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Effectiveness of Calcium and Vitamin D, With and Without Collagen Peptide, in Enhancing Bone Mineral Density on Postmenopausal Women with Osteopenia: A Randomized Controlled Trial

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ABSTRACT

Background: Osteopenia is the precursor stage to osteoporosis and is a significant problem for women. The interaction of collagen peptide supplementation with calcium and vitamin D supplementation may give some ways through which bone health could be enhanced. **Objective:** To determine the efficacy of calcium, Vitamin D3, and collagen peptide supplementation in enhancing bone health. **Study Design:** Randomized controlled trial. **Settings:** King Abdullah Hospital, Mansehra and Shaheena Jamil Hospital, Abbottabad Pakistan. **Duration:** Study duration was between March 2024 to May 2024. **Methods:** Thirty postmenopausal women over the age of 60 years volunteered in this single-blinded randomized controlled trial and were divided into two groups by simple random sampling technique. Group A (n=15) received bioactive collagen peptide along with calcium lactate, and vitamin D3. Group B ((n=15) served as a control group and received Calcium and Vitamin D3 only. Serum procollagen type I N-propeptide was assessed at baseline and after 3 months. **Results:** The results showed a decrease in procollagen type I N-propeptide level from baseline 61.3±14.7 to 53.0±14.1 ($p<0.001$) post 3 months in group A. Group B showed the mean rise from 58.8±23.2 to 56.2±23.1 to ($p=0.310$). **Conclusion:** These findings reflected decreased PINP level after 3 months using calcium, vitamin D, and collagen peptide medication. Adding collagen peptides to calcium and vitamin D supplements may increase their beneficial effects on bone metabolism.

Keywords: *Bone mineral density, Collagen peptide, Osteoporosis, Osteopenia, Vitamin D3.*

INTRODUCTION

Osteopenia, identified by bone mineral density (BMD) being lower than usual but not yet reaching the osteoporosis threshold, is a severe health condition in postpartum women.[1] It is a precursor to osteoporosis, a significant public health disease linked to increased fracture risk, morbidity, and mortality.[2-3] The decrease in estrogen after menopause causes osteoporosis rather than bone formation, resulting in less bone mass.[4-5] Interventions to improve or maintain bone density are essential for preventing osteoporosis and its related complications.[6] At present, more than 34 million adult Americans suffer from osteopenia. It only remains to be seen to rise sharply over the next decade with the increase in age. The population over 65 in the United States is expected to rise to over 20% from 2010 to 2030. The anticipated population growth affected by osteopenia by 2020 was 47 million Americans. 30% of postmenopausal women in the US have been defined as osteoporotic, and 50% are osteopenic. This relative occurrence became magnified at the age of 80 and shifted, with 27% of the women having osteopenia and 70% who have osteoporosis.[7-8] Asia has average t-scores as compared to other regions globally. According to the data obtained in Australia, the incidence of osteopenia was recorded in 42% of male participants and 51% of female subjects.

India, in 2005, documented an average of 52% prevalence of osteopenia.[9]

For several years, calcium and vitamin D have been essential in bone metabolism and may be crucial for bone health. Calcium is built into bones and is required in adequate amounts to maintain bone health. Vitamin D helps absorb calcium in the small intestines and ensures enough calcium to satisfy the needs of bone mineralization.[10] Without these nutrients, it negatively impacts the bones and causes osteoporosis. Thus, the calcium and vitamin D interventions are deemed appropriate measures supporting postpartum women's needs. The most relevant biomarkers for bone metabolism include osteopenia, among other diseases that are related to it. The most significant protein constituting the bone matrix is collagen type I, which generates PINP as a byproduct. During the generation of bone, osteoblasts or bone-producing cells generate collagen type I. Consequently, this leads to the release of PINP into the blood. This, thus, serves as a measure of bone production activity.

Multiple clinical trials have recently reported the effect of bioactive collagen peptides (CP) on bone health.[11-12] It is the major non-collagenous protein and contributes crucially to the bones' desired shape and strength. CP is a product of hydrolyzed collagen, which increases the rate of osteoblasts and enhances bone matrix formation. Consequently, some clinical works and clinical trials indicate that it can have a positive impact on the bone metabolic process, which makes it effective when used alongside calcium and vitamin D.[13]

Combining CP with calcium and vitamin D supplements offers many ways to improve bone health. These components can solve the problem of mineralization and calcium homeostasis, while CP can help form the organic matrix of bones, which can have a joint effect on BMD. However, the clinical effectiveness of this combination in improving BMD in women with postpartum osteopenia is still scarce.

