Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

ASSOCIATION BETWEEN CALCIUM AND VITAMIN D LEVELS IN PATIENTS WITH HEPATITIS C INFECTION

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DOI: https://doi.org/10.5281/zenodo.22302343

Keywords
Hepatitis C virus; Vitamin D deficiency; Serum calcium; Liver disease; Calcium homeostasis

Article History
Received: 31 January 2026
Accepted: 16 March 2026
Published: 30 March 2026

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Abstract

Background: *Hepatitis C virus (HCV) infection is a significant public health problem globally and nationally, particularly in Pakistan, where its prevalence remains high. Chronic HCV infection primarily affects the liver and may alter vitamin D metabolism, potentially affecting calcium homeostasis. This study was conducted to determine serum calcium and vitamin D levels in patients with HCV infection, compare these levels with healthy controls, and assess the association between serum calcium and vitamin D levels.*

Objective: *The objective of this study was to compare serum calcium and vitamin D levels between patients with Hepatitis C virus (HCV) infection and healthy controls and to determine the association between serum calcium and vitamin D levels among HCV-infected patients.*

Methods: *A comparative cross-sectional study was conducted among 242 participants from Peshawar, including 121 HCV-positive patients and 121 healthy controls. Serum calcium levels were measured using a photometric method, while serum vitamin D levels were determined using an immunofluorescence technique. Data were analyzed using SPSS version 27.*

Results: *HCV-positive patients had significantly lower serum calcium levels (9.07 ± 0.82 mg/dL) compared with healthy controls (9.29 ± 0.59 mg/dL; $p = 0.019$). Serum vitamin D levels were also significantly lower among HCV-positive patients compared with healthy controls ($p < 0.001$). A moderate positive correlation was observed between serum vitamin D and calcium levels among HCV patients ($r = 0.377$, $p < 0.001$).*

Conclusion: *HCV infection was associated with significantly lower serum calcium and vitamin D levels. The moderate positive correlation between vitamin D and calcium suggests that disturbances in vitamin D status may be associated with altered calcium homeostasis in HCV patients. Routine assessment of vitamin D and calcium status may help identify metabolic abnormalities early and support appropriate management to reduce potential complications.*

INTRODUCTION

Hepatitis C virus (HCV) infection remains a major global health concern and is an important cause of chronic liver disease (1). Persistent HCV infection can lead to progressive hepatic injury, fibrosis, cirrhosis, and hepatocellular carcinoma, contributing substantially to morbidity and mortality (2). The burden of HCV is particularly significant in developing countries, including Pakistan, where a considerable proportion of the

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

population has been exposed to the virus and many individuals continue to have active infection (3). The liver plays an essential role in numerous metabolic processes, including the metabolism and storage of nutrients and vitamins. Chronic HCV infection can impair normal hepatic function and may consequently affect the metabolism of several essential nutrients (4). Among these, vitamin D has gained considerable attention in patients with chronic liver disease. Vitamin D undergoes important metabolic conversion in the liver, and impaired hepatic function may therefore contribute to reduced circulating vitamin D levels. Several studies have reported a high prevalence of vitamin D deficiency among patients with HCV infection, suggesting a possible relationship between vitamin D status, hepatic disease, and immune function (5). Vitamin D is also essential for maintaining calcium homeostasis. It facilitates intestinal absorption of calcium and contributes to the maintenance of appropriate serum calcium concentrations. Therefore, inadequate vitamin D status may adversely affect calcium metabolism (6). In patients with chronic liver disease, disturbances in vitamin D metabolism may occur alongside abnormalities in calcium and parathyroid hormone regulation. Such disturbances may have important clinical consequences, particularly for skeletal health, and may contribute to conditions such as osteopenia and osteoporosis (7). The relationship between vitamin D and calcium may be particularly relevant in patients with HCV because chronic liver disease can influence nutrient metabolism in addition to its direct effects on hepatic function. Factors such as impaired vitamin D metabolism, inadequate nutritional intake, malabsorption, and reduced exposure to sunlight may further contribute to abnormalities in vitamin D and calcium status. Consequently, assessment of these biochemical parameters may provide useful information regarding metabolic alterations among individuals with chronic HCV infection (8). Although vitamin D deficiency has been widely investigated in patients with HCV, comparatively less attention has been given to the association between vitamin D and calcium levels in this population (9). Previous research has often examined vitamin D and calcium as separate biochemical parameters, while their interrelationship in HCV-infected individuals remains less clearly established (10). This represents an important research gap because vitamin D and calcium are physiologically interconnected and abnormalities in one may influence the other.

