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The association of combined vitamin C and D deficiency with bone mineral density and vertebral fracture

Lei He[1,2,3†], Kishor chhantyal[4†], Zihao Chen[1,2,3†], Ruijue Zhu[1,2,3] and Liangming Zhang[1,2,3*]

Abstract

Purpose Both vitamin C and D deficiencies are extremely common in clinical practice, especially in elderly population. Unfortunately, the role of vitamin C deficiency in osteoporosis related consequences is often neglected. The aim of the present study is to analyse if combined vitamin C and D deficiency would have an association with bone mineral density (BMD) and osteoporotic vertebral fracture (OVF).

Methods Ninety-nine post-menopausal female patients admitted in the department of spine surgery of third affiliated hospital of Sun Yat-sen University were enrolled in the study. The participants were divided into four groups; vitamin D deficiency alone (comparator group), vitamin C deficiency alone and combined vitamin C and D deficiency as experimental group. The levels of vitamin C, vitamin D, calcium, phosphorous, BMD and condition of OVF were analysed.

Results There were statistically significant differences between the groups in terms of vitamin C and D levels. In terms of lumbar BMD, significant differences were observed between vitamin D deficiency alone and combined vitamin C and D deficiency. Only the combined vitamin C and D deficiency had a significant negative association with lumbar BMD and T-score. Similarly, combined vitamin C and D deficiency had a significant positive association with lumbar osteoporosis. None of the groups had any significant association with OVF. Combined vitamin C and D deficiency was found to be significantly associated with lower lumbar BMD and osteoporosis.

Conclusion Combined vitamin C and D deficiency results in lower bone mineral density and higher risk of osteoporosis. We believe that existence of deficiencies of both vitamins could have a synergistic effect. Therefore, we recommend that vitamin C and D should be routinely measured in clinical practice.

Keywords Vitamin D, Vitamin C, Bone mineral density, Osteoporosis, Vertebral fractures

†Lei He, Kishor chhantyal and Zihao Chen contributed equally to this work.

*Correspondence:
Liangming Zhang
zhanglm36@mail.sysu.edu.cn
[1]Department of Spine Surgery, The Third Affiliated Hospital, Sun Yat-Sen University, No. 600 Tianhe Road, Guangzhou 510630, China
[2]Guangdong Provincial Center for Quality Control of Minimally Invasive Spine Surgery, No. 600 Tianhe Road, Guangzhou 510630, China
[3]Guangdong Provincial Center for Engineering and Technology Research of Minimally Invasive Spine Surgery, No. 600 Tianhe Road, Guangzhou 510630, China
[4]Emergency department, Doncaster and Bassetlaw Teaching Hospitals NHS Foundation Trust, Doncaster, England

Introduction

Osteoporosis is a progressive inevitable systemic skeletal disease due to low bone mass and degeneration of bone microarchitecture. There is an increase in the fragility of the bone and susceptibility to fracture, which has led to exponential increase in the global health burden [1, 2]. It is more prevalent in postmenopausal women and Postmenopausal osteoporosis (PMO) has become major public health issue. Various antiresorptive medications, which reduces bone resorption and anabolic medications that increases bone formation, have been used in the management of osteoporosis [3, 4]. Antiresorptive agents such as bisphosphonates have been the first-line therapy as they have proven to be effective in reducing the global fracture risk with good safety profile [5]. One meta-analysis of drugs used in postmenopausal osteoporosis showed higher bone mineral density at different sites with different treatments [6]. However, bone tissue homeostasis is maintained by the dynamic balance between resorption and ossification. Hence, biochemical markers of Bone turnover analysis have been advocated in monitoring treatment of the postmenopausal osteoporosis [7, 8]. In the development of osteoporosis, both genetic and biological risk factors play important role. However, such factors are non-modifiable, and therefore, factors such as diet are of great interest to develop the strategies for slowing down the progression of the condition [9].

