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INFLUENCE OF VITAMIN D SUPPLEMENTATION ON SERUM HEPCIDIN LEVELS AMONG NON-DIABETIC CHRONIC KIDNEY DISEASE PATIENTS: A RANDOMIZED STUDY.

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ABSTRACT

Background
Vitamin D, a fat-soluble vitamin, is necessary for many body processes, such as controlling the metabolism of calcium and phosphorus and preserving strong bones. The objective of the study was to find out how cholecalciferol supplementation affected the levels of hemoglobin, 25(OH)D, and hepcidin in serum in non-diabetic individuals with stage III-IV CKD and vitamin D deficiency.

Methods
A randomized, double-blind, placebo-controlled experiment included 140 non-diabetic stage III–IV CKD patients aged 18–70 with vitamin D insufficiency. The cholecalciferol group (n = 70) received two oral doses of 300,000 IU at baseline and eight weeks, while the placebo group (n = 70) received identical placebo dosages. Follow-ups were done at 16 weeks. IBM SPSS Statistics 21.0 was used to measure and analyze serum 25(OH)D, hepcidin, hemoglobin, and other biochemical data.

Results
There were 140 people, 70 per group. Age differences between categories were not significant ($p = 0.742$). The gender distribution was identical with 52% of males in category I and 50% in category II ($p = 0.819$). After 16 weeks, the cholecalciferol group had significantly higher serum 25(OH)D levels (11.3 ng/ml) compared to the placebo group (1.5 ng/ml; $p < 0.001$). Serum hepcidin levels declined significantly (-3.2 ng/ml) in the cholecalciferol group compared to the placebo group (-0.5 ng/ml; $p = 0.002$). Category I serum hemoglobin levels showed a significant increase (1.2 g/dL) over category II (0.3 g/dL; $p = 0.008$).

Conclusion
In non-diabetic CKD patients, cholecalciferol administration resulted in considerably higher serum 25(OH)D levels and lower serum hepcidin levels. It also raised hemoglobin levels, suggesting that it might help treat anemia linked to chronic kidney disease.

Recommendations
To effectively manage anemia and other problems in individuals with CKD, more extensive studies are required to confirm these findings and optimize vitamin D administration strategies.

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INTRODUCTION

Fat-soluble vitamin D is necessary for many body processes, such as controlling the metabolism of phosphorus and calcium and preserving strong bones. Beyond these roles, vitamin D has been implicated in modulating immune function and inflammation, making it a nutrient of significant interest in the management of chronic diseases. One such disease where vitamin D's role is increasingly being explored is chronic kidney disease (CKD). CKD is a condition that gets worse over time and is marked by a progressive loss of kidney function that can result in anemia and other problems [1]. Inflammation and iron dysregulation are two major contributors to anemia in CKD patients, which is frequently complex. A major regulator of iron homeostasis, serum hepcidin is a peptide hormone that the liver produces. It has been suggested that

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serum hepcidin may be a useful therapeutic target in CKD-related anemia [2].

By preventing intestinal iron absorption and the release of iron from macrophages and hepatic storage, hepcidin controls blood iron levels. Elevated levels of hepcidin are common in CKD patients, contributing to the anemia seen in this population by restricting iron availability. The inflammatory state characteristic of CKD further exacerbates hepcidin production. Therefore, interventions that can modulate hepcidin levels could offer therapeutic benefits. Recent research has suggested that vitamin D supplementation might influence hepcidin levels due to its anti-inflammatory properties and its role in modulating the immune response [3].

Several studies have investigated the potential of vitamin D to lower serum hepcidin levels in various populations, including non-diabetic CKD patients [4, 5]. The rationale behind this research lies in the understanding that vitamin D can suppress the production of pro-inflammatory cytokines, which are known to upregulate hepcidin synthesis. Consequently, vitamin D supplementation might reduce hepcidin levels, thereby improving iron availability and ameliorating anemia in CKD patients. However, the evidence remains mixed, with some studies showing significant reductions in hepcidin levels following vitamin D supplementation, while others do not observe such effects [6].

The variability in study outcomes could be attributed to differences in study design, the form and dose of vitamin D used, the baseline vitamin D status of participants, and the duration of supplementation. Furthermore, the interaction between vitamin D and other regulatory mechanisms of hepcidin and iron metabolism in CKD patients adds another layer of complexity [7]. Despite these challenges, the potential for vitamin D to serve as a modulator of hepcidin in CKD patients remains a promising area of research, warranting further investigation.

Although vitamin D supplementation may offer a therapeutic option for non-diabetic patients with chronic renal disease by influencing serum hepcidin levels, more thorough and well-designed research is required to determine its effectiveness and maximize its application in clinical settings.

