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Research Article

Evaluation of Collagen Triple Helix Repeat Containing-1 protein in Postmenopausal Women with Osteoporosis

Isam Noori Salman [1], Noor Ulhuda G. Mohammed[2*], Rand Jalal Abd Al-Ghanny[3], Baydaa Ahmed Abed[1], Samara ali mutar[2]

[1] National Diabetes Center, Mustansiriyah University.
[2] Department of Chemistry, College of Science for Women, University of Baghdad
[3] Ministry of Higher Education and Scientific Research
* Corresponding author's email: noorulhuda.g@csw.uobaghdad.edu.iq

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ABSTRACT

Background: The collagen triple helix repeat containing 1 (CTHRC1) protein has been connected to decreased levels of vitamin D and calcium, as well as obesity. This study aimed to investigate the relationship between CTHRC1 and osteoporosis in post-menopausal women and compare with healthy subjects.
Subjects and Methods: A cross-sectional study consisted of 86 women were enrolled in this study and divided into three groups based on the results of dual-energy densitometry (DXA): 30 women with osteoporosis, 30 women with osteopenia, and 26 healthy women. Data on demographic and clinical features and laboratory values of Calcium (Ca) and Vitamin D3 (V.D3) were recorded.
Results: Women with osteoporosis had significantly increased levels of CTHRC1 (P = 0.0001) compare to HS groups. The CTHRC1 showed negative correlation with Ca and V.D3 value at (r= -0.453, P=0.001 and r= -0.415, P= 0.022) respectively in osteopenia patients. CTHRC1 alone show excellent discrimination power (AUC= 0.9) in identifying women with osteoporosis.
Conclusions: Serum CTHRC1 is a valid biomarker that can distinguish women with osteoporosis from HS group with high accuracy. Low calcium and vitamin D levels in this age group may be linked to an increase in the CTHRC1 levels and this could make it a Therapeutic target in future studies.

Introduction

Osteoporosis is a disorder in which the replacement of lost bone mass is not balanced by producing new bone. Mostly, it affects post-menopausal women (1,2).

The drop in estrogen levels during menopause is one of the main risk factors for the development of osteoporosis in women (3). Surgery related to the stomach like bariatric surgery or corticosteroid medication, can interfere with the process of rebuilding bone over time (4).

The human protein has an N-terminal hydrophobic signal peptide of 30 amino acids that commands Collagen triple helix repeat containing-1 protein CTHRC1 to secrete (5,6).

Since CTHRC1 is absent from healthy arteries, it is clear that the protein functions specifically in the healing of wounds and encourages the remodelling of arteries in the event of arterial injury (7,8). Mechanistically, elevated CTHRC1 levels are linked to a marked reduction in the mRNA and protein levels of collagen type I

and type III, which enhance migration and decrease collagen deposition (9,10).

The CTHRC1 dimer or trimer may be encouraged by the CTHR domain. By secreting extra factors, osteoblasts and osteocytes also regulate the differentiation of osteoblasts and osteoclasts (11–13). Significantly, owing to the regulation of osteoblast–osteoclast cross-talk, CTHRC1 has been identified as a critical coupling factor that connects bone resorption to bone formation. Studies on mice have provided unambiguous in vivo evidence of the critical CTHRC1's regulatory function in bone homeostasis. Because of this, it has been shown that decreased bone mass and decreased bone formation in mice with loss of CTHRC1 function are caused by impaired coupling processes. On the other hand, in transgenic animals, overexpression of CTHRC1 stimulated the formation of new bone, increasing bone mass. The exact function of secreted CTHRC1 in bone biology and its cellular source are still up for debate (7,14).

In both calcium homeostasis and bone metabolism, vitamin D is essential. Sufficient consumption of calcium and vitamin D promotes bone health and lowers the risk of developing osteoporosis and osteopenia (15). Therefore, this study aimed to estimate validity of CTHRC1, Ca, and Vitamin D_3 levels in women with osteoporosis, osteopenia and compare them with those in HS.