In animal models, vitamin C has been consistently suggested to mediate osteoclast differentiation [10, 11]. This was associated with an increase in receptor activator of NF-kB ligand (RANKL) expression. In concordance with these findings, vitamin C deficiency resulted in an increased differentiation of osteoclasts. [12, 13]. Most studies on vitamin C deficiency are in agreement with those reported in most previous findings, which indicated that osteoclastogenesis in vitamin C deficiency is stimulated by the up-regulation of the RANKL/RANK pathway [14]. RANKL expression was reduced in vitamin C-deficient mice when supplemented with vitamin C [12]. Based on these reports, we can assume that the vitamin C might play a role in osteoclastogenisis via the RANK/RANKL pathway in human. The role of vitamin C in osteoblastogenesis has also been reported in some studies. In vitamin C-deficient mice, a decrease in the number of osteoblasts and suppressed osteoblast differentiation was observed [15]. In agreement with these findings, there was an increase in the number of osteoblasts following vitamin C treatment [13]. Moreover, when human tissues were studied using osteoblast-like cell cultures, there was enhanced osteoblast proliferation and differentiation with the addition of vitamin C [16].

Due to the role of vitamin D in calcium homeostasis, vitamin D deficiency has been reported to have a positive association with low bone mineral density (BMD) and increased the fracture risks [1]. The 1, 25$(OH)_2$D, active metabolite of vitamin D, is responsible for intestinal calcium absorption, bone calcium resorption and renal calcium reabsorption to maintain calcium homeostasis and promote skeletal mineralization [17]. When its levels are consistently low, often results in skeletal pathologies such as rickets and osteomalacia. Similarly, vitamin C is an essential nutrient found in citrus and soft fruits. It has been linked to bone health, especially the bone structure. In animal studies, vitamin C deficiency resulted in a marked reduction in bone formation [18], and likewise, superoxide-induced bone loss in mice was restored by oral administration of 1% vitamin C, which was shown by significant improvements in BMD, bone weight, bone strength and collagen cross-links [19]. In the last two decades, observational and intervention studies have investigated a potential role for vitamin C in osteoporosis and fracture prevention; however, an overall consensus of the results of published studies does not exist.

Both these vitamin deficiencies are extremely common in clinical practice, especially in elderly population. Unfortunately, the role of vitamin C deficiency in osteoporosis related consequences is often neglected. On this background, we hypothesized that a combined vitamin C and D deficiency would be more detrimental to bone health. Therefore, the aim of the present study is to analyse if combined deficiency of these vitamins would be have an association with BMD and osteoporotic vertebral fracture (OVF).

Materials and methods

Between January of 2018 and January of 2019, a total of 99 post-menopausal female patients with osteoporosis or OVF admitted in the department of spine surgery of third affiliated hospital of Sun Yat-sen University were enrolled in the study. The exclusion criteria included (1) age < 48 years; (2) patients under vitamin D, vitamin C and calcium supplement in the recent six months; (3) patients with suspected hyperparathyroidism; (4) patients under steroid; (5) patients with chronic diseases such as liver cirrhosis, renal failure, hypertension, and diabetes. Those with malignancy were also excluded from the study. This study was approved by the ethical committee of the third affiliated hospital of Sun Yat-sen University and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

They were divided into four groups; vitamin C deficiency alone, with vitamin D deficiency alone, combined vitamin C and D deficiency, and a control group with normal vitamin C and D level. We defined vitamin D deficiency as serum level less than 20ng/ml [1] and vitamin C deficiency as serum level less than 34μmol/ml [20]. Fasting venous blood samples were collected

in the morning on the day after admission. The samples were then centrifuged and the resulting serum was stored at −80 °C for later analysis. Serum vitamin D and vitamin C levels were analyzed using a fluorometric assay by LK3000V kit (LANBIAO, Tianjin, China). Serum calcium and phosphorus concentration were examined by HITACHI 7060 Automatic Biochemical Analyzer (Hitachi Ltd., Japan). BMD was measured at lumbar (L1-L4), femur neck, and hip using Dual-energy x-ray absorptiometry by Hologic Discovery DEXA system (Hologic, Bedford, MA) scan. The vertebral fracture was analysed using anteroposterior and lateral X-ray film. Each X-ray images was evaluated by two experienced spine surgeon (>10 years of experience) independently to assess whether it contained a vertebral fracture. Additionally, data were collected regarding patients' smoking status, sun exposure, and history of falls.

