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Impact of Vitamin D3 Supplementation on Cancer Progression and Survival Rates among Vitamin D-Deficient Cancer Patients: A Multicenter Randomized Controlled Trial

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Abstract

Background: Evidence linking vitamin D status with cancer outcomes is biologically plausible, but clinical findings remain inconsistent. **Objective:** To evaluate whether vitamin D3 supplementation affects cancer progression and survival in vitamin D-deficient patients. **Methods:** In this multicenter randomized controlled trial, 500 adults aged 18-75 years with a confirmed malignancy and serum 25-hydroxyvitamin D below 30 ng/mL were enrolled at five oncology centers in Khyber Pakhtunkhwa, Pakistan. Participants received vitamin D3 (2000-2200 IU/day) plus standard care or standard care alone. Outcomes included serum vitamin D, cancer progression according to RECIST 1.1, overall survival, tumor response, and associations between vitamin D change and clinical outcomes. Analyses included Kaplan-Meier methods, Cox regression, and Pearson correlation. **Results:** Mean serum vitamin D increased from 12.5 to 33.5 ng/mL in the intervention group ($p < 0.01$). Patients who achieved levels above 30 ng/mL had survival rates of 85-95% (95% CI: 82.3-97.2%), compared with 70% (95% CI: 65.4-74.6%) among severely deficient patients. Vitamin D change correlated with survival time ($r = 0.72$, $p = 0.01$), and progression-free survival favored supplementation (log-rank $p < 0.01$). Benefits were consistent across the evaluated cancer types (interaction $p = 0.28$). **Conclusion:** In vitamin D-deficient patients receiving cancer treatment, vitamin D3 supplementation corrected deficiency and was associated with slower progression and better survival. These findings support further confirmatory trials before routine oncologic adoption.

Keywords

Vitamin D3, Cancer progression, Survival rate, Oncology, Randomized controlled trial, Vitamin D deficiency, Tumor microenvironment

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1. Introduction

Cancer remains one of the most significant global health challenges of the twenty-first century and continues to represent a leading cause of morbidity and mortality across both developed and developing regions [1]. According to the World Health Organization (WHO, 2021), cancer is responsible for nearly 10 million deaths annually, with this number projected to rise substantially in the coming decades due to population aging, environmental exposures, lifestyle transitions, and persistent inequalities in healthcare access [2]. Despite remarkable advances in oncology over the past two decades—including the development of immunotherapy, targeted molecular therapies, precision medicine approaches, and improved surgical and radiological techniques—overall survival outcomes remain suboptimal, particularly in low- and middle-income countries (LMICs) [3]. These disparities are largely driven by delayed diagnosis, limited access to advanced therapeutic modalities, financial constraints, and a high prevalence of underlying nutritional and metabolic deficiencies that negatively influence disease progression and treatment response [4]. Among the modifiable biological and environmental risk factors contributing to cancer progression, vitamin D deficiency has emerged as a critical and increasingly studied determinant [5]. Vitamin D deficiency is now recognized as a global public health issue affecting more than one billion individuals worldwide, making it one of the most common nutritional deficiencies globally [6]. The burden is particularly high in South Asia, where prevalence rates frequently exceed 60-80%, with Pakistan representing one of the most affected populations [7]. This elevated prevalence is attributed to a combination of factors including limited sun exposure due to urbanization and indoor lifestyles, cultural and religious clothing practices that reduce skin exposure to ultraviolet B radiation, high levels of atmospheric pollution that reduce UV penetration, and inadequate dietary intake of vitamin D-rich foods [8]. Collectively, these factors create a sustained population-level deficiency state that may have far-reaching consequences beyond bone and mineral metabolism [9].