Therefore, the present study was designed to compare serum calcium and vitamin D levels between patients with HCV infection and healthy controls and to determine the association between serum calcium and vitamin D levels among patients with HCV infection (11). Understanding this relationship may contribute to a better assessment of metabolic abnormalities in HCV patients and may provide useful information for the early identification and management of nutritional and mineral disturbances associated with chronic liver disease.

METHODOLOGY

This comparative cross-sectional study was conducted at the Medical Laboratory Technology (MLT) facility of City University of Science and Information Technology (CUSIT), Peshawar. Ethical approval was obtained from the appropriate ethical review committee of CUSIT before commencement of data collection. The study objectives, blood sample collection procedure, laboratory investigations, and participants' right to withdraw from the study at any time without any charges were explained to all eligible participants. Written informed consent was obtained from each participant before enrollment. The study population comprised adults aged 18–55 years from Peshawar, including patients diagnosed with hepatitis C infection and healthy controls. Stratified random sampling was used to select participants for the two study groups according to the predefined inclusion and exclusion criteria until the required sample size was achieved. The sample size was calculated using RaoSoft based on a 95% confidence level, 5% margin of error, a population size of 4.76 million according to the 2023 census, and an HCV prevalence of 19.48% reported by Naz et al. (2019). Participants were included if they were aged 18–55 years and, for the case group, had a diagnosis of

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ISSN: (e) 3007-1607 (p) 3007-1593

HCV infection. Individuals with other liver diseases, including hepatitis B or liver cancer, were excluded.

Approximately 3–5 mL of venous blood was collected from each participant using a sterile disposable syringe and standard venipuncture technique. Blood samples were appropriately labeled, transferred into gel tubes, and allowed to clot before centrifugation. The separated serum was used for the estimation of calcium and vitamin D levels. Patients in the HCV group were confirmed on the basis of polymerase chain reaction (PCR) reports. Prior to biochemical analysis, PCR-confirmed samples were re-screened for HCV antibodies using a rapid immunochromatographic test (ICT), and only samples positive by both PCR records and ICT were included in the case group. Serum calcium concentration was measured using the photometric method on a Mindray BA-88A semi-automatic biochemistry analyzer according to the laboratory standard operating procedure and reagent-kit instructions. Serum vitamin D levels were determined using the i-CHROMA II immunofluorescence analyzer based on the competitive immunofluorescence principle. The primary study variables were HCV infection status, serum calcium level, and serum vitamin D level. HCV infection status was treated as the grouping variable, while calcium and vitamin D were analyzed as continuous biochemical variables.

Data was entered and analyzed using SPSS version 27. Descriptive statistics were used to summarize continuous variables, including age, serum calcium, and vitamin D levels, using mean, standard deviation, minimum, and maximum values. The normality of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The Independent Samples *t*-test was used to compare normally distributed continuous variables between HCV-positive patients and healthy controls, including age and serum calcium levels. Because serum vitamin D levels showed a non-normal distribution, the Mann–Whitney *U* test was used to compare vitamin D levels between the two groups. A *p*-value of <0.05 was considered statistically significant, while *p*-values of <0.01 were considered highly significant.

RESULTS

It is a comparative cross-sectional study, to evaluate the relationship between the levels of calcium and vitamin D in patients with Hepatitis C Virus (HCV) infection. These were divided into 242 individuals whose HCV status was known (121 individuals positive and 121 healthy control individuals). Laboratory tests had been conducted in order to determine the level of serum vitamin D and calcium. The chapter provides demographic profile of the participants, descriptive statistics of the biochemical parameters, comparison of the results between the groups, and correlation results.

4.1 Particulars of the participants selected according to demographic attributes.

There were 242 participants who were enrolled in the study with 121 participants each, i.e., as a case and control group. The average age of the participants was 43.00 + 9.42 years. The mean age among cases was 43.29 ± 9.29 years, while controls had a mean age of 42.71 ± 9.57 years. Independent sample t-test indicated that there was no statistically significant difference in the age distribution in cases and controls ($p = 0.634$), which shows that cases and controls are similar to each other.