Subjects and Methods

A cross-sectional study was carried out in this regard from June 2023 to September 2023. This study included 86 subjects in total (divided into three groups based on the results of DXA: 30 women with osteoporosis, 30 women with osteopenia, and 26 seemingly HS at the Yarmouk Hospital. The inclusion criteria werean age range of 56–62 years old and no history of oral corticoid therapy or calcium or vitamin D supplementation within the previous six months. Every participant had to undergo a DXA scan to identify whether they had osteoporosis, osteopenia, or were healthy. The subjects' weight and height were measured.Total body weight divided by height squared was used to calculate the body mass index (BMI) (kg/m2) (16).

The DXA was used to measure the BMD at the lumbar spine (L2-L4) level. The BMD is expressedas g/cm^2. The DXA scan results were displayed as a "T-score," or the standard deviation (SD) of each participant from the young adult mean. By comparing the T-score and Z-score with reference values, the diagnosis of osteopenia and osteoporosis was made in compliance with the WHO criteria and National Osteoporosis Foundation (NOF) guidelines (17). A T-score of less than -1.0 but more than -2.5 at any site was classified as osteopenic, and a T-score of less than -2.5 was classified as osteoporotic (18). On the day of the bone densitometry, blood samples were obtained and transferring the sample into a regular tube and allowing it to clot at room temperature. Subsequently, the serum was extracted by centrifuging the sample at 3000 r.p.m. Serum was divided into aliquots and kept in the hospital at -80 °C until analysis. Serum calcium levels (Linear chemical, Spian) were assessed by colorimetric method, vitamin D levels (Cobas kit, Roche, Germany) were examined using electrochemiluminescent immunoassay, and the levels of CTHRC1 (Mybiosource, USA) kit were ascertained using the ELISA plate reader.

The study was approved by local ethnics Mustansiriyah University/National diabetes Center Ethics Committee and the Baghdad University/ College of Science for Women Ethics Committee. each participant provided informed consent.

Statistical Analysis

The statistical package for the social sciences SPSS (ver. 25), MedCalc (ver. 20.027), and GraphPad Prism (ver. 8) were used for statistical analysis. The conformity of continuous parameters to the normal distribution was evaluated using the Kolmogrov-Smirnov/ Shapiro-Wilk tests. The parameters that did not show normal distribution were expressed as median and interquartile range [IQR] values. Variables of groups were comparedusing Kruskal-Wallis tests. Spearman was used for correlation analysis. The predictive value of CTHRC1 was evaluated by measuring the area under the curve (AUC) in the receiver operating characteristic curve (ROC). The optimal cut-off value was obtained by calculating the Youden index. Significant result was those with p-value equal to or less than 0.05

Results

Characteristic features in patients and healthy subjects

Out of the 86 women who underwent screening, divided Based on the DXA scans, the patients were divided into three groups: 30 women with osteoporosis (T-score ≤ -2.5), 30 women with osteopenia (T-score: -1 to -2.5), and 26 HS (T-score > -1),. Differences between the groups in terms of age were negligible. 60 had low vitamin D levels (≤ 25 mmol/l) in comparison to the HS group. Table 1 illustrates that there were significant differences between the groups in all clinical measures indicating bone resorption, calcium, and vitamin D3.

Serum CTHRC1 in patients and HS groups

The serum CTHRC1 levels in HS and patients with osteopenia orosteoporosis are presented in table 1. The CTHRC1 levels found a statistically significant difference increase between osteoporosis, osteopenia patients and the HS group (P=0.001),with 93.97 (69.41-148.62) ng/ml, 35.34 (27.27-39.09) ng/ml, and 17.75 (16.99-18.16) ng/ml,respectively, as shown in table 1.

Table 1: Comparison of characteristic features between study groups

Parameter	Osteoporosis (N=30)	Osteopenia (N=30)	HS (N=26)	P-value
Age (year) *	58.03 ± 3.057	56.38 ± 4.639	56.92 ± 4.971	0.947
BMI (Kg/m^2) *	28.786 ± 5.427	30.905 ± 4.397	32.23 ± 5.42	0.072
Ca (mg/dl) †	7.99 (7.81-8.91)	8.11 (7.94-8.725)	9.2 (8.51-9.755)	0.0001
D3 (mg/dl) †	6.44 (4.00-9.33)	12.47 (9.83-13.00)	72.13 (57.85-79.63)	0.0001
DXA(T score %)*	-2.8967± 0.504	-1.63 ± 0.341	-0.12 ± 0.072	0.0001

All data are represented as mean±SD * and median (IQR) †.