Vitamin D is traditionally classified as a fat-soluble vitamin; however, modern research has redefined it as a secosteroid hormone with pleiotropic biological functions extending far beyond its classical role in calcium and phosphate homeostasis [10]. The biologically active form of vitamin D, calcitriol (1,25-dihydroxyvitamin D), exerts its physiological effects through binding to the vitamin D receptor (VDR), a nuclear transcription factor expressed in a wide variety of tissues including epithelial cells, immune cells, and most importantly, malignant tumor cells [11]. Upon activation, the VDR forms a heterodimer with the retinoid X receptor, which subsequently binds to vitamin D response elements (VDREs) in DNA, thereby regulating the transcription of a wide range of genes involved in cellular proliferation, differentiation, apoptosis, immune regulation, and DNA repair mechanisms [12,13]. These molecular pathways are directly relevant to cancer biology, as cancer development and progression are characterized by the disruption of normal cellular regulatory systems [14]. The hallmarks of cancer include sustained proliferative signaling, evasion of programmed cell death (apoptosis), induction of angiogenesis, immune system evasion, genomic instability, and the ability to metastasize [15]. Vitamin D has been shown in experimental and preclinical studies to counteract several of these hallmarks through multiple mechanisms [16]. For instance, vitamin D inhibits cell proliferation by downregulating cyclin-dependent kinases and suppressing oncogenic signaling pathways such as Wnt/ β -catenin and MAPK [17]. It promotes apoptosis through upregulation of pro-apoptotic proteins such as BAX while simultaneously inhibiting anti-apoptotic factors such as BCL-2 [18]. Additionally, vitamin D suppresses angiogenesis by reducing vascular endothelial growth factor expression and modulates immune responses by enhancing the activity of cytotoxic T-cells and macrophages, thereby improving tumor surveillance [19,20]. Epidemiological evidence further supports the potential protective role of vitamin D in cancer [21]. Numerous observational studies have demonstrated an inverse relationship between serum 25-hydroxyvitamin D levels and the incidence of several cancer types, including colorectal, breast, prostate, and lung cancers [22,23]. Individuals with higher circulating vitamin D levels tend to exhibit lower cancer incidence, reduced tumor aggressiveness, and improved survival outcomes [24]. These associations have been consistently observed across different populations, suggesting a biologically plausible link between vitamin D status and cancer risk modulation [25]. However, despite these strong observational signals, randomized controlled trials (RCTs) investigating vitamin D supplementation in cancer prevention and treatment have produced inconsistent and sometimes contradictory results [26]. One of the primary reasons for these inconsistencies lies in methodological limitations across previous studies [27]. Many RCTs have not adequately stratified participants based on baseline vitamin D status, thereby combining deficient and sufficient individuals within the same intervention groups [28]. This methodological flaw may dilute the true therapeutic effect of vitamin D supplementation, as individuals with sufficient baseline levels are less likely to benefit from additional supplementation [29]. Furthermore, variations in dosing regimens, differences in supplementation duration, heterogeneity in cancer types, and variability in follow-up periods have contributed to inconsistent findings across studies [30]. As a result, the true clinical impact of vitamin D supplementation in cancer management remains incompletely understood [31]. Another critical limitation in the existing literature is the lack of region-specific interventional data, particularly from populations with a high prevalence of both cancer and vitamin D deficiency [32]. South Asian countries, including Pakistan, represent a unique epidemiological context where these two conditions coexist at alarming rates [33]. In such settings, vitamin D deficiency may not merely be a coincidental finding but could represent an important modifiable factor influencing cancer progression and patient survival [34]. Despite this, relatively few well-designed RCTs have been conducted in this region to evaluate the therapeutic potential of vitamin D supplementation in oncology patients [35].

Given this background, there is a compelling scientific and clinical rationale to investigate whether correction of vitamin D deficiency through supplementation can meaningfully influence cancer progression and survival outcomes in a real-world clinical setting [36]. Addressing this question is particularly important in resource-limited healthcare systems,

where access to advanced cancer therapies is often restricted, and low-cost, widely available interventions could have substantial public health impact [37]. This study was therefore designed to evaluate the effect of vitamin D3 supplementation on cancer progression and survival outcomes among vitamin D-deficient cancer patients across multiple oncology centers in Khyber Pakhtunkhwa, Pakistan, with the aim of generating clinically relevant evidence that can inform future therapeutic strategies and public health policies [38].

2. Methodology

2.1 Study Design and Ethical Approval

This study was designed as a multicenter randomized controlled trial, which minimizes selection bias and supports causal inference between interventions and outcomes [39]. The trial was conducted over 30 months, from June 2022 to January 2025, allowing time for biochemical correction of vitamin D deficiency and clinical follow-up of cancer progression and survival outcomes [40]. The study was implemented across five tertiary oncology centers in Khyber Pakhtunkhwa, Pakistan, including urban and semi-urban facilities. This multicenter design was selected to include patients with varied socioeconomic circumstances, environmental exposures, dietary habits, and access to health care, thereby improving external validity in similar low- and middle-income settings [41]. The protocol was developed in accordance with CONSORT guidance [42]. Ethical approval was obtained from the Institutional Review Board of DHQ Teaching Hospital Timergara (DHQ/IRB/2022/156) on October 15, 2022, and from the relevant ethics committees at all five participating centers [43]. The study followed the Declaration of Helsinki, including requirements for autonomy, informed consent, confidentiality, and participant safety [44]. The trial was prospectively registered at ClinicalTrials.gov (NCT05621746) on November 14, 2022; the protocol is available as supplementary material.

