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Association of Vitamin D and Serum Calcium Status with Recurrent Low Back Pain in Patients with Normal Radiological Findings: A Case-Control Study

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Abstract

Background

Low back pain is a common and disabling musculoskeletal condition. Although structural abnormalities are frequently investigated using radiological imaging, a substantial proportion of patients experience recurrent symptoms despite apparently normal radiological findings. Vitamin D and calcium have important roles in bone mineralization, calcium homeostasis, neuromuscular function and musculoskeletal health. Their contribution to recurrent low back pain in patients without demonstrable radiological abnormalities remains clinically relevant, particularly in populations at increased risk of vitamin D deficiency.

Objective

To assess and compare serum 25-hydroxyvitamin D and serum calcium concentrations among patients with recurrent low back pain and normal lumbosacral radiographs and age- and sex-matched healthy controls.

Methods

A prospective observational case-control study was conducted at Habsara Wellness Centre from 15 December 2024 to 15 June 2025 under the academic affiliation of Guru Kashi University. The study included 375 participants, comprising 125 patients with recurrent low back pain and 250 age- and sex-matched healthy controls. Cases were adults aged 20 to 60 years with recurrent low back pain and normal lumbosacral radiographs. Participants with metabolic bone disease, malignancy, spinal trauma, autoimmune disease, infection, pregnancy or lactation, recent calcium or vitamin D supplementation, and selected disorders affecting calcium metabolism were excluded. Serum 25-hydroxyvitamin D and calcium were measured using automated laboratory methods. Vitamin D deficiency was defined as a serum 25-hydroxyvitamin D concentration below 20 ng/mL. Group comparisons were performed using the statistical methods specified in the dissertation, with statistical significance defined as $p < 0.05$.

Results

Vitamin D deficiency was substantially more frequent among patients with recurrent low back pain than among controls. Among female patients, 72% had vitamin D concentrations below 20 ng/mL, compared with 18% of female controls. Among male patients, 65% were reported to be deficient compared with 15% of male controls. The reported differences were highly significant ($p < 0.001$). Mean serum vitamin D concentrations were 14.2 ± 4.5 ng/mL among female patients and 19.5 ± 5.8 ng/mL among male patients, compared with 34.5 ± 6.2 ng/mL and 36.8 ± 5.9 ng/mL among female and male controls, respectively. Serum calcium concentrations were also lower among patients. Female patients had a reported mean calcium concentration of 8.6 ± 0.4 mg/dL and male patients 9.1 ± 0.3 mg/dL, compared with 9.6 ± 0.5 mg/dL and 9.4 ± 0.3 mg/dL among female and male controls, respectively. The reported difference in calcium between groups was statistically significant ($p < 0.01$). A moderate inverse

correlation between vitamin D and CRP was reported in the overall dissertation analysis, although inflammatory markers are outside the primary scope of the present manuscript.

Conclusion

Patients with recurrent low back pain and normal radiological findings demonstrated substantially lower serum vitamin D concentrations and lower serum calcium concentrations than healthy controls. The findings support an association between vitamin D deficiency, lower serum calcium status and recurrent low back pain in this study population. Further longitudinal and interventional studies incorporating parathyroid hormone, bone mineral density, dietary intake, sunlight exposure and standardized pain assessment are required to determine causality and clarify the clinical significance of these biochemical abnormalities.

1. Introduction

Low back pain is one of the most common musculoskeletal conditions worldwide and represents a major cause of disability, reduced productivity and healthcare utilization. It is a heterogeneous clinical syndrome involving mechanical, degenerative, inflammatory, metabolic, psychological and lifestyle-related factors. Although imaging is frequently used to investigate persistent symptoms, radiological abnormalities do not always explain the presence or severity of pain. A considerable proportion of patients are therefore classified as having nonspecific low back pain when no clear structural explanation is identified.