This clinical trial was to identify the effects of vitamin D and calcium supplements, either alone or in combination with collagen peptide, on bone mineral density in postpartum women diagnosed with osteopenia. By comparing BMD, bone turnover markers, and other outcomes between the two groups, this study aims to provide preliminary evidence as to whether adding collagen peptide can increase the effectiveness of conventional medicine in improving bone health.

METHODS

The study was a single-blinded, randomized controlled trial, carried out at King Abdullah Hospital Mansehra and Shaheena Jamil Hospital Abbottabad. The research plan was properly presented by the Ethics Committee of Frontier Medical and Dental College having reference number 3390 and was registered in the clinical registry having reference number NCT06464718.

Using the World Health Organization software program "Sample Size Determination in health sciences", the sample size for the experimental portion of the study was determined. After determining the sample size, the "Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study" was considered. With a 90% confidence level, 80% test power and a mean difference of 11.0, the following calculation was used to determine the sample size of 30.

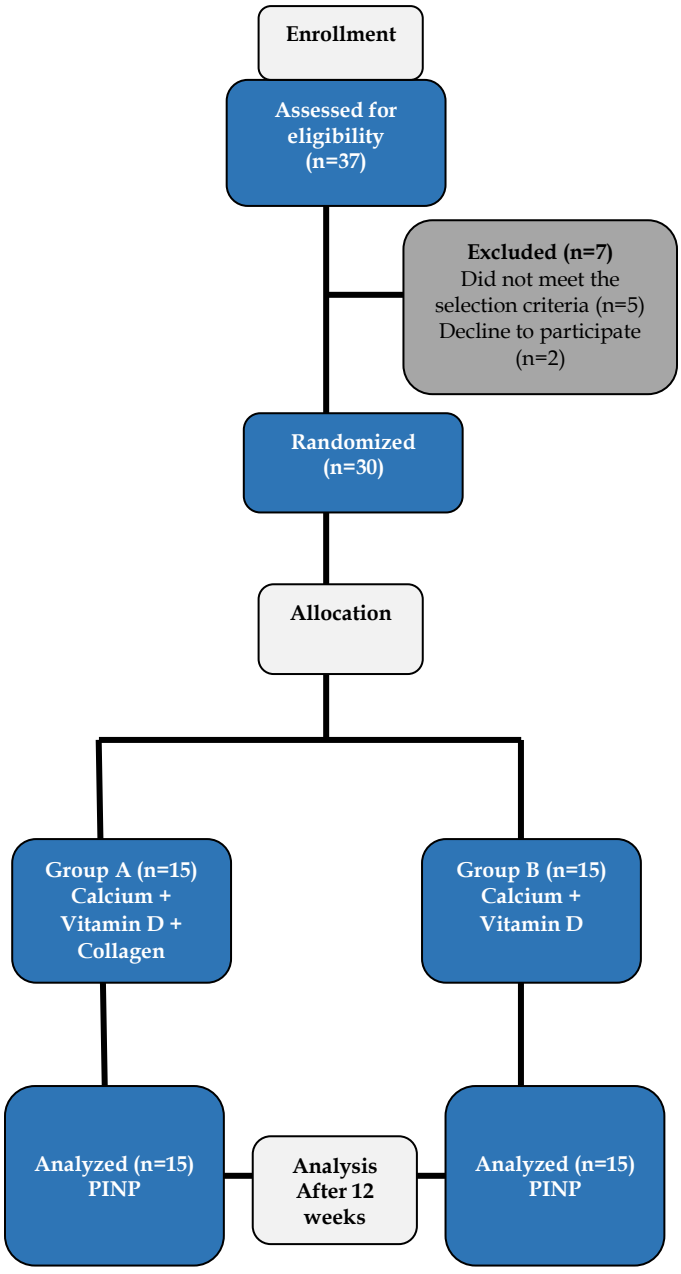
Postmenopausal patients with a T-score measurement based on DXA within the osteopenic category, i-e (-1. 0>T-score>-2. 5) either at Lumber spine or femur were recruited from the gynecological OPD of King Abdullah Hospital Mansehra and Shaheena Jamil Hospital Abbottabad. Exclusion criteria were clinical fracture, current smokers, biochemical history of primary hyperparathyroidism, and use of any medication that might affect bone turnover or BMD including antiresorptive agents, estrogens, and systemic corticosteroids Further exclusion made to decrease variability included participants who had secondary causes of osteoporosis such as thyrotoxicosis and those who did not present themselves for follow up, thereby providing only baseline measurement.[14]

Participants were recruited through the envelop method of a simple random sampling technique into mutually exclusive groups. All participants signed the informed consent and had an independent and optional right to refuse to continue participating in a study at any date or based on the researcher's decision, and the subject's departure was not compensated.

