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The Role of Galectin-3 as a Biomarker of Osteoporosis and its Correlation with Bone Mineral Density

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Abstract

This study investigates the potential link between serum levels of Galectin-3 (Gal-3) and bone health in postmenopausal women with osteoporosis. As Gal-3 is known to be expressed in bone cells and may influence bone remodeling, we aim to quantify serum Gal-3 levels in this population and examine its correlation with bone mineral density (BMD). In cross-sectional study, 45 postmenopausal women with OP and 45 healthy control group (HS), aged between 43-50 years, were registered. Patients and HS were categorized based on T score on dual-energy X-ray absorptiometry (DXA). Serum levels of calcium (Ca), Vitamin D_3, and Gal-3 were measured both groups. The concentration of serum Gal-3 concentration was significantly increased in OP patients compared to the HS at ($P< 0.001$). Additionally, serum Gal-3 levels was positively correlate with BMD of the lumber spine (L_1-L_4) levels T score (r= 0.689, P=0.0001). Depended on the receiver operating characteristic curve (ROC), the criterion value of the Gal-3 was >11.905 ng/ml. In this study, OP postmenopausal women showed an increase in Gal-3, which is positively correlated with BMD of T-score on DXA. These results suggest a diagnostic role of Gal-3 in OP patients as well as its role in monitoring disease severity. Furthermore, this study is the first to assess serum Gal-3 concentration in patients with OP.

Keywords: Galectin-3, osteoporosis, Bone mineral density, DXA, Vitamin D3.

دور الكالاكتين-3 كمؤشر حيوي لهشاشة العظام وارتباطه بكثافة المعادن في العظام

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الخلاصة

تبحث هذه الدراسة في الارتباط المحتمل بين مستويات الجالاكتين-3 (Gal-3) في المصل وصحة العظام لدى النساء بعد انقطاع الطمث المصابات بهشاشة العظام. ونظرًا لأنه من المعروف أن الجالاكتين-3 يتم التعبير عنه في خلايا العظام وقد يؤثر على إعادة تشكيل العظام، فإننا نهدف إلى تحديد مستويات الجالاكتين-3 في المصل في هذا السكان وفحص ارتباطه بكثافة المعادن في العظام .(BMD) في دراسة مقطعية، تم تسجيل 45 امرأة بعد انقطاع الطمث مصابات بهشاشة العظام و45 امرأة من مجموعة التحكم السليمة(HS) ، تتراوح أعمارهن بين 43 و50 عامًا. تم تصنيف المرضى و HS بناءً على درجة T على قياس امتصاص الأشعة السينية ثنائية الطاقة .(DXA) تم قياس مستويات الكالسيوم (Ca) وفيتامين D3 و Gal-3في المصل في كلتا المجموعتين. زاد تركيز تركيز الجالاكتين-3 في المصل بشكل ملحوظ في مرضى OP مقارنةً بـ HS عند $P < 0.001$) بالإضافة إلى ذلك، ارتبطت مستويات Gal-3 في المصل بشكل إيجابي بـ BMD لمستويات T-score في العمود الفقري القطني($r=0.689$ ، $P = 0.0001$)واعتمادًا على منحنى خاصية التشغيل للمستقبل(ROC) ، كانت قيمة المعيار لـ <Gal-3 11.905نانوغرام/مل. في هذه الدراسة، أظهرت النساء المصابات بالتهاب المفاصل الروماتويدي بعد انقطاع الطمث زيادة فيGal-3 ، وهو ما يرتبط بشكل إيجابي بـ BMD لـ T-score على .DXA تشير هذه النتائج إلى الدور التشخيصي لـ Gal-3 في مرضى التهاب المفاصل الروماتويدي بالإضافة إلى دوره في مراقبة شدة المرض. وعلاوة على ذلك، تعد هذه الدراسة الأولى التي تقيم تركيز Gal-3 في المصل لدى مرضى التهاب المفاصل الروماتويدي.

1. Introduction

Osteoporosis (OP) is a systemic skeletal disorder, characterized by reduced bone mineral density (BMD) [1]. It is primarily caused by factors such as hormonal changes, lack of calcium, vitamin D3 deficiency, certain medications like steroids, and genetics [2-6]. Postmenopausal women are more significantly affected by OP than men [7]. Currently, individuals with OP are diagnosed by measuring BMD via dual energy X-ray absorptiometry (DXA), a spectral imaging technique that involves radiological [8]. Therefore, it is crucial to identify biomarkers that can be measured in the serum of these patients, rather than relying solely on radiological methods for diagnosing and screening OP.