The research data does not have any missing values. Continuous data were indicated with mean±standard deviation (SD) while categorical data were indicated with number and percentage (%). One-way ANOVA was used to compare the means of continuous characteristics among the four groups, and Bonferroni test was used in post-hoc comparisons. Categorical data were tested using Chi-square test or Fisher's exact text (if expected value≤5 was found). To compare the T score and BMD among groups, two-way ANOVA was used to confirm the main effect and interaction effect with the design vitamin C deficiency (or not) cross vitamin D deficiency (or not). The post-hoc comparison was also Bonferroni test. Univariate and adjusted logistic regression models were also used to estimate the risk of group to OVF and osteoporosis, the adjusted covariates were patient's age and BMI. A $P<0.05$ would be recognized as reaching significance of each test, two-tailed. All above analyses were performed using IBM SPSS Version 25 (SPSS Statistics V25, IBM Corporation, Somers, New York).

Results

Patient's clinical characteristics

A total of 99 post-menopausal female patients participated in this study, including 29 patients with vitamin C deficiency alone, 15 with vitamin D deficiency alone, 24 with combined vitamin C and D deficiency, and 31 normal subjects as control group. Patient's clinical characteristics by group (normal, vitamin C deficiency, vitamin D deficiency, and vitamin C and D deficiency groups) were listed in Table 1, including age, height, weight, BMI (Body Mass Index), SBP (Systolic Blood Pressure), DBP (Body Mass Index), vitamin C level, vitamin D level, calcium level, and phosphorus level. The average age of all participant was 66.71±10.81 year, and the BMI was 22.46±2.65 kg/m^2.

As indicated in Table 1, significant differences were only found in vitamin C and vitamin D levels. According to the post-hoc results of vitamin C and vitamin D levels, it seems rational to the corresponding deficiency in each group. In addition, Sun exposure is readily accessible in southern China due to abundant sunlight year-round. None of the patients had a history of smoking, and 89% of those with OVF reported a history of falls. These results confirm the comparativeness among vitamin C deficiency, vitamin D deficiency, and vitamin C and D deficiency groups.

T score and BMD

Table 2 indicated the results of T score and BMD among four groups, including the sites of lumbar, femur neck, and total hip. In two-way ANOVA analysis, it was found that significant main effects were found in vitamin D deficiency of lumbar T-score and vitamin C deficiency of lumbar BMD. In post-hoc comparisons, it was found that lumbar T-scores were significantly lower in vitamin D and C+D deficiency groups than normal group (both $P<0.05$); the total hip T-score of vitamin C deficiency group was lower than normal group ($P=0.40$); lumbar BMD was significantly lower in vitamin C and C+D

Table 1 Patient's clinical characteristics among all groups

Parameters	Normal ($n=31$)	Vitamin C deficiency alone ($n=29$)	Vitamin D deficiency alone ($n=15$)	Combined vitamin C and D deficiency ($n=24$)	*P*
Age, year	67.77±8.92	69.10±11.24	69.60±6.39	66.71±10.81	0.763
Height, m	1.56±0.07	1.54±0.09	1.56±0.07	1.54±0.07	0.673
Weight, kg	57.35±9.13	52.53±11.92	58.23±11.46	52.96±7.01	0.115
BMI, kg/m2	23.70±3.92	21.99±3.63	24.05±4.19	22.46±2.65	0.164
SBP, mmHg	134.23±14.33	123.76±12.60	129.33±12.36	133.25±18.56	0.057
DBP, mmHg	78.26±10.92	77.59±8.43	81.67±11.11	81.71±6.83	0.283
Vitamin C, μmol/L	39.51±5.34	31.03±1.59 [a]	40.81±6.57 [b]	31.34±1.70 [ac]	<0.001
Vitamin D, ng/ml	29.05±6.45	31.14±10.15	15.17±3.17 [ab]	15.63±3.25 [ab]	<0.001
Calcium	9.35±0.48	9.29±0.53	9.27±0.38	9.20±0.45	0.693
Phosphorous	3.44±0.52	3.47±0.54	3.74±0.55	3.54±0.63	0.347

[a], $P<0.05$ compared to normal group; [b], $P<0.05$ compared to vitamin C deficiency group; [c], $P<0.05$ compared to vitamin D deficiency group