In terms of gender distribution, 57.9% (n=140) make up of males and 42.1% (n=102) make up of females. There was no major gender difference between case and the control groups ($p = 1.000$).

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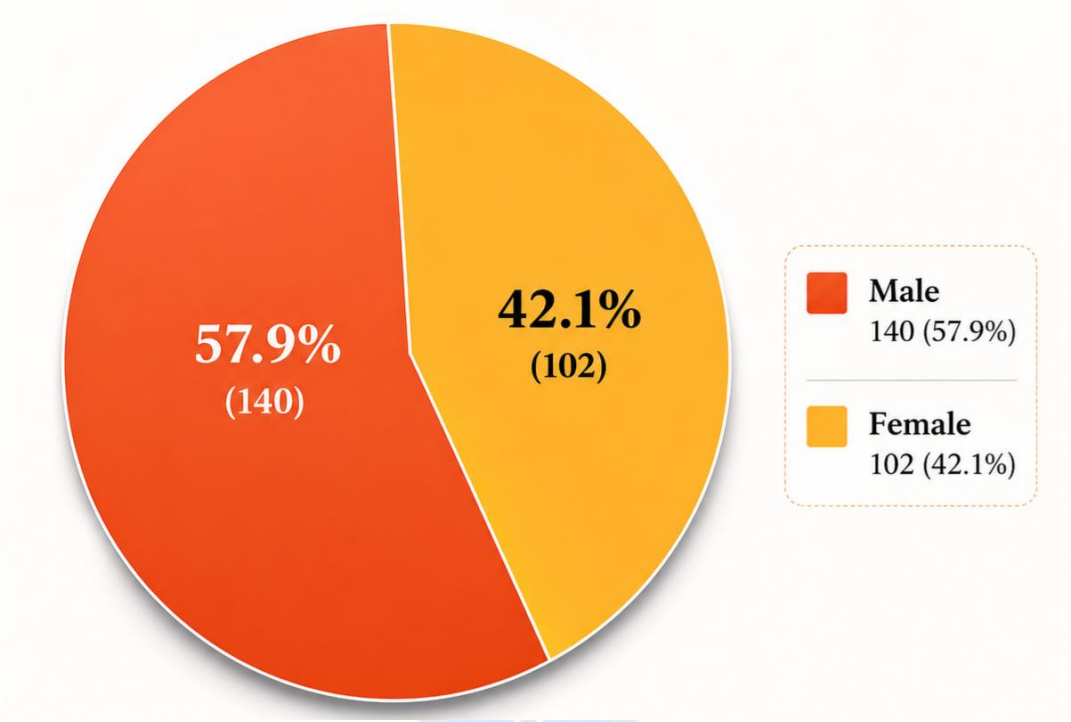


Figure 1 Gender distribution of study participants

4.2 Descriptive Analysis of Vitamin D and Calcium levels.

The mean serum vitamin D level amongst all the participants was 59.27. + 37.96 ng/dL, with the mean calcium level being 9.18 + 0.72mg/dL.

Table 1 Descriptive Statistics of the Study Variables.

Variable	Mean +SD	Minimum	maximum
Age	43.00 ± 9.42	23	55
Vitamin D	59.27 ± 37.96	6.14	100
Calcium	9.18 ± 0.72	7.6	10.3

4.3 Comparative Analysis of Cases vs. Controls.

4.3.1 Calcium Levels:

The distribution of calcium status differed between HCV patients and healthy controls. Among the 121 HCV patients, 30 (24.8%) were calcium deficient, while 91 (75.2%) had normal calcium levels. Among the 121 healthy controls, 13 (10.7%) were calcium deficient and 108 (89.3%) had normal calcium levels. In addition, the mean serum calcium level among HCV patients (9.07 ± 0.82 mg/dL) was significantly lower than that of the controls (9.29 ± 0.59 mg/dL). An independent samples t-test indicated that this difference was statistically significant ($p = 0.019$).