The duration of the study was between March 2024 to May 2024. Thirty females aged over 60 years with postmenopausal status chosen using the inclusion criteria after BMD measurement with the help of DXA at LS and femur were enrolled. Data on demographic details and medical history was obtained at the baseline and the subjects also filled out a short survey as to their calcium intake. These participants were assigned to the study's supplementation and informed of what was expected from them at baseline and then followed up later. For adherence assessment, participants were checked at three months self-reported on their compliance, persistence, health status and any adverse effects and with the other medications being taken. Compliance of patients were assessed based on the patient's drug intake rate, ranging from 0-49 %, 50-74 %, and 75-100% and a reminder was made of continuous medication adherence.

Participants were divided into two groups through simple random sampling (Figure 1). Group A comprised of 15 subjects who were given a daily sachet containing 5g of Bioactive collagen peptides (Fortibone). Electrolyte mix containing 500 mg calcium as calcium lactate, 4 MCg calcitriol and selenised yeast rich in vitamin D3 (400IU) (Colabone®, Vivapharm SA). Group B served as control consisted of 15 subjects who consumed a sweet formed from a chewable tablet with 1. A calcium compound containing 25 g calcium carbonate (500mg elemental calcium) and vitamin D3 400IU taken daily, these are potent supplements that are often used in practice, as per the researchers' recommendations.[15]

Figure 1: Illustrating framework of interventional strategies



Blood samples were taken from all participants at the baseline and three months following an overnight fast. Tests conducted on these samples included measurements of N-MID-osteocalcin (N-MID-OC), intact parathyroid hormone (iPTH), total calcium, 25-hydroxy-vitamin D [25(OH)D], and total procollagen type I N-terminal propeptide (total-P1NP) to investigate potential secondary causes of osteoporosis.

The blood samples obtained were processed by centrifugation within one hour at a speed of 3000 rpm for 10 minutes, then divided into smaller portions and stored at -80°C pending further analysis. The serum levels of 25(OH) D and N-MID-OC were measured using an ECLIA immunoassay technique on a Cobas e411 instrument.

The analysis of data involving categorical variables compared two groups' cross-tabulations and was conducted deploying the Pearson 'chi-square test'. The Wilcoxon signed-rank test was used to determine the changes in P1NP level within each group because the data in these sets were not normally distributed. Variables were documented as continuous data with means and standard deviations rounded to the nearest whole number where appropriate to enhance comparability. All the statistical tests was done with the help of SPSS 20 statistical analysis software.

RESULTS

The baseline demographics between two Groups - study participants of Group A (n=15) and Group B (n=15). The average age was 63.2 ± 5.3 and 61.9 ± 6.5 respectively. BMI was 27. 8 ± 4 in Group A and 26.2 ± 3.6 in Group B. The participants in Group A consumed an average of 786 ± 417 mg of dietary calcium per day while the participants in the Group B consumed 651 ± 348 mg of calcium per day, not showing any possible difference ($p = 0.3$).

The results presented in Table 1 demonstrate significant within-group differences in Group A for all measured variables, including Bone Mineral Density (BMD), osteocalcin, and PINP, with substantial improvements observed post-intervention ($p < .001$). Notably, the mean BMD in Group A showed a marked improvement, accompanied by a significant increase in osteocalcin and decrease in PINP levels. In contrast, Group B exhibited smaller changes, with BMD showing no statistically significant improvement ($p = .526$), while osteocalcin and PINP displayed moderate yet statistically significant increases ($p < .01$). Overall, Group A demonstrated more pronounced improvements in bone health markers compared to Group B.

Table 1: Shows within group differences among variables

		Paired Differences					df	Sig. (2-tailed)
		Pre Mean ± SD	Post Mean ± SD	Mean Difference ± SD	95% Confidence Interval of the Difference			
					Lower	Upper		
Group A	BMD	-1.90±0.39	-1.07±0.25	-0.82±0.42	-1.06112	-.59221	14	.000
	Osteocalcin	14.53±1.18	17.60±1.88	-3.06±1.79	-4.05877	-2.07456		.000
	PINP	61.3±14.7	53.0±14.1	-12.20±4.97	-14.95463	-9.44537		.000
Group B	BMD	-1.78±1.11	-1.58±0.36	-2.0±1.18	-.85891	.45891		.526
	Osteocalcin	14.13±1.18	15.66±0.97	-1.53±1.80	-2.53423	-.53243		.005
	PINP	58.8±23.2	56.2±23.1	-6.73±2.68	-8.20	-5.26		.310

Table 2 highlights the between-group differences for Bone Mineral Density (BMD), osteocalcin, and PINP. The findings reveal a statistically significant difference in BMD between the two groups, with a mean difference of 0.50±0.10 ($p < 0.001$), favoring Group A. Similarly, osteocalcin levels showed a significant mean difference of 1.93±0.54 ($p = 0.001$), indicating greater improvement in Group A. PINP levels also exhibited a significant mean difference of 5.93±2.47 ($p = 0.023$), further emphasizing the enhanced effect of the intervention in Group A compared to Group B. These results suggest that the intervention in Group A led to superior outcomes in all measured bone health markers compared to Group B.