Table 2: A comparision of serum CTHRC1 levels among osteoporosis, osteopenia and HS group.

Parameter	Osteoporosis (N=30)	Osteopenia (N=30)	HS (N=26)	P-value
CTHRC1 (mg/dl) *	93.97(69.41-148.62)	35.34(27.27-39.09)	17.75 (16.99-18.16)	0.0001

All data is represented as median (IQR) *.

Correlation of CTHRC1 with study parameter

The CTHRC1 showed negative correlation to the Ca and D3 value at (r= -0.453, P=0.001 and r= -0.415, P= 0.022) respectively in osteopenia patients, as shown in Table 2, while the rest of the variables did not show a correlation with CTHRC1 in both groups.

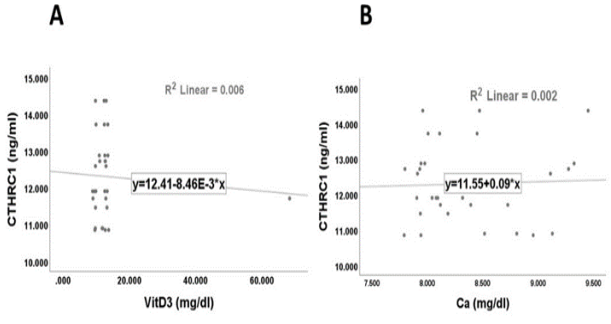

Figure 1: Spearman correlation of serum CTHRC1 in patient study groups, A. show Scatter/Dot between CTHRC1 and V.D3 in osteopenia patients at r= -0.453.P= 0.011. B. show Scatter/Dot between CTHRC1 and Ca in osteopenia patients at r= -0.415, P= 0.022.

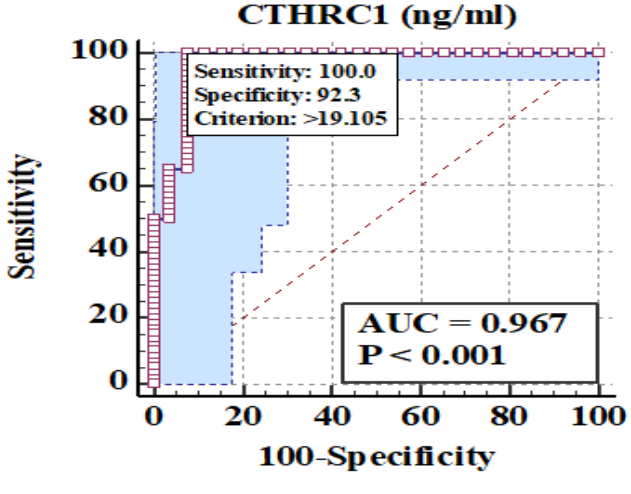

Figure 2: ROC curve for serum CTHRC1 for the prediction of Osteoporosis

ROC curve of CTHRC1 in osteoporosis women

Using the ROC analysis, statistically significant and optimal cut-off values of roughly >19.105 were determined to test the predictive power of the CTHRC1 values in the diagnosis of osteoporosis (Fig. 3, Table 3). The sensitivity to diagnose osteoporosis from the CTHRC1 value was 100% based on the cut-off values and the DXA-defined definition, while the specificity was calculated to be 92.3% at the Youden index equal to 0.92.

Table 3: Criteria to assess the diagnostic power of serum CTHRC1.

Area under the ROC curve (AUC)	0.967
Standard Error	0.0235
95% Confidence interval	0.905 to 0.994
Significance level P (Area=0.5)	<0.0001
Optimal criterion	>19.105
95% Confidence interval	>18.653 to >19.105
Sensitivity	100.00
Specificity	92.31
+PV	96.8
-PV	100.00

Discussion

Bone is a very active and complex tissue and the target of various endogenous and exogenous factors. Osteoporosis is the most common bone disorder, characterised by low BMD (19). Prevention and recognition of osteoporosis are first-step measures to lessen the impact of this condition.

To our knowledge, this is the first study to estimate the role of CTHRC1 in osteopenia and osteoporosis sample of women after menopause,as well as the interaction between this protein and serum calcium and vitamin D.

Bone mass is controlled by continuous remodelling, which is based on the balance between osteoblastic bone formation and osteoclastic bone resorption (20,21).