The absence of demonstrable structural pathology does not necessarily indicate the absence of biological abnormalities. Increasing attention has been directed toward metabolic and nutritional factors that may influence musculoskeletal function and pain. Among these, vitamin D has attracted considerable interest because of its established role in calcium homeostasis, skeletal mineralization and neuromuscular function. Vitamin D receptors are expressed in several tissues relevant to musculoskeletal health, and vitamin D deficiency has been associated with muscle weakness and various forms of musculoskeletal pain.

Vitamin D facilitates intestinal calcium absorption and contributes to the maintenance of normal calcium concentrations and bone mineralization. Persistent deficiency can adversely affect skeletal and neuromuscular function. In severe deficiency states, impaired mineralization may lead to osteomalacia, while less severe deficiency may be associated with nonspecific musculoskeletal symptoms.

Vitamin D deficiency is common in South Asian populations. India has a substantial burden of hypovitaminosis D despite abundant sunlight, with multiple environmental, behavioral and dietary factors contributing to inadequate vitamin D status. The dissertation population was particularly relevant in this context because the study was conducted in Kashmir, a region characterized by seasonal variation in sunlight exposure and dietary and lifestyle factors that may influence vitamin D status.

Serum calcium is closely linked to vitamin D metabolism. Although serum calcium is tightly regulated and may remain within conventional reference intervals despite alterations in vitamin D status, relatively lower calcium concentrations may accompany vitamin D deficiency. Calcium is also essential for muscle contraction and neuromuscular signaling, providing a biologically plausible pathway through which altered mineral status could contribute to musculoskeletal symptoms.

Previous studies have examined the association between vitamin D and nonspecific low back pain, but findings have not been entirely consistent across populations. Differences in study design, vitamin D thresholds, patient characteristics, geographical location and assessment of confounding factors may contribute to variation in reported associations. The dissertation therefore focused on a clinically relevant subgroup, namely patients with recurrent low back pain in whom routine radiological assessment did not identify an obvious structural abnormality.

The present manuscript focuses specifically on the biochemical component of the original dissertation, namely serum vitamin D and calcium status. The hematological and inflammatory findings are reserved for a separate manuscript.

2. Aim

The aim of this study was to assess serum 25-hydroxyvitamin D and serum calcium concentrations in patients with recurrent low back pain and normal radiological findings and to compare these parameters with those of healthy controls.

3. Objectives

Primary Objective

To assess and compare serum calcium and vitamin D concentrations in patients with recurrent low back pain and normal radiological findings and age- and sex-matched healthy controls.

Secondary Objective

To examine differences in serum vitamin D and calcium concentrations according to sex among patients and controls.

4. Materials and Methods

4.1 Study Design and Setting

This was a prospective observational case-control study conducted over six months, from 15 December 2024 to 15 June 2025, at Habsara Wellness Centre. The study was conducted under the academic affiliation of the Department of Health and Allied Sciences, Guru Kashi University. Ethical approval was obtained from the Institutional Ethics Committee of Guru Kashi University.

4.2 Study Population

The study comprised 375 participants:

- 125 patients with recurrent low back pain, designated as the case group.
- 250 healthy individuals without chronic musculoskeletal complaints, designated as the control group.

Controls were age and sex matched to the case population. Cases were adults between 20 and 60 years of age with recurrent low back pain and normal lumbosacral radiographs.

4.3 Eligibility Criteria

Inclusion criteria for cases

Participants were eligible if they:

1. Were aged 20 to 60 years.

2. Had recurrent low back pain.

3. Had normal lumbosacral radiographs.

4. Were willing to participate and provide informed consent.

Inclusion criteria for controls

Controls were healthy individuals without chronic musculoskeletal pain and were age and sex matched to cases.

Exclusion criteria

Participants were excluded if they had:

• Diagnosed metabolic bone disease, including osteoporosis or osteomalacia.

• Spinal trauma.

• Malignancy.

• Autoimmune disease.

• Infection.

• Pregnancy or lactation.

• Recent calcium or vitamin D supplementation.

• Known thyroid, renal or liver disorders affecting calcium metabolism.

4.4 Sample Collection

Venous blood samples were collected under aseptic conditions using standard BD Vacutainer systems. Five milliliters of venous blood were collected from each participant. Three milliliters were collected into plain tubes for serum-based investigations, including vitamin D and calcium estimation.

