (0.97-2.58)

Data are expressed as mean±SD. $p < 0.05$: significant, PGRN: progranulin.

Correlations regarding to D3 and PGRN levels among KOA patients are shown in table 5. A considerable negative correlations ($p < 0.05$) was found between serum vitamin D3 and age, WC, BMI, FSG, TC, TAG, LDL-C, VLDL, and ALP levels. While, there was significant positive correlations between serum vitamin D3 with HDL-C, Ca^{+2}, and Mg^{+2}. Furthermore, significant positive correlations ($p < 0.05$) were found between PGRN with age, WC, BMI, FSG, TC, TAG, LDL-C, VLDL, and ALP levels. Whereas, significant negative correlations (p= 0.0001) were detected between PGRN and serum HDL-C, Ca^{+2}, Mg^{+2}, and vitamin D3.

Table 5: Correlations according to D3 and PGRN in postmenopausal group

Variables	Vitamin D3 (ng/mL)		PGRN (ng/mL)	
	r	p	r	p
Age (Years)	-0.38	0.650	0.280	0.620
WC (cm)	-0.61	0.045	0.730	0.001
BMI (Kg/m^2)	-0.52	0.040	0.830	0.043
FSG (mg/dL)	-0.87	0.001	0.750	0.001
TC (mg/dL)	-0.83	0.001	0.870	0.001
TAG (mg/dL)	-0.938	0.001	0.950	0.001
HDL-C (mg/dL)	0.822	0.001	-0.932	0.01
LDL-C (mg/dL)	-0.980	0.001	0.690	0.001
VLDL (mg/dL)	-0.930	0.001	0.950	0.01
Ca^{+2} (mg/dL)	0.662	0.01	-0.530	0.05
Mg^{+2} (mg/dL)	0.530	0.042	-0.542	0.05
ALP (U/L)	-0.620	0.001	0.430	0.072
Vitamin D (ng/mL)	-	-	-0.982	0.001

$p < 0.05$: significant, WC: waist circumference, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FSG: fasting serum glucose, TC: total cholesterol, TAG: triacylglycerol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, VLDL: very low density lipoprotein, Ca^{+2}: calcium, ALP: alkaline phosphatase.

DISCUSSION

Osteoarthritis is the greatest mutual form of arthritis, characterized by cartilage loss, thereby, leading to the functional failure (17). An increase in WC results in a similarly significant elevation in mortality risk, regardless of BMI. For WC < 90 cm for men and < 70 cm for women, an elevation in WC of 5 cm rises mortality by 7%

in men and 9% in women (18). Though, current data show that an elevation in WC, with no alterations in BMI, is also a risk element for OA. High BMI is a significant contributor to death and disability-adjusted life years, and its burden has significantly increased in last year's (19). Ranges of overweight and obese, frequently celebrated as a BMI of 25-29.9 kg/m^2 and BMI ≥ 30 kg/m^2, respectively, have been approved as a vital adjustable risk influence for the incidence and progress of OA (20).

A positive relationship between the risk of KOA and BMI was found in a study of individuals aged 45–85 years, and this association was found to be strength with a higher BMI, which is supported by previous data (21, 22). Furthermore, a dose–response relationship between BMI and the clinical and functional concerns of KOA was found in a larger cross-sectional study, proposing that approaches for treating KOA should differ depending on the severity of obesity (23).

A hyperglycemic situation elevates the creation of reactive oxygen species and oxidants and stimulates matrix catabolism. In these situations, cellular transport of glucose becomes serious, which, if changed as in DM, donates to excess oxidative stress and tissue damage and accelerates OA; this was broadly elucidated formerly (24). The influences of high glucose may be related with the impaired function of ATP-sensitive K^+ channels, which couples glucose transporters channels to intracellular ATP/ADP levels and membrane potential (25). High concentrations of glucose in tissues also accelerate the formation of advanced glycation end products (AGEs). The AGEs signal through RAGE (receptors for AGEs) and other receptors and produce numerous adverse effects on chondrocytes as in inflammation and cytokine-mediated catabolism. Moreover, cross-linking of collagen by AGEs modifies the biomechanical possessions of tissues and may also prevent extracellular matrix turnover by constraining access to proteolytic sites (26, 27).

All OA patients in this study have dyslipidemia. Mechanisms through which lipid metabolism might lead to OA are indistinct. There is indication that higher HDL-C levels in the synovial fluid have a protective influence, vascular insults disturbing the bone marrow next to the cartilage, and the toxic influence of cholesterol at the joint itself might donate to the progress of OA (28). High HDL-C concentrations appear in control persons with improved physical status and lesser occurrence of comorbid disorders, which might also lead to a lesser occurrence and prevalence of OA.