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ISSN: (e) 3007-1607 (p) 3007-1593

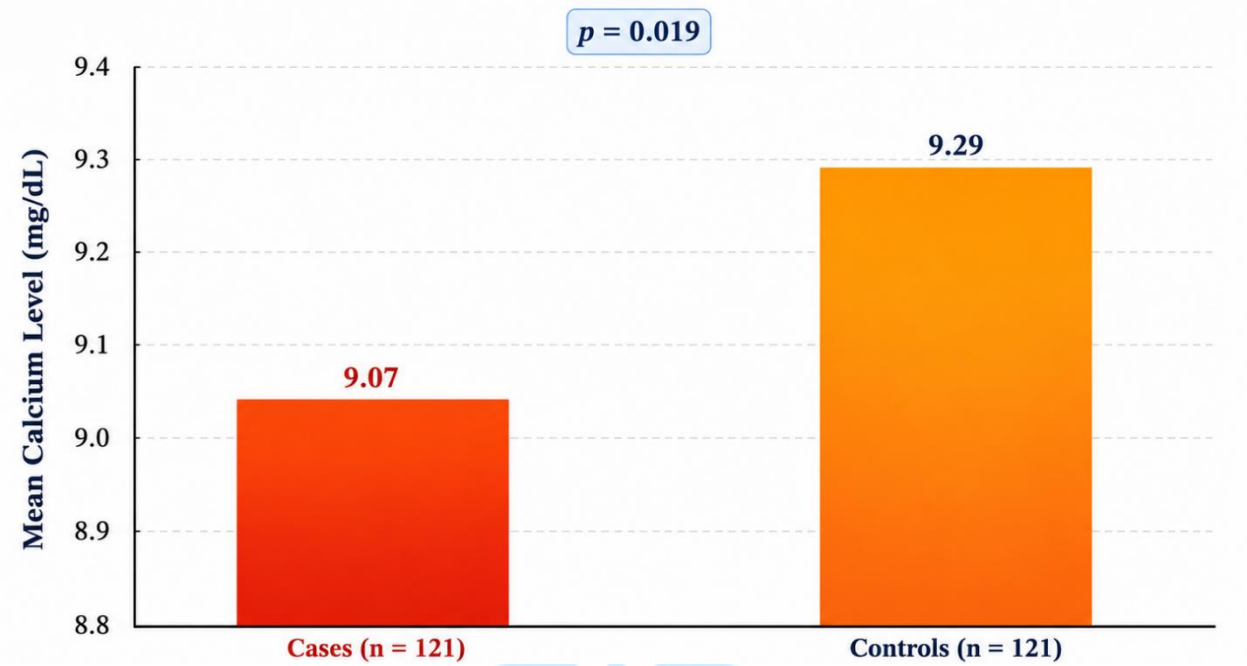


Figure 2 Comparison of mean calcium levels between cases and controls

4.3.2 Vitamin D Levels:

The distribution of vitamin D status among study participants showed a higher prevalence of deficiency in Hepatitis C patients compared to healthy controls. Among the 121 HCV patients, 70 (57.9%) were vitamin D deficient, 5 (4.1%) were insufficient, and 46 (38.0%) had sufficient vitamin D levels. In contrast, among the 121 healthy controls, none (0.0%) were vitamin D deficient, 4 (3.3%) were insufficient, and 117 (96.7%) had sufficient vitamin D levels. Furthermore, vitamin D data were not normally distributed, the Mann-Whitney U test was used. The test showed a highly significant difference between HCV patients and controls (U = 977, Z = -11.885, p < 0.001). The higher mean rank in controls (173.93) compared to cases (69.07) indicates that healthy controls had significantly higher Vitamin D levels than HCV patients.