4.5 Laboratory Analysis

Serum vitamin D was assessed as 25-hydroxyvitamin D using the Agappe Mispa i3 automated system. The dissertation describes the assay as based on an immunoturbidimetric approach.

Serum calcium was estimated using the Meril CliniQuant fully automated biochemistry analyzer using the Arsenazo III colorimetric method. In this method, calcium reacts with Arsenazo III to form a colored complex, with the measured optical signal corresponding to the calcium concentration.

The dissertation reports that laboratory instruments were calibrated weekly and internal quality control was performed daily using control samples.

4.6 Definition of Vitamin D Deficiency

For the study, serum 25-hydroxyvitamin D below 20 ng/mL was considered deficient, 20 to 30 ng/mL was considered insufficient and concentrations above 30 ng/mL were considered sufficient.

4.7 Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 23.0. Continuous variables were expressed as mean ± standard deviation. The dissertation specifies independent-samples t-tests for quantitative comparisons and chi-square testing for categorical variables. Pearson correlation was specified for relationships between continuous variables, while the statistical notes indicate that Mann-Whitney U and Spearman correlation were used when distributional assumptions were not met. Statistical significance was defined as $p < 0.05$.

4.8 Ethical Considerations

Ethical approval

was obtained from the Institutional Ethics Committee of Guru Kashi University. Written informed consent was obtained from all participants, and confidentiality of participant information was maintained.

5. Results

5.1 Study Population

A total of 375 participants were included in the study, comprising 125 patients with recurrent low back pain and 250 healthy controls. Among the 125 patients, 74 were female and 51 were male, corresponding to approximately 59% and 41%, respectively. The majority of participants were reported to belong to the 30 to 50 year age range.

Table 1
Sex distribution of study participants

Group	Female	Male	Total
Recurrent LBP cases	74	51	125
Healthy controls	Age and sex matched	Age and sex matched	250

5.2 Serum Vitamin D Concentrations

A marked difference in vitamin D status was observed between patients and controls.

Among female patients, 72% were reported to have serum 25-hydroxyvitamin D concentrations below 20 ng/mL. The mean concentration was 14.2 ± 4.5 ng/mL.

Among male patients, 65% were reported to have vitamin D deficiency, with a mean concentration of 19.5 ± 5.8 ng/mL.

In comparison, vitamin D deficiency was reported in 18% of female controls and 15% of male controls. Mean concentrations were 34.5 ± 6.2 ng/mL among female controls and 36.8 ± 5.9 ng/mL among male controls. The difference between patients and controls was reported as highly statistically significant, $p < 0.001$.

Table 2
Serum vitamin D concentrations according to sex

Parameter	Female LBP patients	Male LBP patients	Female controls	Male controls
Vitamin D, ng/mL, mean ± SD	14.2 ± 4.5	19.5 ± 5.8	34.5 ± 6.2	36.8 ± 5.9
Vitamin D deficiency	72%	65%	18%	15%

Between-group comparison

$p < 0.001$.

5.3 Serum Calcium Concentrations

Serum calcium concentrations were lower among patients with recurrent low back pain than among controls.

Female patients had a mean serum calcium concentration of 8.6 ± 0.4 mg/dL, while male patients had a mean concentration of 9.1 ± 0.3 mg/dL. Female controls had a mean concentration of 9.6 ± 0.5 mg/dL and male controls 9.4 ± 0.3 mg/dL.

The dissertation reports that the intergroup difference in serum calcium was statistically significant, with $p < 0.01$.

Table 3
Serum calcium concentrations according to sex

Parameter	Female LBP patients	Male LBP patients	Female controls	Male controls
Calcium, mg/dL, mean ± SD	8.6 ± 0.4	9.1 ± 0.3	9.6 ± 0.5	9.4 ± 0.3

Between-group comparison

$p < 0.01$.