Table 2: Shows between group comparison among variables

	Mean difference ±SD	95% of Confidence Interval	df	p-value
BMD	0.50±0.10	0.28 to 0.72	28	0.000
Osteocalcin	1.93±0.54	0.811 to 3.05		0.001
PINP	5.93±2.47	0.866 to 10.99		0.023

DISCUSSION

In the menopause process, women become vulnerable to developing osteoporosis. Despite the medical treatments that are available to control and cure osteoporosis, there is growing use of control measures and remedies such as nutritional supplements, particularly calcium, vitamin D and collagen hydrolyzate. The study investigated changes in P1NP levels of type I procollagen between two groups over three months. Mean P1NP levels at baseline were similar in groups A and B (61.3 ± 14.7 and 58.8 ± 23.2, respectively) and did not differ ($p = 0.201$). However, while P1NP levels decreased in three months in group A ($p<0.001$), the change in group B was not significant ($p=0.310$). The percentage reduction in P1NP was greater in group A (-13.1 ± 12.3) compared to group B (-2.1 ± 12.6) ($p = 0.011$); this indicates a greater decrease in P1NP in group A. Similar results were reported in multiple clinical trials. In a study conducted by Zdzieblik D, the effects of collagen supplementation on bone mineral density, bone geometry, and bone turnover among post-menopausal women were analyzed. The findings revealed enhancements in trabecular and cortical parameters evaluated through peripheral tibial quantitative computed tomography. Moreover, the group receiving supplementation with calcium, vitamin D, and CP demonstrated the prevention of BMD loss after 12 months.[16] Another study examined the impact of collagen supplementation on individuals with osteopenia. The participants were segregated into two groups, one receiving vitamin D and calcium supplementation alone, while the other received supplementation with collagen peptide in addition to vitamin D and calcium. The findings revealed a decrease in P1NP and CTX levels within three months of supplementing calcium, vitamin D, and bioactive collagen peptides in postmenopausal women with osteoporosis. Conversely, bone markers remained unchanged in the absence of calcium, vitamin D, and collagen peptide supplementation.[17] Likewise, in a clinical trial on post-menopausal women analyzing the effects of specific collagen peptides (SCP) vs. control on BMD and bone formation. SCP consumption increased BMD in postmenopausal women with primary, age-related reduction of BMD. Additionally, SCP supplementation has been associated with changes in bone structure indicating increased bone formation and decreased bone loss.[18] Elam *et al*. reported on the efficacy of long-term calcium collagen chelates in mitigating bone loss in postmenopausal women with osteopenia. Thirty-nine women were divided into two groups: one received 5 grams of calcium collagen chelate, providing 500 mg of elemental calcium and 200 IU of vitamin D (1,25-dihydroxyvitamin D3), while the control group received 500 mg of calcium. Bone mineral density (BMD) was measured using DEXA scan at the start of the study, and then at 6 and 12 months. At 12 months, women receiving CC had significantly reduced total body BMD loss compared to the control group (CC: ≤1.33% and ≤0.33% and control group: ≤3.75% and ≤2.17); 035 a.).[19] These findings endorse the use of calcium collagen chelate (CC)

as an effective measure to decrease bone loss in postmenopausal women with osteopenia.

CONCLUSION

These findings reflected decreased PINP levels after 3 months of using calcium, vitamin D, and collagen peptide medication. Adding collagen peptides to calcium and vitamin D supplements may increase their beneficial effects on bone metabolism.

LIMITATIONS

The study had several limitations, including a small sample size, and short follow-up duration, which limit the statistical power and generalizability of findings. Additionally, the study lacked controls for dietary and lifestyle factors that could influence bone health and did not assess other markers of bone health beyond bone mineral density.

SUGGESTIONS / RECOMMENDATIONS

Future research should focus on the proper dosage and the results of long-term treatment, which can frequently be required to prevent osteopenia and osteoporosis.

CONFLICT OF INTEREST / DISCLOSURE

There was no conflict of interest.