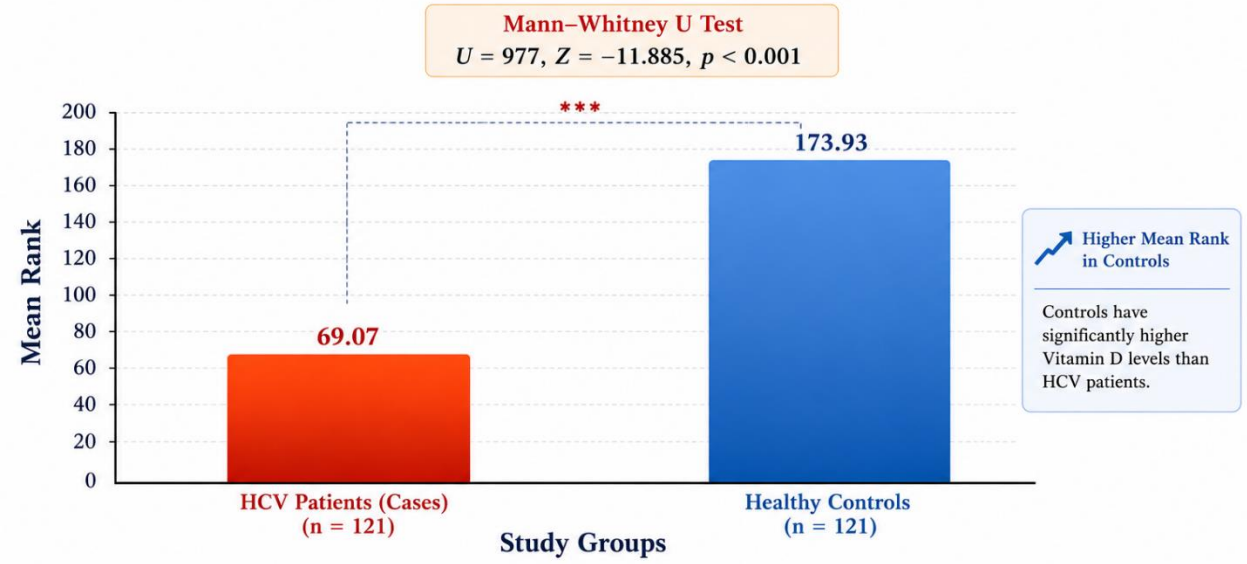


Figure 3 Comparison of mean vitamin D levels between cases and controls

Volume 4, Issue 3, 2026

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Table 2 Comparison of Cases and Controls of Ca and Vit D Levels.

Parameter	Cases	Controls	Statistical Test	P-value
Calcium	9.07 ± 0.82	9.29 ± 0.59	Independent t-test	0.019
Vitamin D	69.07	173.93	Mann-Whitney U test	<0.001

4.4 HCV Status Distribution

The case group was composed of all HCV positive participants and the control group comprised of all HCV negative participants in the case. Among the cases, 70 (57.9%) were males and 51 (42.1%) were females. Similarly, among the controls, 70 (57.9%) were males and 51 (42.1%) were females. An analysis using chi-square proved the existence of highly significant difference between groups ($p < 0.001$).