5.4 Overall Biochemical Comparison

The dissertation provides an overall summary of the principal biochemical findings, reporting mean serum vitamin D of 16.8 ± 5.1 ng/mL among patients compared with 35.4 ± 6.0 ng/mL among controls. Mean serum calcium was reported as 8.8 ± 0.5 mg/dL among patients and 9.5 ± 0.4 mg/dL among controls.

Table 4
Overall comparison of vitamin D and calcium

Parameter	LBP patients, n = 125	Controls, n = 250	p-value
Vitamin D, ng/mL	16.8 ± 5.1	35.4 ± 6.0	< 0.001
Calcium, mg/dL	8.8 ± 0.5	9.5 ± 0.4	< 0.01

5.5 Vitamin D and Calcium Relationship

The study interpreted the lower calcium concentrations observed among patients in the context of the substantially lower vitamin D concentrations. The dissertation proposes that reduced vitamin D status may contribute to reduced intestinal calcium absorption and altered musculoskeletal function.

However, the present study design does not establish that vitamin D deficiency causes low back pain or that lower calcium concentrations directly cause pain. The observed findings should therefore be interpreted as associations.

6. Discussion

The present case-control study examined serum vitamin D and calcium concentrations among patients with recurrent low back pain despite normal radiological findings. The principal finding was a substantially higher prevalence of vitamin D deficiency among patients compared with healthy controls. Serum calcium concentrations were also lower among patients, although the reported values generally remained within or close to conventional laboratory reference intervals.

The vitamin D findings were particularly striking. The mean vitamin D concentration among female patients was 14.2 ng/mL, while the corresponding value among male patients was 19.5 ng/mL. Both values were below the study's deficiency threshold of 20 ng/mL. In contrast, mean vitamin D concentrations among controls were substantially higher. The reported prevalence of deficiency was 72% among female patients and 65% among male patients, compared with 18% and 15% among female and male controls, respectively.

These observations are consistent with the biological importance of vitamin D in musculoskeletal health. Vitamin D participates in calcium absorption, skeletal mineralization and neuromuscular function. Vitamin D receptors are also expressed in skeletal muscle and other tissues relevant to pain biology. Vitamin D deficiency may therefore plausibly contribute to musculoskeletal symptoms through several mechanisms, including impaired mineral homeostasis and altered neuromuscular function.

The findings are also relevant to the Indian context. Vitamin D deficiency is widely recognized in India despite substantial sunlight availability. Factors affecting vitamin D status include season, duration of sun exposure, clothing, dietary intake and lifestyle. Previous work cited in the dissertation has specifically described seasonal vitamin D deficiency in Kashmir.

The lower vitamin D concentrations observed among female patients deserve particular attention. Female patients had a mean concentration of 14.2 ng/mL compared with 19.5 ng/mL among male patients. Several factors may potentially contribute to this difference, including differences in outdoor activity, sunlight exposure, dietary intake and hormonal status. However, these variables were not quantitatively assessed in the present study. Consequently, the sex difference should be regarded as an observed pattern rather than attributed to a specific causal mechanism.

Serum calcium was also lower among patients. Female patients had a mean concentration of 8.6 mg/dL compared with 9.6 mg/dL among female controls, while male patients had a mean concentration of 9.1 mg/dL compared with 9.4 mg/dL among male controls. The reported overall difference was statistically significant.

The relationship between vitamin D and calcium is biologically plausible because vitamin D promotes intestinal calcium absorption and contributes to maintenance of calcium balance. Nevertheless, serum calcium is tightly regulated and may remain within the conventional reference range even when vitamin D status is abnormal. Therefore, the lower calcium concentrations observed in this study should not automatically be classified as clinical hypocalcemia.

The dissertation describes these lower concentrations as "functional hypocalcemia." For a journal manuscript, however, this terminology should be used cautiously because functional hypocalcemia is not established solely by a low-normal total serum calcium concentration. Additional measurements, such as albumin-corrected calcium, ionized calcium, parathyroid hormone and phosphate, would provide a more complete assessment of calcium homeostasis.