Table 2 The comparisons of T score and BMD among all groups

Parameters	Normal ($n=31$)	Vitamin C deficiency alone ($n=29$)	Vitamin D deficiency alone ($n=15$)	Combined vitamin C and D deficiency ($n=24$)	*P*
T score					
Lumbar	-2.82 ± 1.06	-3.30 ± 1.20	-3.61 ± 0.95 [a]	-3.84 ± 0.93 [a]	0.005
Femur neck	-2.51 ± 0.94	-2.99 ± 1.26	-2.85 ± 0.76	-2.97 ± 1.15	0.298
Total hip	-2.18 ± 1.15	-2.80 ± 1.18 [a]	-2.71 ± 1.35	-2.58 ± 1.02	0.191
BMD					
Lumbar	0.66 ± 0.13	0.58 ± 0.15 [a]	0.65 ± 0.12	0.55 ± 0.13 [ac]	0.010
Femur neck	0.57 ± 0.10	0.52 ± 0.14	0.53 ± 0.10	0.51 ± 0.12	0.189
Total hip	0.68 ± 0.14	0.60 ± 0.16 [a]	0.62 ± 0.16	0.64 ± 0.13	0.219

[a], $P<0.05$ compared to normal group; [b], $P<0.05$ compared to vitamin C deficiency group; [c], $P<0.05$ compared to vitamin D deficiency group

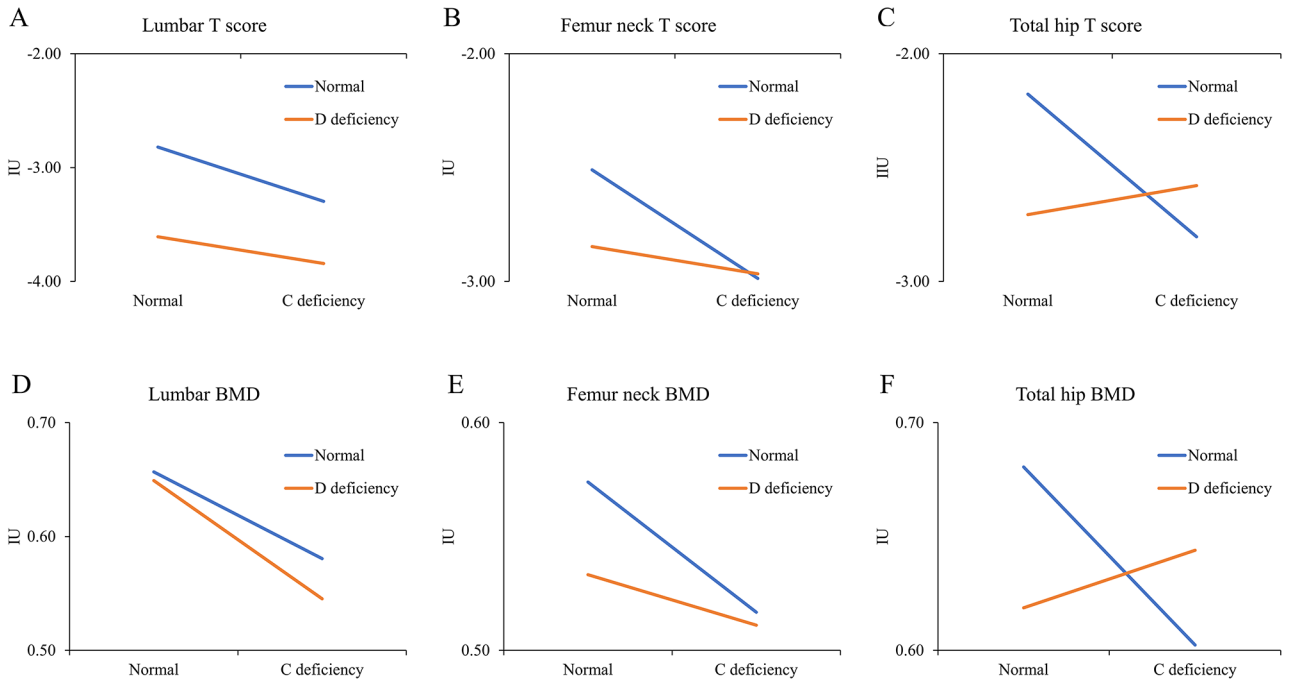

Fig. 1 The mean plots of T score, including lumbar (**A**), femur neck (**B**), total hip (**C**); and the mean plots of BMD, including lumbar (**D**), femur neck (**E**), and total hip (**F**)