Galectin-3 (Gal-3) is a member of the β-galectin family and is expressed in different tissues, including the bone [9]. It plays a role in regulating the cell bone differentiation and function. Additionally, Gal-3 has been shown to impact osteoclastogenesis and osteoblast-osteoclast interactions [10]. Previous studies have indicated that Gal-3 contributes to bone cell maturation, function, and bone remodelling. Thus, identify Gal-3 as a potential therapeutic target agent [11].

Other important reports indicate that Gal-3 mediates the differentiation and bone resorption of osteoclasts, which are regulated by 1,25-Dihydroxyvitamin D [12]. Furthermore, previous studies have shown that Gal-3 plays a role in osteoarthritis, atherosclerosis, diabetic and cardiovascular complications, and other inflammatory diseases [13-15]. Serum Gal-3 levels increased in a few inflammatory disorder such as Rheumatoid arthritis [13], heart diseases [16], cancer [17], and osteoarthritis [18]. To our knowledge, the concentration of Gal-3 in the serum of OP patients has not yet been investigated. Therefore, this study aimed to assess the levels of Gal-3 in OP patients, and its correlation with BMD.

2. Materials and Method

2.1 Study sample

The study involved 90 postmenopausal women aged between 43-50 years, divided into two groups based on DXA scan results. The first group consisted of 45 women diagnosed

with OP without comorbidities according to the World Health Organization (WHO) criteria and national OP foundation guidelines [19]. The second group included 45 with normal DXA served as healthy subjects (HS). Samples were collected from Al-Yarmouk hospital in Baghdad from February 2024 to April 2024. The study protocol received approval from the local ethical comment in Sciences College for women in Baghdad University and local ethical comment in Al-Yarmouk hospital. In addition, written informed consent was taken from all the individuals.

Exclusion criteria included patients with cardiovascular, rheumatoid, cancer, inflammatory, lung, liver, kidney diseases, hematological and musculoskeletal diseases. A systematic questionnaire was employed to gather demographic information for each group, including age, Body mass index (BMI), medical history, duration of disease, etc.

2.2 Bone mineral density (BMD)

The BMD was measured using DXA at the lumber spine (L_1-L_4) levels [20]. The T scores results were recorded for all patients and HS. Individuals with T score less than -2.5 were diagnosed with OP while subjects with T score more than -1 were diagnosed as normal DXA (HS) by consultant physician.

2.3 Blood Sample collection

Blood samples were collected from all subjects in the morning, without using a tourniquet. After a blood clotting within 15 min., Serum was obtained after centrifugation at 3500 rpm. Serum was divided into aliquots and stored at -80 °C until analysis.

2.4 Biochemical tests

Serum Ca level was assessed using standard colorimetric method using (Linear chemicals, Spain). Serum Vitamin D3 (Vit.D3) was measured by electrochemiluminescence immunoassay method (Elecsye and cobas analyzers, Germany). The Cal-3 Level was determined by sandwich ELISA for quantitative method in human serum. Gal-3 kits were supplied from BioSource, USA).

2.5 Statistical analysis

All data were analyzed using Statistical package for the social sciences (SPSS ver.25), GraphPad Prism (ver. 8), and Medcalc (ver. 20.027). Normal distribution of all parameters was assessed by Kolmogorov Smirnov test. Continuous normally distributed parameters were tested by the student independent t-test. Continuous normally distributed parameters were expressed as (Mean ± SD). Correlation analysis was done using Pearson's correlation method. Receiver operating characteristic curve (ROC) analysis was performed to determine the diagnostic of Gal-3 in discriminate OP and HS groups. In addition to determined cut-off value of Gal-3 by Youden index.

For Gal-3 at *P*-value of < 0.0001 and effective sample size of 0.76 (adjusted R^2 =0.757) we got an observed statistical power of 1.000 and this indicates that we have 100% chance of detecting the true difference between the OP patients and the HS. Statistical significance was obtained at $P< 0.05$ for all the analysis.

3. Results

3.1 Demographics characteristic and laboratory findings of study groups

As shown in Table 1; there were no statistical differences in age and serum Ca between the patients with OP and HS group ($P > 0.05$). However, Vitamin D3 levels were significantly lower in OP compared to HS group ($P < 0.05$). In patients with OP, T score mean value was (-2.1±0.515) which is significantly decreased compared to HS group.