The combination of low vitamin D and relatively lower calcium among patients with normal radiological findings raises an important clinical question. Patients whose radiographs do not demonstrate an obvious structural explanation for recurrent pain may nevertheless have potentially relevant metabolic abnormalities. Vitamin D assessment may therefore have value in selected patients, particularly in populations with a high background prevalence of deficiency.

However, the present findings should not be interpreted as evidence that vitamin D deficiency is the sole cause of recurrent low back pain. Low back pain is multifactorial, and vitamin D status may be associated with other factors that were not measured in this study. Physical activity, body composition, dietary calcium and vitamin D intake, sunlight exposure, socioeconomic status, occupational loading and psychological factors may all influence both vitamin D status and low back pain.

The study also did not incorporate standardized pain severity or disability instruments such as the Visual Analog Scale or Oswestry Disability Index. Consequently, although the study demonstrates biochemical differences between cases and controls, it cannot determine whether lower vitamin D or calcium concentrations were associated with greater pain intensity or functional disability. The dissertation itself identifies this as an important limitation.

Another important limitation is the absence of parathyroid hormone and bone mineral density measurements. These investigations would have helped distinguish simple variation in serum calcium from clinically meaningful disturbances in mineral metabolism.

Finally, because the study was observational and cross-sectional in nature, temporal sequence and causality cannot be established. It remains possible that recurrent pain and reduced physical activity influenced vitamin D status rather than vitamin D deficiency being responsible for the development of pain.

7. Clinical Implications

The findings suggest that vitamin D status may warrant consideration in selected patients with recurrent low back pain when routine radiological investigations do not identify a clear structural abnormality.

The particularly high prevalence of vitamin D deficiency observed among the cases supports the potential usefulness of biochemical assessment in populations with a high background prevalence of hypovitaminosis D.

However, the present study does not demonstrate that supplementation will necessarily relieve low back pain. Clinical treatment decisions should therefore be based on established indications for vitamin D and calcium supplementation rather than on the association demonstrated in this observational study.

8. Strengths

Several features strengthen the study.

First, the study included 375 participants, with a 1:2 case-to-control ratio. Second, controls were age and sex matched to cases. Third, the study focused specifically on patients with recurrent low back pain and normal radiological findings, a clinically relevant subgroup. Fourth, vitamin D and calcium were measured using laboratory-based analytical methods, and the dissertation reports regular calibration and internal quality control.

The study also provides sex-stratified biochemical findings, allowing differences between male and female participants to be described.

9. Limitations

The following limitations should be considered when interpreting the findings:

1. The observational design prevents determination of causality.

2. The study was conducted at a single center, limiting generalizability.

3. Standardized pain intensity and disability scores were not collected.

4. Dietary intake, physical activity and sunlight exposure were not quantitatively assessed.

5. Parathyroid hormone, phosphate, alkaline phosphatase and bone mineral density were not measured.

6. The study did not include longitudinal follow-up to determine whether correction of vitamin D deficiency influenced pain recurrence.

7. Potential confounding variables such as BMI and occupational physical loading were not comprehensively analyzed.

These limitations are consistent with those acknowledged in the original dissertation.

10. Conclusion

In this case-control study of patients with recurrent low back pain and normal radiological findings, serum vitamin D concentrations were substantially lower and vitamin D deficiency was considerably

more prevalent than among healthy controls. Serum calcium concentrations were also lower among patients, with the greatest difference observed among female participants.

The findings indicate an association between vitamin D deficiency, lower serum calcium status and recurrent low back pain in the studied population. They support consideration of metabolic and nutritional factors when evaluating patients with persistent or recurrent low back pain in whom radiological investigations do not provide an obvious structural explanation.

Nevertheless, the findings should be interpreted as associations rather than evidence of causation. Larger multicenter studies with standardized pain and disability measurements, dietary and lifestyle assessment, parathyroid hormone and bone mineral density measurements, and longitudinal follow-up are required to determine whether correction of vitamin D deficiency or altered calcium status improves clinical outcomes.

11. Declarations

Ethics Approval
The study received approval from the Institutional Ethics Committee of Guru Kashi University before commencement.