Table 3 The incidence of OVF and osteoporosis among all groups

Parameters	Normal ($n=31$)	Vitamin C deficiency alone ($n=29$)	Vitamin D deficiency alone ($n=15$)	Combined vitamin C and D deficiency ($n=24$)	*P*
OVF	21 (67.74%)	22 (75.86%)	13 (86.67%)	17 (70.83%)	0.530
Osteoporosis					
Lumbar	16 (51.61%)	19 (65.52%)	13 (86.67%)	21 (87.50%)	0.010
Femur neck	19 (61.29%)	18 (62.07%)	10 (66.67%)	16 (66.67%)	0.967
Total hip	13 (41.94%)	17 (58.62%)	8 (53.33%)	14 (58.33%)	0.541
Any-site	28 (90.32%)	26 (89.66%)	13 (86.67%)	23 (95.83%)	0.748

deficiency groups than normal group (both $P<0.05$); the lumbar BMD of C+D deficiency group was significantly lower than simply D deficiency group ($P=0.021$); the total hip BMD of vitamin C deficiency group was lower than normal group ($P=0.44$). Figure 1 also indicates the mean plots of T score and BMD at all sites, similar patterns were observed in T-score and BMD at the same site.

OVF and osteoporosis

Table 3 shows the incidences of OVF and osteoporotic among all groups, osteoporotic includes lumbar, femur neck, total hip, and any-site. The incidence rates of all deficiency groups were 73.74% OVF, 69.70% lumbar osteoporosis, 63.64% femur neck osteoporosis, 52.53% total hip osteoporosis, and 90.91% any-site osteoporosis.

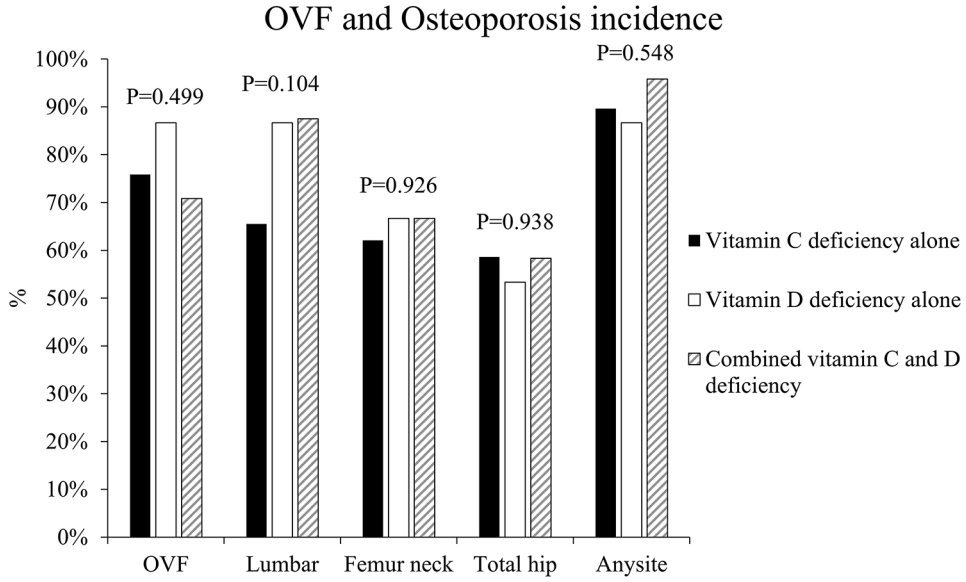

Fig. 2 The incidences of OVF and osteoporosis among vitamin C deficiency, vitamin D deficiency, and combined vitamin C and D deficiency groups