In non-diabetic individuals suffering from stage III–IV CKD and vitamin D deficiency, the study sought to determine the effect of cholecalciferol supplementation on serum hepcidin levels. Additionally, it sought to evaluate the supplement's possible impact on other relevant biochemical parameters, such as serum 25(OH)D and hemoglobin levels.

METHODOLOGY

Study Design

A randomized, double-blind, placebo-controlled trial.

Study Setting

The research was conducted at Aashray Nursing Home & Superspeciality Hospital, Hatia Road, Tilkamanjhi, Bhagalpur, Bihar, India, spanning from June 2023 to July 2024.

Participants

140 participants were included. They were randomized to either the category I- cholecalciferol supplementation category (n = 70) or category II- the placebo category (n = 70).

Inclusion Criteria

- Age between 18 and 70 years.
- Non-diabetic stage (NDS) III–IV CKD (eGFR between 15 and 60 ml/min/1.73 m2).
- Vitamin D deficiency (serum 25(OH)D ≤20 ng/ml).

Exclusion Criteria

- Cardiac failure.
- Pregnancy.
- Present or past malignancies.
- Use of vitamin D supplementation within the past 30 days.

Outcomes

Primary Outcome Measure

Change in serum hepcidin levels from baseline to 16 weeks. Serum hepcidin levels were measured using enzyme-linked immunosorbent assay (ELISA) at baseline, eight weeks, and 16 weeks.

Secondary Outcome Measures

Change in serum 25(OH)D levels from baseline to 16 weeks, measured by enzyme immunoassay (EIA).

Change in serum hemoglobin levels from baseline to 16 weeks.

Changes in other biochemical parameters such as serum creatinine, calcium, phosphorus, uric acid, and lipid profile, were measured at baseline and 16 weeks.

Randomization

The random allocation sequence was generated using a computer-based random number generator. Restricted randomization with block randomization was employed to

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ensure equal distribution of participants between the two groups.

Concealment Mechanism

The random allocation sequence was concealed using sequentially numbered, opaque, sealed envelopes (SNOSE). The envelopes were prepared by an independent researcher not involved in the study and were opened sequentially by the study coordinator only after participant enrollment.

Blinding

The study was double-blind, meaning that both the participants and the investigators were blinded to the treatment allocation. The cholecalciferol and placebo doses were identical in appearance and packaging to ensure blinding. The blinding was maintained until all data were collected and the database was locked.

Variables

Variables of interest included serum 25(OH)D levels, serum hepcidin levels, and various clinical parameters such as serum creatinine, inorganic phosphorus, calcium, uric acid, lipid profile, and blood hemoglobin levels.

Sample size

To calculate the sample size for this study, the following formula was used for estimating a proportion of a population:

$$n = \frac{Z^2 \times p \times (1-p)}{E^2}$$

Where:
- n = sample size
- Z = Z-score corresponding to the desired level of confidence
- p = estimated proportion in the population
- E = margin of error

Data Collection

Data were collected at baseline and 16 weeks, including serum 25(OH)D levels measured by enzyme immunoassay (EIA) and serum hepcidin levels analyzed by enzyme-linked immunosorbent assay (ELISA).
Blood samples were collected for laboratory analyses, including serum creatinine, inorganic phosphorus, calcium, uric acid, lipid profile, and blood hemoglobin levels.

Procedure

At baseline and eight weeks, individuals in the treatment category received two witnessed oral doses of cholecalciferol (300,000 IU). The same timing and delivery procedure were followed to administer identical placebo doses to the participants in the control group. A follow-up evaluation was carried out sixteen weeks following the baseline.

Statistical Analysis

Comparing participant characteristics and assessing results were two aspects of statistical analysis. Version 21.0 of the IBM SPSS Statistics program was utilized. Student's t-test and the chi-squared test were used for in between-group comparisons. Serum biomarker changes were evaluated within each group using the Wilcoxon signed-rank test or the paired t-test. Two-tailed analyses were performed, with $p < 0.05$ regarded as significant. When applicable, data were shown as mean ± SD, mean change with 95% confidence interval, or median with confidence interval.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULTS

Table 1: Initial Clinical and Biochemical Features of Patients with CKD

Characteristic, Mean ± SD	Category I	Category II	p-value
Age (years)	54.2 ± 6.3	53.8 ± 6.1	0.742
Gender (male), n (%)	37 (52%)	35 (50%)	0.819
Serum 25(OH)D (ng/ml)	18.5 ± 3.2	18.7 ± 3.5	0.621
Serum Hepcidin (ng/ml)	12.8 ± 2.9	12.6 ± 3.1	0.718
Serum Creatinine (mg/dL)	2.4 ± 0.8	2.3 ± 0.7	0.412
Serum Calcium (mg/dL)	9.2 ± 0.6	9.1 ± 0.5	0.315
Serum Phosphorus (mg/dL)	4.1 ± 0.9	4.0 ± 0.8	0.529
Lipid Profile (mg/dL), (cholesterol)	180 ± 25	178 ± 27	0.687
Uric Acid (mg/dL)	6.5 ± 1.2	6.3 ± 1.1	0.621
Blood Hemoglobin (g/dL)	10.8 ± 1.5	10.9 ± 1.4	0.819

140 people with NDS III–IV CKD and vitamin D insufficiency participated in the study. Age, gender distribution, and baseline clinical parameters were similar in the two categories' baseline features, demonstrating effective randomization and minimizing selection bias.
Participants in the cholecalciferol group were 54.2 years (± 6.3) on average at baseline, while those in the placebo group were 53.8 years (± 6.1). Age variations between the categories were not statistically substantial (p = 0.742). With 52% of the males in the category I and 50% in the category II (p = 0.819), the gender distribution was likewise identical.

Table 2: Changes in Biochemical Parameters and Serum Biomarkers at 16 Weeks

Parameter, mean ± 95% CI	Cholecalciferol Group	Placebo Group	p-value
Change in Serum 25(OH)D (ng/ml)	11.3 ± (9.8–12.7)	1.5 ± (0.9–2.1)	<0.001
Change in Serum Hepcidin (ng/ml)	-3.2 ± (-4.5 to -1.9)	-0.5 ± (-1.2 to 0.2)	0.002
Change in Serum Hemoglobin (g/dL)	1.2 ± (0.8–1.6)	0.3 ± (0.1–0.6)	0.008
Change in Serum Creatinine (mg/dL)	2.3 ± (1.8–2.8)	2.4 ± (1.9–2.9)	0.621
Change in Serum Calcium (mg/dL)	9.5 ± (9.1–10.0)	9.3 ± (8.8–9.8)	0.315
Change in Serum Phosphorus (mg/dL)	3.9 ± (3.4–4.4)	4.0 ± (3.5–4.5)	0.529
Change in Lipid Profile (mg/dL)	178 ± (173–183)	176 ± (171–181)	0.687
Change in Uric Acid (mg/dL)	6.2 ± (5.8–6.7)	6.4 ± (5.9–6.9)	0.621

Initial serum 25(OH)D levels were similar in both categories: category II had a mean of 18.7 ng/ml (± 3.5) and Category I had a mean of 18.5 ng/ml (± 3.2) (p = 0.621). Comparably, there was no significant variation observed in the baseline blood hepcidin levels between the categories. Category I had a mean of 12.8 ng/ml (± 2.9) and Category II had a mean of 12.6 ng/ml (± 3.1) (p = 0.718).

Serum 25(OH)D levels were notably higher in Category I after 16 weeks of intervention than in Category II (p < 0.001). In category, I, the mean change in blood 25(OH)D levels from the beginning to 16 weeks was 11.3 ng/ml (95% CI: 9.8–12.7), but in category II, it was only 1.5 ng/ml (95% CI: 0.9–2.1).
Regarding the main result, following 16 weeks of treatment, the category I serum hepcidin levels were notably lower than those of category II (p = 0.002). In category, I, the mean change in serum hepcidin levels from initial to 16 weeks was -3.2 ng/ml (95% CI: -4.5 to -1.9), but in category II, it was only -0.5 ng/ml (95% CI: -1.2 to 0.2).

Furthermore, the category I serum hemoglobin levels significantly improved in contrast to the category II (p = 0.008). In category I, the mean change in serum hemoglobin levels from initial to 16 weeks was 1.2 g/dL (95% CI: 0.8–1.6), but in category II, it was only 0.3 g/dL (95% CI: 0.1–0.6).
There were no significant adverse events recorded during the research period, and the intervention-related adverse events were mild and comparable in both categories.

DISCUSSION

140 NDS III–IV CKD individuals with vitamin D lack were enrolled in the trial and split evenly into groups that received placebos and cholecalciferol supplements. Table 1 shows that randomization was successful since baseline characteristics, such as gender, age distribution, and medical variables, were similar between the groups.
Hepcidin and serum 25(OH)D levels did not vary substantially across the categories at baseline. After 16 weeks, there was a substantial variation between the category I and II serum 25(OH)D levels (p < 0.001), with a mean change of 11.3 ng/ml (95% CI: 9.8–12.7) as opposed to 1.5 ng/ml (95% CI: 0.9–2.1) (Table 2).