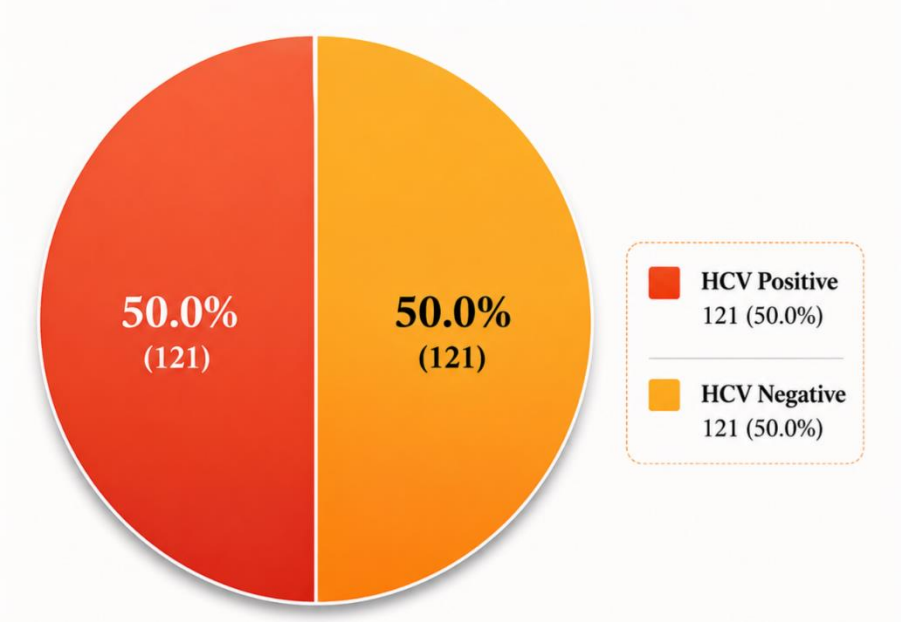


Figure 4 Distribution of HCV status among study participants

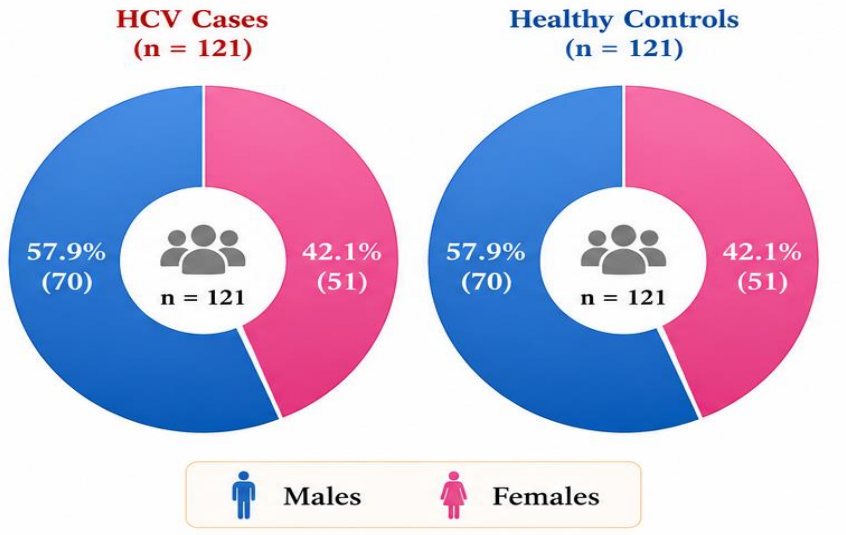


Figure 5 Distribution of Participants by gender in Cases and Controls

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4.5 Correlation Analysis

The analysis based on Spearman correlation showed:

- Significant positive correlation between the HCV infection and the lower level of vitamin D (r = -0.766, p < 0.001).
- Minor negative association between age and the level of vitamin D (r = -0.247, p < 0.001)
- Positive moderate correlation between the level of vitamin D and calcium (r = 0.377, p < 0.001).

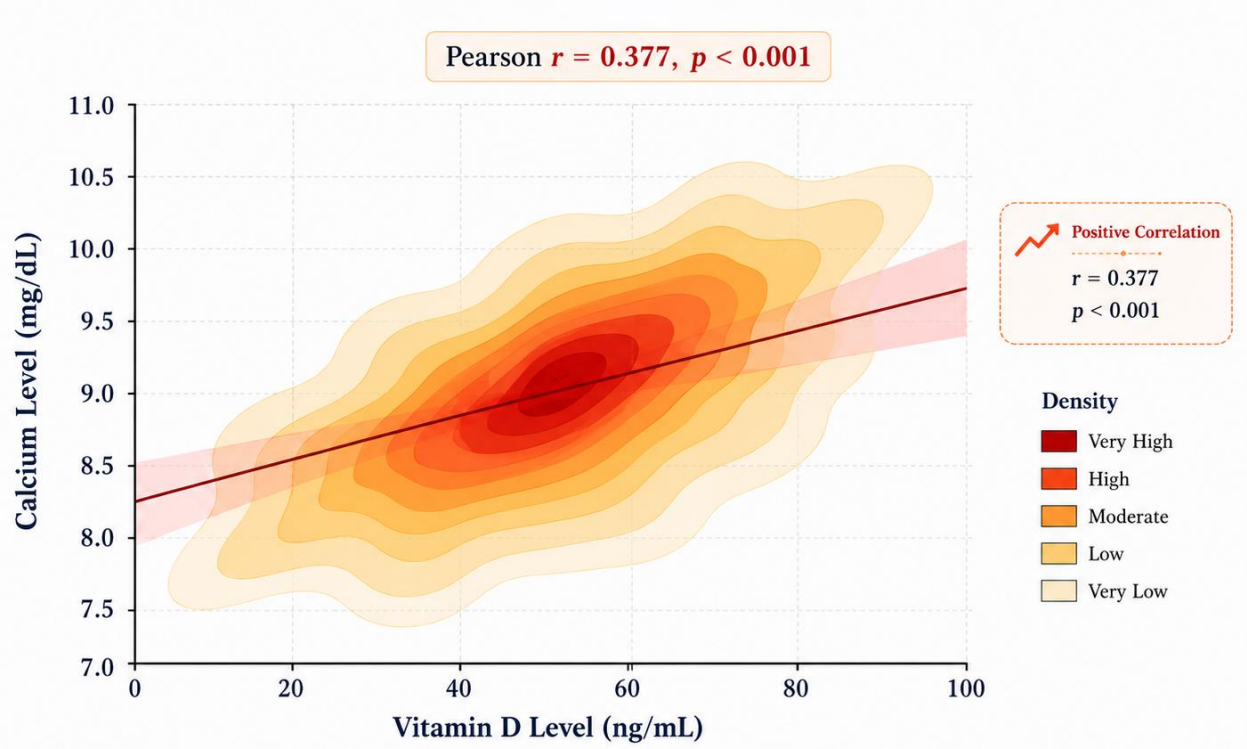


Figure 6 Correlation between serum vitamin D and calcium levels among study participants