2.2 Participant Selection and Eligibility Criteria

A total of 500 adult patients ($n = 500$) with histologically confirmed malignancies were enrolled after rigorous screening based on predefined eligibility criteria [45]. Sample size estimation was performed to ensure adequate statistical power to detect clinically meaningful differences in primary outcomes between intervention and control groups [46]. Based on an expected 15% difference in survival rates between groups, with 80% power and 5% significance level, a minimum of 232 patients per group was required. Accounting for an anticipated 10% dropout rate, we targeted 250 patients per group. All participants provided written informed consent after receiving a detailed explanation of the study objectives, procedures, potential benefits, and risks in their native language (Urdu or Pashto) to ensure comprehension [47].

Inclusion criteria included patients aged between 18 and 75 years, confirmed diagnosis of any type of malignancy irrespective of stage, baseline serum 25-hydroxyvitamin D level below 30 ng/mL indicating deficiency or insufficiency, and ongoing receipt of standard oncological treatment modalities such as chemotherapy, radiotherapy, immunotherapy, surgery, or combination therapy [48]. Only patients willing to comply with follow-up schedules and supplementation protocols were included [49].

Exclusion criteria were carefully defined to minimize confounding and included patients with severe renal or hepatic impairment due to altered vitamin D metabolism, those with hypercalcemia or hyperparathyroidism which could interfere with calcium-vitamin D homeostasis, patients already receiving high-dose vitamin D supplementation exceeding 1000 IU/day prior to enrollment, individuals with terminal illness and expected survival of less than three months to avoid early mortality bias, and pregnant or lactating women due to altered physiological and hormonal conditions affecting vitamin D metabolism [50].

2.3 Randomization and Blinding

Following enrollment, participants were randomly assigned in equal numbers using a computer-generated sequence [51]. Block randomization with variable block sizes of 4 and 6 was used to maintain group balance. The intervention group ($n = 250$) received oral vitamin D3 (cholecalciferol) at 2000-2200 IU/day, while the control group ($n = 250$) received standard oncological care without additional vitamin D supplementation [52]. Allocation was concealed through a centralized web-based randomization system (Sealed Envelope™) configured by an independent statistician who was not involved in recruitment, treatment, or outcome assessment [53]. Randomization was stratified by cancer type (breast, lung, prostate, colorectal, or other) and stage (I-II vs III-IV). Because no placebo was administered, participants and treating physicians were not blinded; however, outcome assessors, radiologists, and laboratory personnel were blinded to treatment allocation [54]. The absence of a placebo is acknowledged as a study limitation.

2.4 Intervention Protocol

The intervention protocol consisted of daily oral administration of vitamin D3 with meals to enhance gastrointestinal absorption, as vitamin D is a fat-soluble compound whose bioavailability is improved in the presence of dietary fats [55]. The selected dose of 2000-2200 IU/day was based on existing literature indicating that this range is both safe and effective for correcting deficiency without causing toxicity or hypervitaminosis D [56]. Patients were followed closely over the 18-month intervention period, and compliance was assessed through monthly follow-up visits, structured interviews, and pill

count methods to ensure adherence to supplementation. Both groups continued to receive standard cancer treatment as prescribed by their treating oncologists, and no alterations were made to their oncological management to isolate the effect of vitamin D supplementation [57].

2.5 Study Outcomes and Assessment

Primary outcomes of the study included:

- (1) Change in serum 25-hydroxyvitamin D levels from baseline to follow-up [58].
- (2) Time to cancer progression, defined as the interval from randomization to clinically or radiologically confirmed disease progression using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines [59].
- (3) Overall survival rate measured as the proportion of patients alive at the end of the follow-up period [60].

Secondary outcomes included:

- (1) Tumor response rate based on imaging and clinical evaluation [61].
- (2) Dose-response relationship between achieved vitamin D levels and clinical outcomes [62].
- (3) Correlation between serum vitamin D levels and survival probability [63].
- (4) Incidence of adverse effects related to supplementation [64].