Additionally, there is a significant increase in BMI in patients with OP compared to HS group ($P< 0.05$).
Serum Gal-3 levels were significantly higher in OP compared to HS at ($P\leq0.001$). Demographic characteristic and serum markers are reported in Table 1

Table 1: Demographics characteristic and laboratory findings

Parameter	OP N=45	HS N=45	*P*-value
Age (year)	49.73± 6.113	49.87± 6.084	0.983
BMI (kg/m^2)	33.96±3.223	24.29±1.06	0.001
Disease duration (year)	4.5±1.365	-	-
Ca (mg/dl)	8.764±0.8723	8.763±0.7989	0.998
Vit. D_3 (mg/dl)	27.253±34.1253	55.516± 12.912	0.02
T-score (g/cm^2)	-2.1±0.515	-0.6433±0.313	0.001
Gal-3 (ng/ml)	33.96 ± 7.985	12.02 ±1.203	0.001

3.2 Correlation between parameters in study groups

Correlation testing revealed that serum Gal-3 levels were positively correlated with the T score of bone mineral density (BMD) at the lumbar spine (L1-L4) (r = 0.689, P = 0.0001). However, no correlation found between Gal-3 with Ca (r= 0.145 , P= 0.444), and Gal-3 with Vit.D3 (r=0.339 , P=0.067). as show in table 2 and figure 1.

Table 2: Correlation Coefficient between Gal-3 and laboratory findings

Parameters	r	*P*
T-score (g/cm^2)	0.689	0.0001
Ca (mg/dl)	0.145	0.444
Vit. D_3 (mg/dl)	0.339	0.067

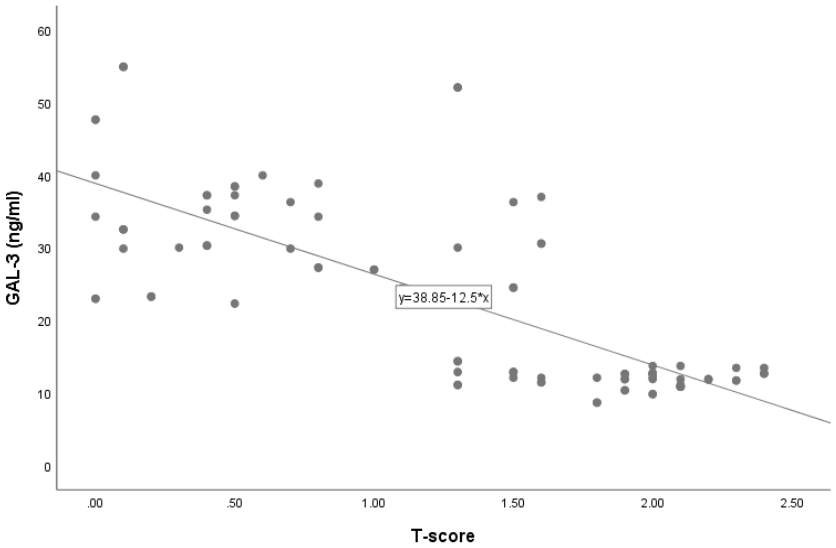

Figure 2: Correlation Coefficient between Gal-3 and T score.

3.3 Receiver operating characteristic curve (ROC)

To assess the potential of Gal-3 as a diagnostic marker or disease-screening tool and to identify the optimum value of the cut-off of the above serum proteins concentration that has the highest specificity and sensitivity for diagnosing a disease, a receiver operating

characteristic (ROC) curve method was used. The area under the curve (AUC) was found to be 1.000, indicating that Gal-3 effectively distinguishes OP patients from HS. According to the Youden index, the optimal cut-off of >11.905 ng/ml, as shown in Figure 1 and Table 2.