Table 4 Logistic regression results of group to OVF and osteoporosis

	OVF		Lumbar osteoporosis		Femur neck osteoporosis		Total hip osteoporosis		Any-site osteoporosis	
Parameter	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Univariate model										
Group		0.584		0.022		0.968		0.546		0.796
Normal	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
Vitamin C deficiency alone	1.50 (0.48 to 4.66)	0.487	1.78 (0.63 to 5.04)	0.277	1.03 (0.36 to 2.93)	0.951	1.96 (0.70 to 5.48)	0.199	0.93 (0.17 to 5.02)	0.931
Vitamin D deficiency alone	3.10 (0.58 to 16.41)	0.184	6.09 (1.17 to 31.63)	0.031	1.26 (0.35 to 4.61)	0.723	1.58 (0.46 to 5.47)	0.468	0.70 (0.10 to 4.69)	0.710
Combined vitamin C and D deficiency	1.16 (0.36 to 3.68)	0.806	6.56 (1.62 to 26.61)	0.008	1.26 (0.41 to 3.85)	0.681	1.94 (0.66 to 5.71)	0.230	2.46 (0.24 to 25.31)	0.448
Adjusted model										
Group		0.609		0.024		0.853		0.750		0.781
Normal	ref.	-	ref.	-	ref.	-	ref.	-	ref.	-
Vitamin C deficiency alone	1.34 (0.42 to 4.29)	0.626	1.70 (0.59 to 4.91)	0.328	0.69 (0.21 to 2.26)	0.538	1.56 (0.47 to 5.17)	0.465	0.45 (0.07 to 3.08)	0.414
Vitamin D deficiency alone	3.09 (0.57 to 16.69)	0.189	6.24 (1.19 to 32.67)	0.030	1.24 (0.29 to 5.29)	0.773	1.63 (0.39 to 6.81)	0.503	0.70 (0.08 to 6.01)	0.749
Combined vitamin C and D deficiency	1.09 (0.33 to 3.59)	0.882	6.29 (1.54 to 25.80)	0.011	1.06 (0.30 to 3.68)	0.928	1.94 (0.56 to 6.73)	0.299	1.31 (0.10 to 16.53)	0.835
Age, year	1.02 (0.97 to 1.07)	0.473	1.00 (0.95 to 1.05)	0.866	1.07 (1.01 to 1.12)	0.012	1.08 (1.03 to 1.14)	0.003	1.06 (0.97 to 1.17)	0.212
BMI, kg/m2	0.94 (0.82 to 1.08)	0.410	0.97 (0.85 to 1.11)	0.638	0.80 (0.69 to 0.93)	0.004	0.79 (0.68 to 0.92)	0.003	0.71 (0.54 to 0.93)	0.013

Significantly higher incidence rates of lumbar osteoporosis were found in D and C+D deficiency groups than the other two groups. The incidences among all three deficiency groups did not significantly differ (all $P>0.05$) (Fig. 2).

Risk estimation using OR

Table 4 further demonstrates the logistic regression results of group to OVF and osteoporosis, including univariate group and the model adjusted with patient's age and BMI. As indicated, no significant was found in OVF. For lumbar osteoporosis, vitamin D deficiency alone group and combined vitamin C and D deficiency group had significantly higher risk than other groups, and the

combined vitamin C and D deficiency group had the highest OR in both univariate and adjusted models. For femur neck and total hip osteoporosis, higher age and lower BMI were associated to risk; only lower BMI were associated to any-site osteoporosis.

Discussion

Osteoporosis has a multifactorial etiology. Due to its role in calcium homeostasis, vitamin D is one of many. Several studies have shown that vitamin D deficiency and decreased BMD have positive association with, albeit some have found no association at all [21]. Observational studies have suggested associations between vitamin D deficiency and secondary hyperparathyroidism, elevation of bone turnover markers and bone loss. Similarly, vitamin C plays a role in collagen formation, osteoclastogenesis and osteoblastogenesis [22]. We assumed that low levels of vitamin C might play some roles in osteoporosis. Therefore, principal objective of this study was to analyse whether combined vitamin C and D deficiency was associated with bone health parameters.

In a study of the relation of ascorbic acid to BMD and self-reported fractures among US adults, dietary vitamin C intake was independently associated with BMD among premenopausal women. In postmenopausal women, who did not have a history of smoking or estrogen use, serum ascorbic acid was associated with lower BMD [23]. Another community-based cohort of 277 postmenopausal women who taking a regular dose of vitamin C 100 to 5,000 mg daily, showed that BMD at ultradistal and midshaft radius, hip, and lumbar spine were approximately 3% higher. The study also found that the women taking both estrogen and vitamin C had significantly higher BMD levels at all sites [24]. A few studies have reported a positive association between vitamin C and BMD, but the sample size were relative small [25]. Our study has also shown significantly lower lumbar BMD levels among vitamin C deficiency than those with normal levels.