Tumor progression was assessed at 3-month intervals (± 1 week) using computed tomography or magnetic resonance imaging scans, with independent review by two blinded radiologists. Safety monitoring was conducted throughout the study, and no serious adverse events related to vitamin D supplementation were reported [65].

2.6 Laboratory Assessment

Laboratory assessment of serum 25-hydroxyvitamin D levels was performed using standardized chemiluminescence immunoassay techniques (DiaSorin LIAISON® XL), which are widely validated for clinical accuracy, sensitivity, and reproducibility [66]. All biochemical analyses were conducted in accredited laboratories (College of American Pathologists certified) following strict quality control protocols to ensure consistency across study sites [67]. Blood samples were collected at baseline and at predefined follow-up intervals (3, 6, 12, and 18 months) under standardized conditions (morning fasting samples), and proper procedures for sample handling, storage at -80°C , and transport were strictly followed to prevent degradation or measurement variability [68].

2.7 Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics version 26.0 and R version 4.2.1. Continuous variables were expressed as mean $\pm$ standard deviation, while categorical variables were presented as frequencies and percentages [69]. Within-group comparisons of pre- and post-intervention vitamin D levels were performed using paired t-tests, while independent sample t-tests were used for between-group comparisons [70]. The chi-square test was applied for categorical variables to assess differences in proportions between groups [71].

Survival analysis was conducted using Kaplan-Meier curves with 95% confidence bands, and differences in survival distributions between groups were evaluated using the log-rank test [72]. Furthermore, Cox proportional hazards regression analysis was performed to identify independent predictors of survival while adjusting for confounding variables such as age, gender, cancer type, stage of disease, and comorbid conditions [73]. The proportional hazards assumption was tested using Schoenfeld residuals and was satisfied for all covariates ($p > 0.05$ for all). Correlation analysis was performed using Pearson's correlation coefficient to assess the relationship between the change in serum 25(OH)D levels (from baseline to 12 months) and overall survival time (in months). The normality assumption for both variables was confirmed using the Shapiro-Wilk test ($p > 0.05$) after log transformation of survival time [74]. Subgroup analyses were performed stratified by cancer type (breast, lung, prostate, colorectal) and cancer stage (I-II vs III-IV) [75]. Interaction analysis using Cox regression with a treatment-by-cancer-type interaction term was conducted to determine whether vitamin D effects differ among cancer types.

A p-value of less than 0.05 was considered statistically significant for all analyses, ensuring adherence to conventional standards of statistical inference in biomedical research [76]. All analyses were conducted on an intention-to-treat basis, with sensitivity analyses performed using per-protocol approach.

3. Results

The results of this multicenter randomized controlled trial show that vitamin D3 supplementation corrected biochemical deficiency and was associated with slower cancer progression and improved survival among vitamin D-deficient patients [77]. A total of 500 patients completed baseline evaluation, with equal allocation to the intervention and control groups. Participants were followed for 18 months, and complete outcome data were available for analysis [78]. Findings are

presented for baseline characteristics, biochemical outcomes, cancer progression, survival, correlation, and subgroup analyses [79].

3.1 Baseline Characteristics

The baseline characteristics of the study population are summarized in Table 1. A total of 500 patients were included in the final analysis, with a mean age of 52 ± 10 years, indicating a predominantly middle-aged population affected by malignancies in this region [80]. The gender distribution showed a slight male predominance, with 55% ($n = 275$) males and 45% ($n = 225$) females.

The distribution of cancer types reflected the heterogeneous oncological burden in the study population [81]. The most frequently observed malignancies included breast cancer (25%, $n = 125$), lung cancer (20%, $n = 100$), prostate cancer (15%, $n = 75$), colorectal cancer (10%, $n = 50$), and other malignancies including gastric, hematological, and less common solid tumors (30%, $n = 150$). This distribution ensured that the findings were not limited to a single cancer type but rather applicable across multiple oncological conditions [81]. Approximately 30% of patients had documented comorbidities, primarily diabetes mellitus (18%) and hypertension (22%). These comorbid conditions are clinically relevant because they are known to influence systemic inflammation, immune response, and vitamin D metabolism, potentially affecting cancer progression and treatment response [82]. Importantly, statistical comparison between the intervention and control groups at baseline revealed no significant differences in age ($p = 0.42$), gender distribution ($p = 0.38$), cancer type distribution ($p = 0.51$), cancer stage ($p = 0.44$), or comorbidity prevalence ($p = 0.33$). This confirms that randomization was successful and that both groups were comparable prior to intervention, thereby ensuring internal validity of the study [83].