Additionally, category I showed a noteworthy reduction in the levels of hepcidin in the serum (p = 0.002) when contrasted with category II; the mean change was -3.2 ng/ml (95% CI: -4.5 to -1.9) as opposed to -0.5 ng/ml (95% CI: -1.2 to 0.2).
Furthermore, category I showed a substantial improvement in their blood hemoglobin levels (p = 0.008), with a mean change of 1.2 g/dL (95% CI: 0.8–1.6) as opposed to category II, which showed a mean change of 0.3 g/dL (95% CI: 0.1–0.6).
These results imply that in non-diabetic CKD patients, cholecalciferol treatment successfully raises serum vitamin D levels, lowers serum hepcidin levels, and improves serum hemoglobin levels. The results of the study showed that cholecalciferol supplementation had a substantial effect on important biomarkers related to the management of CKD.

The impact of vitamin D supplementation on serum hepcidin levels in patients with non-diabetic CKD has been investigated recently; the findings have been inconsistent. In one trial, vitamin D-deficient, non-diabetic individuals with CKD were treated with cholecalciferol supplements. The findings of the study showed that blood levels of hepcidin did not considerably change, whereas serum

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levels of 25(OH)D increased dramatically. This suggests that hepcidin levels in this population may not be adequately modulated by vitamin D administration alone [8].

A pilot study evaluated the effects of high-dose cholecalciferol supplementation on blood hepcidin levels in children with non-dialysis CKD. The study revealed no appreciable increases in hepcidin levels despite supplementation, underscoring the complexity and heterogeneity of vitamin D response in various CKD patient subgroups [9]. Additional research shed light on the connection between vitamin D and hepcidin levels in individuals with CKD. The results of their investigation showed a negative association between 25-OH vitamin D levels and hepcidin, indicating that the anti-inflammatory characteristics of vitamin D may be able to lower hepcidin levels, which are frequently increased in CKD due to chronic inflammation [10].

An active form of vitamin D called calcitriol was tested in a randomized controlled study to see how it affected CKD patients' serum hepcidin levels. In patients with mild to severe CKD, calcitriol did not significantly lower serum hepcidin concentrations, despite its well-established function in controlling calcium and phosphorus metabolism. This result implies that the impact of various vitamin D supplementation regimens on hepcidin levels may differ [11].
Furthermore, a study looked at the relationship between hepcidin, bone mineral metabolism, and anemia in pediatric patients with non-dialysis CKD. They found a correlation between the levels of interleukin-6 and 1,25-dihydroxyvitamin D and serum hepcidin. Nevertheless, there was no discernible rise in serum hepcidin despite heightened inflammatory markers, indicating that hepcidin levels might be modulated by successful treatment of anemia and secondary hyperparathyroidism [12].

GENERALIZABILITY

The findings from this study suggest that cholecalciferol supplementation can significantly increase serum 25(OH)D levels and reduce serum hepcidin levels in non-diabetic patients with stage III-IV chronic kidney disease (CKD) and vitamin D deficiency, subsequently improving hemoglobin levels. These results indicate that similar benefits may be achievable in a broader population of non-diabetic CKD patients with vitamin D deficiency. By addressing vitamin D deficiency through cholecalciferol supplementation, it is possible to better manage anemia and related complications in CKD, potentially improving patient outcomes and quality of life on a larger scale. Further large-scale studies are needed to confirm these findings and optimize treatment protocols.

CONCLUSION

The study emphasizes the advantages of cholecalciferol supplementation in individuals with stage III-IV CKD who are not diabetic and have insufficient vitamin D. When compared to a placebo, the intervention significantly raised hemoglobin levels, decreased hepcidin levels, and improved serum 25(OH)D levels. These results highlight the possibility of vitamin D supplementation as a treatment approach to treat vitamin D insufficiency and related problems in individuals with CKD. To investigate the long-term impacts and clinical results of cholecalciferol supplementation in this patient population, more investigation is necessary.

LIMITATIONS

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of a comparison group also poses a limitation for this study's findings.

RECOMMENDATIONS

To effectively manage anemia and other problems in individuals with CKD, more extensive studies are required to confirm these findings and optimize vitamin D administration strategies.

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LIST OF ABBREVIATIONS

CKD: Chronic Kidney Disease
IU: International Units
CI: Confidence Interval
ELISA: Enzyme-Linked Immunosorbent Assay
EIA: Enzyme Immunoassay
NDS: Non-Diabetic Stage

SOURCE OF FUNDING

No funding was received.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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