The role of serum Ca^{+2} concentrations in the evolution of KOA has been demonstrated by studies that have highlighted the effects of Ca^{+2} on chondrocytes in terms of matrix protein synthesis, cytoskeletal remodeling and apoptosis and regulation of proteoglycans involved in static cartilage compression (29). Yazmalar et al., did not obtain statistically significant differences between serum Ca^{+2} levels in KOA patients compared to the control group (30). The same conclusions being reported in a study that looked at the role of serum Ca^{+2} levels in hand OA (31). The present results contradict these studies, the total serum Ca^{+2} levels for patients with KOA was considerably lower equated to the healthy group and thus underline the conclusions of Li et al., which support an inverse association between serum Ca^{+2} values and radiological progression of KOA (32). In contrary, Iraqi study, found a decrease in serum Ca^{+2} and Mg^{+2} levels among osteoporotic patients as compared to the controls, but it was not significant (33).

Although some studies on human subjects have indicated an important role of Mg^{+2} intake in preventing the onset and progression of KOA, they have been contradicted by studies that did not obtain a lower risk of KOA in increased Mg^{+2} intake, or that noticed only modest inverse associations between them (34). On the other hand, Wu and his colleagues, claim a lower risk of KOA in association with higher serum Mg^{+2} values, but more evaluations are needed to confirm this hypothesis (35).

Although serum ALP concentration has been shown as a main biochemical marker for the assessment of disease activity in ankylosing spondylitis and rheumatoid arthritis. According to previous study, there is a positive correlation between serum ALP concentration and OA in a general population study (36). The strict mechanism by which serum ALP concentration associates with OA is not totally defined, but numerous probable causes for this association warrant deliberation. First, serum ALP concentration could be associated with low-grade inflammation, which may cause an inflammatory response in chondrocytes. Emerging analysis have proposed that serum ALP concentration is positively and independently related with inflammatory markers such as C-reactive protein level and leukocyte counts. Additionally, increased ALP expression via inflammatory stimuli is a significant risk factor for the progress of OA (37). This study reflected marked variance in serum ALP between patients and controls, while another study showed that the normal levels of serum ALP in osteoporotic cases reflect different phases of osteoblast in bone formation (38).

The particular role of vitamin D on chondrocytes and bone metabolism has been formerly deliberated (39). Suboptimal of vitamin D levels have contrary influences on Ca^{+2} metabolism, osteoblastic activity, matrix ossification, and bone mineral density. A direct influence of vitamin D metabolites on subchondral bone quality, articular chondrocytes, and primary degenerative variations, thus, increase the vulnerability to OA. A considerable correlation between low vitamin D intake and KOA has been described in various populaces' worldwide (40, 41). Sun exposure is the main source of vitamin D. Another study, notify that without adequate sun exposure, it is not probable to produce the right extent of vitamin D (42). Additionally, means of age in this study between 40-55 years, and this stage of life especially in women is categorized by the decreased production of hormones in the ovaries. Lower levels of estrogen donate to lower vitamin D-binding protein, and accordingly lower 25(OH)D levels in blood. This deficit of 25(OH)D can negatively disturb biological functions, ensuing in several disorders particularly in women. As stated by previous study, women at this age more often undergo laboratory analysis to determine 25(OH)D levels, which is in agreement with previous study (43).

Progranulin is a glycoprotein that has a vital role in systemic autoimmune inflammatory diseases. It interacts with numerous cellular progressions, comprising lysosome role and autophagy, cellular localization of transactive response DNA binding protein-43 (TDP-43), inflammation, cell-morphology,-survival, and-signalling. Its actions seem to be intermediated by binding to a variety of diverse cell membrane proteins (44). Progranulin principally creates from inflammatory cells, epithelial cells, and chondrocytes, and it is complicated in inflammation and cartilage progress and degradation. Additionally, PGRN also contributes in the conversion of immunosuppressive regulatory T cells (Tregs) in autoimmune inflammatory circumstances (45). Guo et al., have noticed that PGRN levels increase moderately in cartilage tissues of OA patients and markedly in that of those with rheumatoid arthritis (46). Similarly, it has been stated that PGRN expression rises in cartilage, synovial, and fat tissues of OA patients. It might be hypothesized that the joint compartment characterizes a major site of PGRN production in those patients and the levels of PGRN were predominantly elevated at sites of local inflammation compared to the blood circulation of OA patients (47). Additionally, researchers have observed that serum PGRN levels elevate in RA and OA patients equated to healthy controls. The present results is an agreement with that of previous two studies (48, 49).

CONCLUSIONS

Inflammation has a vital role in the pathogenesis of OA. Numerous molecules released by the chondrocytes and synoviocytes and infiltrating immune cells. Serum vitamin D concentration is considerably decrease in KOA patients equated to control group. Future researches should evaluate the influence of psychological and social variables on vitamin D levels in OA patients. However, this study demonstrates increased PGRN levels in KOA patients and associates with vitamin D level, disease activity, and inflammation among KOA patients.

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Ethical statement: The study was performed according to the Declaration of Helsinki and approved by the Institute in approval number: 6698 at 15/11/2021.

Conflict of interest: No potential conflicts of interest were recorded about this article.

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