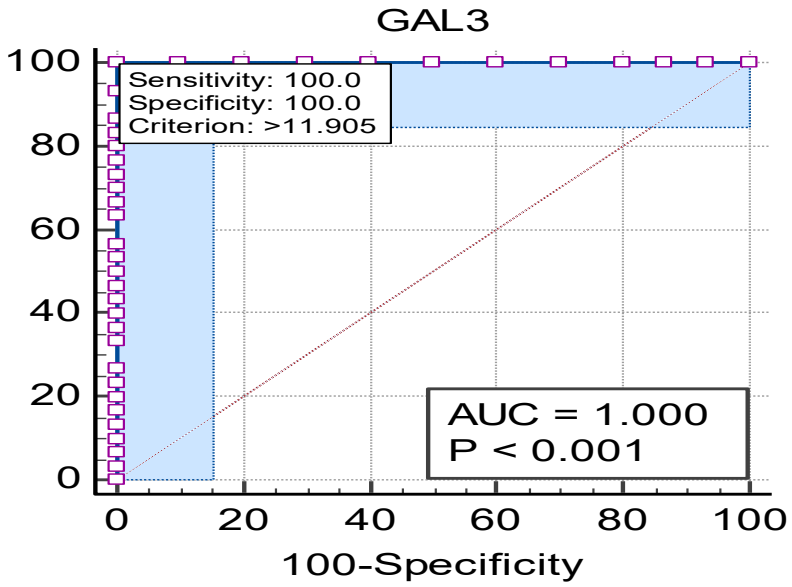

Figure 2: Receiver operating characteristic curve (ROC) analysis of Gal-3 levels for predicting the presence of OP.

Table 2: Validity of Gal-3 to differentiate OP patients from HS

Criterion	Sensitivity	95% CI	Specificity	*P*- value	+PV	-PV
>11.905	100	88.4 - 100.0	100	< 0.001	100	100

4. Discussion

The OP is a widely condition characterized by altered bone remodelling [21]. This disease primarily affects women, particularly following the loss of bone mass which increases after menopause [1]. Various cell signalling approaches related to bone regulation have been suggested [22]. The wingless-related integration site (Wnt / β-catenin) pathway is important for bone accumulation, bone remodelling, and fracture repair by improving the function of osteoblast and reducing bone loss [23]. Notably, a study indicated that Gal-3 increases activation of Wnt / β-catenin signalling [24]. Noteworthy, this agrees with another important study that Gal-3 ability to stabilize and increase β-catenin levels in mesenchymal stem cells differentiating towards the osteogenic lineage [10]. Gal-3 osteogenic activates in bone and vascular tissue [25]. Gal-3 is suggested to be a biomarker of chondrogenic and osteogenic cell lineages and is u*P*-regulated in conjunction with other osteogenic biomarkers through differentiation of bones cell [10]. This study describes a correlation between Gal-3 and T score on DXA in OP patients. Our result showed that Gal-3 increased in the serum of OP postmenopausal women compared to HS group. Correlation between serum Gal-3 and BMD of the lumber spine (L1-L4) levels T-score was observed. Other reports suggest that Gal-3 EVs content might contribute to the reduction of bone formation, development of OP, and increased facture risk in the elderly [26]. These findings indicate that Gal-3 is a possible new biomarker that may be used for diagnosing of OP patients. These results suggest a diagnostic role of Gal-3 in OP patients as well as its role in monitoring disease severity., and this is consistent with the only study conducted to determine the role of Gal-3 in bone remodelling [11]. Furthermore, in this study did not found correlation between Gal-3 and Vit.D_3. Vit.D3

insufficiency is common in Iraqi population [27]. In addition, no correlation we found between Gal-3 and Ca in the current study. Further studies are warranted due to this is the first study. To evaluate the use of Gal-3 as a diagnostic marker or disease-screening tool and to identify the optimum value of the cut-off of the Gal-3 levels. The ROC analysis demonstrated excellent discriminant of OP from HS. In the current study found there is a significant difference in BMI between the groups and this result agreement with other reports and this is due to the fact that most osteoporosis patients take cortisone, and women after menopause tend to be obese. Moreover, Vit.D3 and Ca are essential for maintaining bone health, and their deficiency is an important risk factor for the development of OP. However, this study has some limitations, including a small sample size due to the collection patients from single centre. Additionally, the osteopenia patients lacked in the current study, so they were excluded. In addition, in this study, no bone-specific marker was measured for comparison with Gal-3. This limitation must be avoided in the next study to better validate study conclusion.

Conclusion

In this study, postmenopausal women with OP exhibited elevated serum levels of Gal-3 with and positively correlated with BMD according to T-score on DXA. These results highlighted the diagnostic role of Gal-3 in OP patients as well as its role in monitoring disease severity. Further research involving larger patient groups is needed to investigate the potential role of Gal-3 as therapeutic protein for OP patients

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