FUNDING SOURCE

No funding was provided; the authors arranged it themselves.

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REFERENCES

1. 1. Kurabayashi T, Nagai K, Morikawa K, Kamimura N, Yanase T, Hayashi K. Prevalence of osteoporosis and osteopenia assessed by densitometry in Japanese puerperal women. J Obstet Gynaecol Res. 2021 Apr;47(4):1388-1396.
2. Reid IR, Horne AM, Mihov B, Stewart A, Garratt E, Wong S, et al. Fracture prevention with zoledronate in older women with osteopenia. N Engl J Med. 2018 Dec 20;379(25):2407-2416.
3. Lorentzon M, Johansson H, Harvey NC, Liu E, Vandenput L, McCloskey EV, et al. Osteoporosis and fractures in women: the burden of disease. Climacteric. 2022 Jan;25(1):4-10.
4. Levin VA, Jiang X, Kagan R. Estrogen therapy for osteoporosis in the modern era. Osteoporos Int. 2018 May;29(5):1049-1055.
5. Cheng CH, Chen LR, Chen KH. Osteoporosis due to hormone imbalance: an overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. Int J Mol Sci. 2022 Jan 25;23(3):1376.
6. LeBoff MS, Greenspan SL, Insogna KL, Lewiecki EM, Saag KG, Singer AJ, et al. The clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int. 2022 Oct;33(10):2049-2102.
7. Varacallo MA, Fox EJ, Paul EM, Hassenbein SE, Warlow PM. Patients' response toward an automated orthopedic osteoporosis intervention program. Geriatr Orthop Surg Rehabil. 2013 Sep;4(3):89-98.
8. Falaschi P, Marques A, Giordano S. Osteoporosis and fragility in elderly patients. In: Orthogeriatrics: The management of older patients with fragility fractures. 2021:35-52.
9. Varacallo MA, Seaman TJ, Jandu JS, Pizzutillo P. Osteopenia. 2023 Aug 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
10. Ebeling PR. Vitamin D and osteoporosis. In: Feldman D, Pike JW, Bouillon R, editors. Vitamin D. 4th ed. Academic Press; 2024. p. 411-33.
11. Cao C, Xiao Z, Tong H, Liu Y, Wu Y, Ge C. Oral intake of chicken bone collagen peptides anti-skin aging in mice by regulating collagen degradation and synthesis, inhibiting inflammation and activating lysosomes. Nutrients. 2022 Apr 13;14(8):1622.
12. Zhang W, Chen Y, Liu Q, Zhou M, Wang K, Wang Y, et al. Emerging nanotherapeutics alleviating rheumatoid arthritis by readjusting the seeds and soils. J Control Release. 2022 May 1;345:851-79.
13. Liao W, Chen H, Jin W, Yang Z, Cao Y, Miao J. Three newly isolated calcium-chelating peptides from tilapia bone collagen hydrolysate enhance calcium absorption activity in intestinal Caco-2 cells. J Agric Food Chem. 2020 Jan 12;68(7):2091-8.
14. Zhu L, Xie Y, Wen B, Ye M, Liu Y, Imam KM, et al. Porcine bone collagen peptides promote osteoblast proliferation and differentiation by activating the PI3K/Akt signaling pathway. J Funct Foods. 2020 Jan 1;64:103697.
15. Zdzieblik D, Brame J, Oesser S, Gollhofer A, König D. The influence of specific bioactive collagen peptides on knee joint discomfort in young physically active adults: A randomized controlled trial. Nutrients. 2021 Feb 5;13(2):523.
16. Zdzieblik D, Oesser S, König D. Specific Bioactive Collagen Peptides in Osteopenia and Osteoporosis: Long-Term Observation in Postmenopausal Women. J Bone Metab. 2021 Aug;28(3):207.
17. Lampropoulou-Adamidou K, Karlafti E, Argyrou C, Makris K, Trovas G, Dontas IA, et al. Effect of calcium and vitamin D supplementation with and without collagen peptides on volumetric and areal bone mineral density, bone geometry and bone turnover in postmenopausal women with osteopenia. J Clin Densitom. 2022 Jul 1;25(3):357-72.
18. König D, Oesser S, Scharla S, Zdzieblik D, Gollhofer A. Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study. Nutrients. 2018 Jan 16;10(1):97.
19. Elam ML, Johnson SA, Hooshmand S, Feresin RG, Payton ME, Gu J, et al. A calcium-collagen chelate dietary supplement attenuates bone loss in postmenopausal women with osteopenia: a randomized controlled trial. J Med Food. 2015 Mar 1;18(3):324-31.