Although many studies have evaluated the association between the serum 25(OH)D status and BMD value, the results are still controversial. Some studies reported a positive association between serum vitamin D status and BMD value at various sites, while several other studies did not show any correlation [26–28]. In this study, we found that the lumbar BMD levels were significantly lower than that of the normal group. Similarly, lumbar T-scores were also significantly lower compared to those with normal vitamin D levels. This shows that there is significant association between vitamin D deficiency and lower BMD levels. This is in contradiction to those reported previously. In a long-term prospective study of 669 postmenopausal women, Garnero et al. reported that there was no significant association between serum 25(OH)D and BMD at radius [29]. Recently, a cross-sectional study based on large population demonstrated that 25(OH)D deficiency was not associated with calcaneal BMD [30]. The reasons for these contradicting results still remains unclear. Some studies pointed out that only a very low level of 25(OH)D status was positively associated with BMD [31].

In our study, we observed that BMD at most sites were higher than that in the deficient groups. The lumbar BMD of vitamin C deficiency alone and vitamin C and D deficiency group was significantly lower than in the normal group. Also, the T-score at lumbar site was significantly lower among vitamin D deficient as well as those with both vitamin C and D deficiency. A fascinating finding of our study is that the lumbar BMD was significantly lower in vitamin C and D deficiency group when compared to the vitamin D deficiency alone. This is the first study to report on the lower BMD levels and combined vitamin C and D deficiencies. In clinical practice, vitamin C deficiency is often neglected, partly because its deficiency doesn't have bone related sign and symptoms only. We believe that because of its role in the collagen formation as well as osteoclast differentiation and osteoblastogenesis, it does play a role in osteoporosis development. On the other hand, vitamin D not only promotes bone formation but also bone resorption by increasing the osteoclast activity. We believe that when both these vitamins are below the optimal level, there is a synergistic effect. This is further supported by our result, which a positive association is observed between the lumbar osteoporotic group and combined deficiency group. The condition of combined vitamin C and D deficiency is likely to be a promoting factor for lumbar osteoporosis.

Some reports suggested that vitamin D deficiency increased the muscle weakness and risk of falling in elderly people [32], and supplementation of vitamin D reduced the risk and prevented fragile fractures [33]. Several other studies also reported the vitamin D insufficiency as a risk factor of fragile vertebral fractures [34–36]. Hence, low vitamin D might be an independent determinant of OVF. Similarly, observational data support the hypothesis that high dietary intake and supplementation with vitamin C may reduce the risk of fractures in postmenopausal women [37]. In our study no significant differences among the deficient groups and normal group was found.

Even though this is the first study to report on combined vitamin C and D deficiency with BMD and OVF, there are several limitations of this study. Firstly, only postmenopausal women from inpatient ward were included in this study, and hence the results might not reflect the normal population. Secondly, our sample size is considerably small, and thus, placebo controlled randomized clinical trials are required to further support

our findings. Lastly, there is a lack of data on potentially confounding factors, including intake levels of calcium, vitamin D, and vitamin C, physical activity, and medication use, which could influence the outcomes. Future studies should comprehensively address these factors to enhance the robustness of the findings.

Conclusion

Combined vitamin C and D deficiency results in lower bone mineral density and higher risk of osteoporosis. We believe that existence of deficiencies of both vitamins could have a synergistic effect. Therefore, we recommend that vitamin C deficiency should not be neglected and vitamin C should be routinely measured in clinical practice besides vitamin D.

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Author contributions
L.Z. carried out the study design; R.Z. collected and processed the samples; L.H., K.C., and Z.C. conducted data analyses and wrote the paper. All the authors have read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Code availability
Not applicable.

Declarations

Ethics approval
This study was approved by the ethical committee of the third affiliated hospital of Sun Yat-sen University and have been performed in line with the principles of the Declaration of Helsinki.

Consent for publication
Not applicable.

Informed consent
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare no competing interests.

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