In our study the level of calcium was also lower in the osteoporosis group than the osteopenia group in comparison to the control group; this can mainly be attributed to a poor calcium diet, especially during the post-menopausal period, or may be due to a defect in the parathyroid gland, which would need further evaluation. In addition, both groups with osteoporosis or osteopenia had low levels of vitamin D3. Osteoporosis and osteopenia are associated with low bone density, as vitamin D3 plays a vital role in calcium absorption, and insufficient levels of it can lead to weak bones (22,23).

In subjects with osteopenia and normal BMD, the CTHRC1 levels were significantly increased in those with osteoporosis. These results indicate that CTHRC1 functions as a positive regulator of osteoblastic bone formation by promoting bone mass and may play a significant role in the anabolic strategy used to treat osteoporosis. In vivo and in vitro osteoblast proliferation has been demonstrated to be stimulated by CTHRC1, while osteoclast bone resorption remains unaffected (24).

Bone resorption is necessary for the synthesis of CTHRC1 with $PO4^{-3}$ and Ca^{+2} playing a vital roles as regulators of CTHRC1 expression in mature osteoclasts (6,12), there is a correlation between the calcium level and the collagen triple helix. Placing osteoclasts in an environment rich in extracellular calcium and phosphate can help understand the close relationship between CTHRC1 production and osteoclast attachment to calcified tissue (25,26). Here, it might catalyze the drawing of stromal/osteogenic cells toward bone resorption and subsequent triggering of bone formation (11).

Because osteoblasts with high phenotype of bone mass and stimulated formation of bone exhibit CTHRC1 expression, CTHRC1 may be crucial in the identification and management of bone disorders like osteoporosis, even though more research is required to fully understand its involvement in age-related bone loss (6,12).

Information was compared to that of the general population even though a sizable portion of the subjects were supplemented. Low levels of vitamin D have been linked to BMD in a variety of populations; however, this correlation was only discovered when comparing individuals with osteopenia and osteoporosis, presumably due to the high proportion of those who took supplements.

Based on the findings of the present study, neither age, BMI, nor Vitamin D are correlated to CTHRC1 values and cann't be considered predictive markers of osteoporosis.

In the current study, the calcium levels and T scores were significantly correlation with CTHRC1, based on BMD values, indicating that it may be considered an independent predictor of low BMD.

When comparing the osteoporosis and osteopenia groups to the control group, there were significant differences in BMI. All of the subjects' T-scores revealed a significant difference between the osteoporosis and osteopenia subjects. Further, the HS T-scores, which correlated with total BMI, were lowest in the osteoporosis group. One of the key variables influencing BMD is BMI. Women who have attained menopause and have a low BMI are more vulnerable to osteopenia and osteoporosis. It is believed that increased adipose tissue production of oestrogen and elevated mechanical loading are responsible for the protective effect of obesity on bone. So, BMI can be used as an important index of osteopenia to prevent osteoporosis (27).

Despite these encouraging findings, more long-term intervention-based studies are advised to validate and broaden the associations reported in this research to obtaina complete picture of the correlation between CTRHC1 and bone health.

The limitation of this study that it is a cross-sectional study conducted at a single centre, with a limited number of patients might limit the power of the study. In addition, in our study did not report other bone markers. In addition, the study depended on just women. While several studies have focused on various aspects of the topic, none of them deals with this particular idea discussed in present study. Notably, no previous research has been conducted on the same subject; thus, the results could not be compared.

Conclusion

In our study, serum CTHRC1 levels were significantly higher in women with osteoporosis and negatively correlated with Ca, V.D3 in women with osteopenia. These findings suggest a potential association between CTHRC1 and the bone health of post-menopausal women. This suggests the possibility that targeting the CTHRC1 may serve as a treatment strategy against osteoporosis. The ROC showed that the optimum cut-off value for CTRHC1 was less than 19.105 with 100% sensitivity and 92.3% specificity, which establishes the presence of osteoporosis with a high confidence interval and is compatible with the diagnosis. further studies will require large sample size and obtain date from multicentre to better validate this conclusion.

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Conflict of Interest

The authors declare no conflict of interest**.**

Data availability

Data are available upon reasonable request

ORCID

Isam Salman 0000-0003-2813-3879

Noor Ulhuda Mohammed 0000-0003-3549-1578

Baydaa Abed 0000-0002-8895-7901

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