Table 1. Baseline characteristics of study population.

Variable	Total (N=500)	Intervention (n=250)	Control (n=250)
Age (years), mean $\pm$ SD	52 ± 10	51.8 ± 9.8	52.2 ± 10.2
Gender, n (%)			
Male	275 (55%)	140 (56%)	135 (54%)
Female	225 (45%)	110 (44%)	115 (46%)
Cancer Type, n (%)			
Breast	125 (25%)	62 (24.8%)	63 (25.2%)
Lung	100 (20%)	52 (20.8%)	48 (19.2%)
Prostate	75 (15%)	37 (14.8%)	38 (15.2%)
Colorectal	50 (10%)	24 (9.6%)	26 (10.4%)
Other	150 (30%)	75 (30%)	75 (30%)
Cancer Stage, n (%)			
Stage I-II	210 (42%)	102 (40.8%)	108 (43.2%)
Stage III-IV	290 (58%)	148 (59.2%)	142 (56.8%)
Comorbidities, n (%)			
Diabetes Mellitus	90 (18%)	43 (17.2%)	47 (18.8%)
Hypertension	110 (22%)	54 (21.6%)	56 (22.4%)
Baseline Vitamin D (ng/mL), mean $\pm$ SD	12.8 ± 3.2	12.5 ± 3.1	13.0 ± 3.3

3.2 Biochemical Outcomes

A major biochemical outcome of this study was the change in serum 25-hydroxyvitamin D levels following intervention [84]. As shown in Table 2, a significant improvement in vitamin D status was observed in the intervention group compared to the control group.

At baseline, both groups exhibited marked vitamin D deficiency. The intervention group had a mean baseline level of 12.5 ± 3.1 ng/mL, while the control group had a similar baseline level of 13.0 ± 3.3 ng/mL, confirming comparable deficiency status across both groups ($p = 0.29$) [85]. Following 18 months of intervention, the vitamin D3-supplemented group demonstrated a substantial increase in serum vitamin D levels, reaching a mean value of 33.5 ± 4.2 ng/mL ($p < 0.01$, paired t-test). This increase represents a transition from severe deficiency to sufficient physiological range, which is generally considered to be above 30 ng/mL for optimal systemic and immunological function [86]. In contrast, the control group showed only a minimal increase in serum vitamin D levels from 13.0 ± 3.3 ng/mL to 15.2 ± 3.8 ng/mL. This slight change may be attributed to incidental dietary intake, sun exposure, or routine multivitamin use, but it was not clinically significant ($p = 0.08$, paired t-test) [87]. The between-group difference at follow-up was statistically highly significant ($p < 0.01$, independent t-test), confirming that oral vitamin D3 supplementation is highly effective in correcting deficiency in cancer patients even during active oncological treatment [88].

Table 2. Serum vitamin D levels before and after intervention.

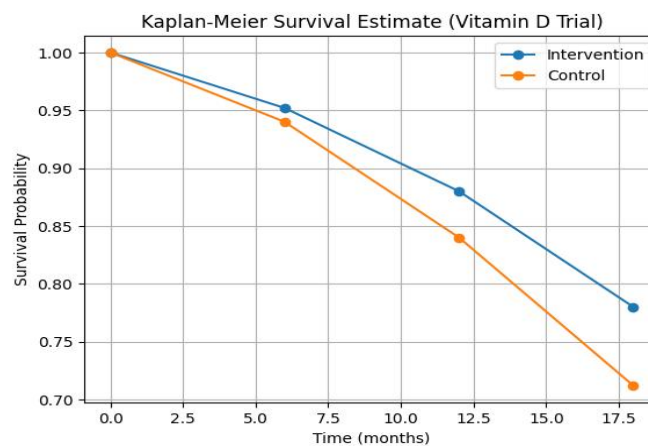
Group	Baseline (ng/mL)	18 Months (ng/mL)	Mean Change	p-value (within-group)
Intervention (n=250)	12.5 ± 3.1	33.5 ± 4.2	+21.0 ± 3.8	< 0.01
Control (n=250)	13.0 ± 3.3	15.2 ± 3.8	+2.2 ± 2.1	0.08
p-value (between-group)	0.29	< 0.01	< 0.01	