DISCUSSION

This study investigated the association between serum calcium and vitamin D levels among patients with Hepatitis C Virus (HCV) infection in Peshawar and compared these parameters with healthy controls. The findings demonstrated lower serum calcium and vitamin D levels among HCV-infected patients compared with healthy individuals. The mean serum calcium level among HCV patients was 9.07 mg/dL, while vitamin D levels were also reduced compared with the control group. These findings are consistent with previous studies, including those reported by Udomsinprasert et al. (2020), Kumar et al. (2021), and Bessong et al. (2023), which reported a high frequency of vitamin D deficiency among individuals with chronic hepatitis and liver disease.

The observed reduction in vitamin D among HCV patients may be related to impaired hepatic metabolism associated with chronic liver disease. The liver plays an important role in vitamin D metabolism, and hepatic dysfunction may consequently contribute to reduced circulating vitamin D levels. Since vitamin D is essential for intestinal calcium absorption and maintenance of calcium homeostasis, reduced vitamin D status may also contribute to alterations in serum calcium levels. The present findings are in agreement with studies by Mihele et al. (2024) and Mutlag et al. (2023), which reported an association between vitamin D and calcium status in patients with liver disease.

The positive relationship observed between serum vitamin D and calcium levels further supports the physiological interaction between these two parameters. Patients with lower vitamin D levels

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ISSN: (e) 3007-1607 (p) 3007-1593

tended to have lower calcium levels, suggesting that disturbances in vitamin D status may be accompanied by alterations in calcium homeostasis among individuals with HCV infection. Assessment of both parameters may therefore provide a more comprehensive understanding of metabolic and nutritional abnormalities in these patients.

These findings are particularly relevant in Peshawar, where HCV represents an important public health concern. Identification of vitamin D and calcium deficiencies may help facilitate early recognition of metabolic abnormalities and potentially reduce the risk of complications related to impaired bone health, including osteopenia and osteoporosis. Further investigation and appropriate clinical management of these deficiencies may contribute to improved health outcomes among patients with chronic HCV infection.

LIMITATIONS

This study has several limitations. First, the cross-sectional design does not allow a causal relationship to be established between HCV infection and alterations in calcium or vitamin D levels. Second, the study was conducted in Peshawar; therefore, the findings may not be generalizable to other populations or geographic regions. Third, potentially relevant factors such as dietary intake, socioeconomic status, sun exposure, and duration of HCV infection were not assessed and may have influenced calcium and vitamin D levels. Finally, the study focused on serum calcium and vitamin D and did not include other potentially relevant markers, such as parathyroid hormone or bone mineral density.

CONCLUSION

This study demonstrated differences in serum vitamin D and calcium levels between patients with HCV infection and healthy controls and identified a moderate positive correlation between serum vitamin D and calcium levels. The findings suggest that HCV infection may be associated with disturbances in vitamin D status and calcium homeostasis. Assessment of these parameters may therefore be useful for identifying metabolic abnormalities and potentially reducing the risk of complications related to bone and mineral health. Further longitudinal and interventional studies are required to clarify the underlying mechanisms and clinical significance of these findings

RECOMMENDATION

Future studies should use longitudinal or cohort designs to investigate changes in calcium and vitamin D levels during the course of HCV infection. Larger multicenter studies involving diverse populations are also recommended to improve the generalizability of the findings. Future research should consider potential influences of sex, nutritional status, socioeconomic factors, sun exposure, and duration of infection. Assessment of additional markers such as parathyroid hormone and bone mineral density may provide a more comprehensive evaluation of mineral and skeletal health. Interventional studies are also recommended to determine whether correction of vitamin D and calcium deficiencies improves clinical and metabolic outcomes in HCV patients.

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