Consent

Written informed consent was obtained from all study participants.

Conflict of Interest

The authors declare that they do not have any conflict of interest in publishing this paper

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Data Availability

Data availability can be made upon the request.

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Figures

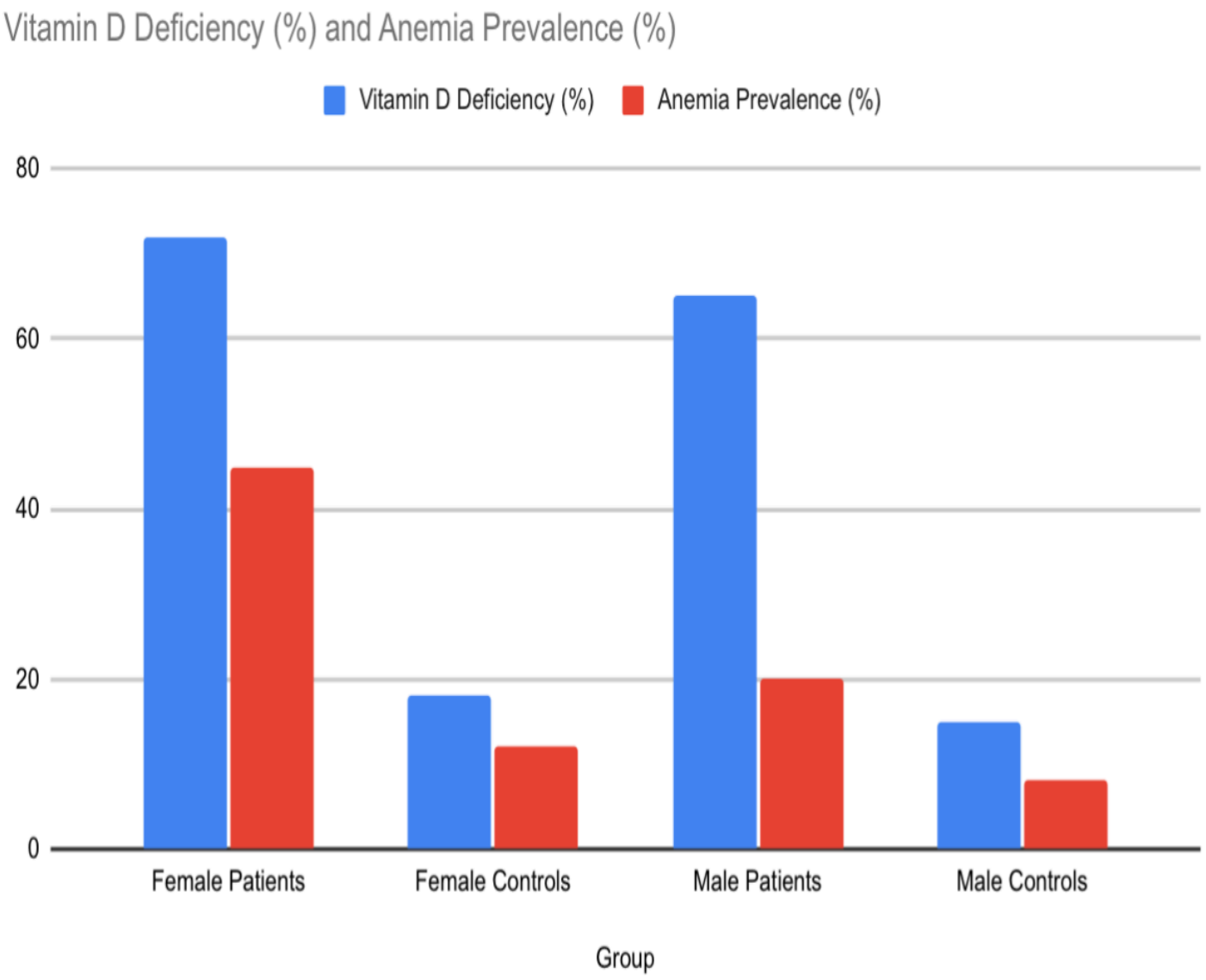


Figure 1

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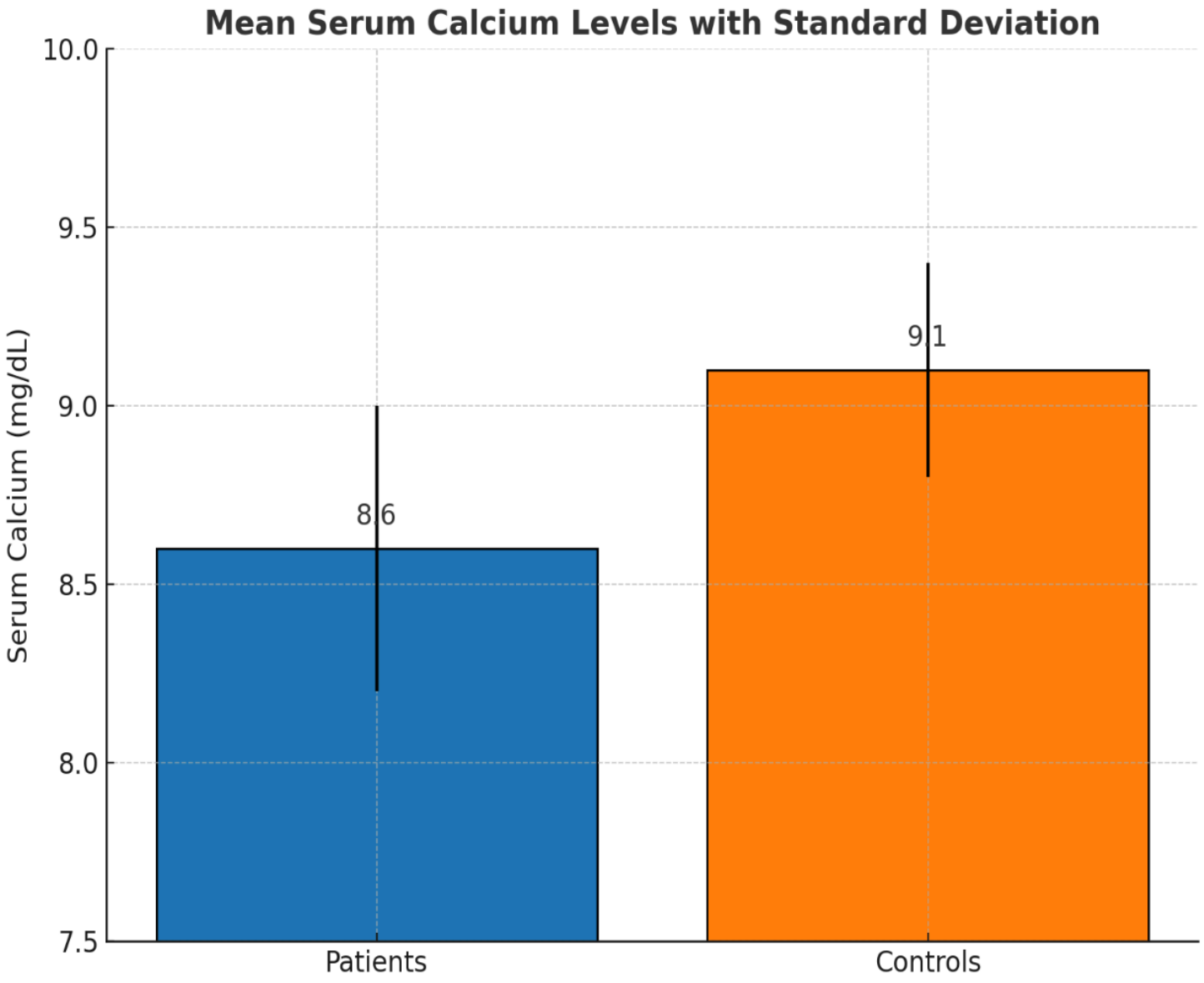


Figure 2

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