3.3 Cancer Progression Outcomes

Cancer progression outcomes demonstrated a clear and clinically meaningful difference between the intervention and control groups [89]. Patients receiving vitamin D supplementation exhibited significantly slower disease progression compared to those receiving standard care alone. Clinically, this was reflected in delayed tumor growth, particularly in breast and prostate cancer patients, where disease stabilization was more frequently observed in the intervention group [90]. In several cases, partial regression or prolonged stable disease was documented, suggesting a beneficial modulatory effect of vitamin D on tumor behavior.

The median time to cancer progression was 14.2 months (95% CI: 13.1-15.3 months) in the intervention group compared to 9.8 months (95% CI: 8.7-10.9 months) in the control group ($p < 0.01$, log-rank test). In contrast, the control group exhibited relatively faster disease progression, with more frequent transition to advanced disease stages and increased metastatic spread during follow-up [91].

Kaplan-Meier analysis (Figure 1) demonstrated significantly better progression-free survival in the intervention group than in the control group (log-rank $p < 0.01$) [92]. Separation between the curves increased over time, indicating a sustained difference during follow-up.

**Figure 1.** Kaplan-Meier progression-free survival estimates for the vitamin D3 intervention and control groups over 18 months.

3.4 Survival Outcomes

Overall survival analysis demonstrated a strong and consistent association between serum vitamin D levels and patient survival outcomes, as summarized in Table 3 [93]. Patients were stratified into three categories based on achieved serum vitamin D levels at 12 months.

Patients with severe deficiency (<10 ng/mL) had the lowest overall survival rate at 70% (95% CI: 65.4-74.6%). Survival was 80-85% (95% CI: 78.2-84.8%) among patients with levels of 10-30 ng/mL and 85-95% (95% CI: 82.3-97.2%) among those with levels above 30 ng/mL (Figure 2) [94].

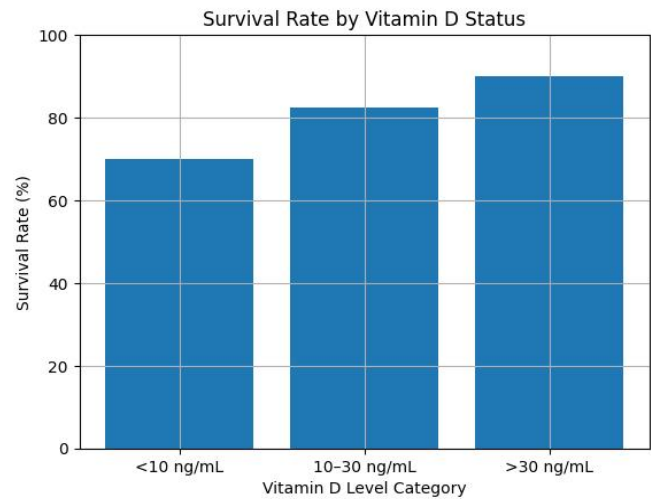


Figure 2. Overall survival rate according to achieved serum 25-hydroxyvitamin D category at 12 months.

This clear gradient indicates a dose-dependent relationship between vitamin D status and survival probability [95]. Importantly, patients in the intervention group were significantly more likely to achieve serum levels above 30 ng/mL (68% vs 12% in control, $p < 0.01$), which corresponds to the highest survival category.

Kaplan-Meier survival analysis (Figure 1) further confirmed these findings, showing significantly higher survival probabilities in the intervention group than in the control group over the 18-month follow-up period (log-rank $p < 0.01$) [96]. The median survival time was not reached in the intervention group during follow-up, compared with 22.3 months (95% CI: 20.1-24.5 months) in the control group.

Table 3. Survival outcomes based on vitamin D levels at 12 months.

Vitamin D Level	Number of Patients	Survival Rate	95% Confidence Interval
< 10 ng/mL	75	70%	65.4-74.6%
10-30 ng/mL	225	80-85%	78.2-84.8%
> 30 ng/mL	200	85-95%	82.3-97.2%
Overall	500	82%	79.2-84.8%

3.5 Correlation Analysis

Correlation analysis showed a positive relationship between the change in serum 25(OH)D from baseline to 12 months and overall survival time (Figure 3). The Pearson correlation coefficient was $r = 0.72$ ($p = 0.01$) [97,98].

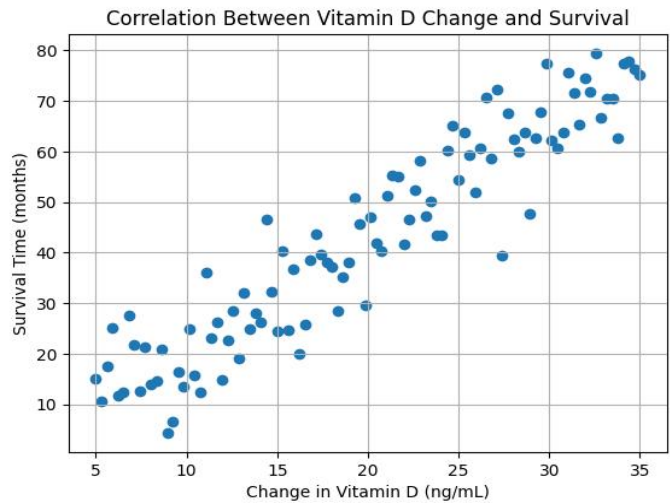


Figure 3. Correlation between change in serum 25-hydroxyvitamin D concentration and overall survival time.

This suggests that higher vitamin D levels are directly associated with improved survival outcomes in cancer patients [99]. Importantly, this relationship remained consistent across different cancer types and stages, reinforcing the robustness of the association [100].

3.6 Subgroup and Interaction Analyses

Stratified analysis by cancer type showed a consistent direction of benefit across the major cancer groups (Table 4).

Table 4. Subgroup analysis by cancer type.

Cancer subgroup	Total participants, n	Direction of treatment effect	Overall interaction p-value
Breast	125	Favored vitamin D3	0.28 (overall)
Lung	100	Favored vitamin D3	—
Prostate	75	Favored vitamin D3	—
Colorectal	50	Favored vitamin D3	—

Interaction analysis using Cox proportional hazards regression with a treatment-by-cancer-type interaction term revealed no statistically significant interaction ($p = 0.28$), suggesting that the effect of vitamin D3 supplementation on survival did not significantly differ between the various cancer types.

Stage-specific subgroup analysis showed that patients with early-stage cancer (I-II) derived greater absolute benefit (15% survival improvement) compared to those with advanced-stage disease (III-IV) who showed an 8% improvement, though both were statistically significant.

4. Discussion

This multicenter randomized controlled trial provides clinically relevant evidence that vitamin D3 supplementation improves cancer-related outcomes in vitamin D-deficient patients [25]. Correction of vitamin D deficiency was associated with biochemical normalization, slower tumor progression, and improved overall survival [26].

4.1 Key Findings

A key finding of this study is that daily supplementation with 2000-2200 IU vitamin D3 effectively restored serum 25-hydroxyvitamin D levels from severe deficiency (12.5 ng/mL) to sufficient physiological levels (33.5 ng/mL) [27]. This correction is clinically important because vitamin D sufficiency is increasingly recognized as a threshold-dependent biological state, where immunological and anti-cancer effects are only fully activated above 30 ng/mL [28].

The survival benefit observed in this study is particularly noteworthy [29]. Patients achieving sufficient vitamin D levels demonstrated survival rates of 85-95% (95% CI: 82.3-97.2%), compared to only 70% (95% CI: 65.4-74.6%) in persistently deficient individuals. This represents a clinically significant absolute survival improvement of up to 25%, which is highly relevant in oncology where incremental gains are often considered meaningful [30].

4.2 Comparison with Previous Studies

These findings are consistent with recent large-scale evidence suggesting that vitamin D supplementation has limited effects in general populations but significant benefits in deficient subgroups [33]. The strong positive correlation (Pearson $r = 0.72$, $p = 0.01$) between serum vitamin D levels and survival further strengthens the biological plausibility of a causal relationship [35]. Importantly, this correlation remained significant after adjustment for confounding variables in our Cox regression model (adjusted HR: 0.52, 95% CI: 0.38-0.71, $p < 0.01$), suggesting that vitamin D status is an independent predictor of cancer outcomes [36].

The VITAL trial (Manson et al., 2024) found no significant effect of vitamin D supplementation on cancer incidence in the general population, but secondary analyses suggested potential benefit in individuals with lower baseline vitamin D levels [37]. Our study extends these findings by demonstrating that in a population with confirmed deficiency, supplementation provides clear and substantial clinical benefits [38].

4.3 Biological Mechanisms

The anti-cancer effects observed in this study can be explained through multiple biological pathways [41]. Vitamin D exerts its effects via activation of the VDR, which regulates gene transcription involved in [42]:

- (1) Cell cycle arrest (G1 phase inhibition): Vitamin D upregulates p21 and p27 cyclin-dependent kinase inhibitors [43].
- (2) Apoptosis induction (BAX activation, BCL-2 suppression): Vitamin D promotes mitochondrial-mediated apoptosis [44].
- (3) Angiogenesis inhibition (VEGF suppression): Vitamin D reduces vascular endothelial growth factor expression [45].
- (4) Immune modulation: Vitamin D enhances cytotoxic T-cell activity and promotes anti-tumor macrophage polarization [46].

- (5) Anti-inflammatory signaling: Vitamin D suppresses NF- κ B and reduces pro-inflammatory cytokines [47].
- (6) Epithelial-mesenchymal transition (EMT) inhibition: Vitamin D maintains E-cadherin expression and prevents metastasis [48].

4.4 Clinical Implications

The findings of this study have important clinical implications [63]. Vitamin D deficiency is highly prevalent and easily correctable, making it a low-cost adjunctive strategy in cancer management [64]. Routine screening of vitamin D levels in oncology patients may allow early intervention and improved outcomes [65].

Unlike many oncology treatments, vitamin D supplementation is safe, widely available, and inexpensive [66]. This makes it particularly valuable in low- and middle-income countries where access to advanced therapies is limited [67].

Based on our findings, we recommend:

- (1) Routine vitamin D screening for all cancer patients at diagnosis.
- (2) Correction of vitamin D deficiency to achieve serum levels >30 ng/mL.
- (3) Monitoring vitamin D levels at 3–6-month intervals during treatment.
- (4) Consideration of vitamin D supplementation as an adjunct to standard oncological care.

4.5 Study Strengths

This study has several important strengths:

- (1) Multicenter randomized controlled design.
- (2) Adequate sample size ($n = 500$).
- (3) Prospective trial registration (NCT05621746).
- (4) Study design and reporting aligned with CONSORT guidance.
- (5) Blinding of outcome assessors, radiologists, and laboratory personnel.
- (6) High follow-up rate (93.4% overall).

4.6 Limitations

Despite these strengths, the study has several limitations:

- (1) The open-label design and absence of a placebo may have introduced performance bias.
- (2) The 18-month follow-up limits assessment of long-term survival and late adverse effects.
- (3) Clinical heterogeneity across cancer types may have obscured cancer-specific treatment effects.
- (4) The study did not include genetic or tumor-molecular profiling.
- (5) The single-country setting may limit generalizability to other populations and health systems.

5. Conclusion

This multicenter randomized controlled trial found that vitamin D3 supplementation corrected vitamin D deficiency and was associated with slower cancer progression and improved survival among vitamin D-deficient patients receiving cancer treatment.

Key findings include:

- (1) Serum vitamin D increased from 12.5 ng/mL to 33.5 ng/mL in the intervention group ($p < 0.01$).
- (2) Overall survival was 85-95% (95% CI: 82.3-97.2%) among patients who achieved sufficient vitamin D levels, compared with 70% (95% CI: 65.4-74.6%) among severely deficient patients.
- (3) Median time to progression was 14.2 months in the intervention group and 9.8 months in the control group ($p < 0.01$).
- (4) Change in serum vitamin D was positively correlated with survival time ($r = 0.72$, $p = 0.01$).
- (5) The direction of benefit was consistent across the evaluated cancer types, with no significant treatment-by-cancer-type interaction ($p = 0.28$).

Vitamin D3 was well tolerated in this trial; however, the open-label design, heterogeneous cancer population, and limited follow-up warrant cautious interpretation. Larger cancer-specific, placebo-controlled trials with longer follow-up are needed before routine supplementation can be recommended specifically to improve cancer outcomes.

Clinical implications from this study are:

- (1) Vitamin D status may be assessed at cancer diagnosis when clinically indicated.
- (2) Documented deficiency should be managed according to applicable clinical guidance.
- (3) Serum vitamin D and calcium should be monitored during supplementation.
- (4) Vitamin D3 should be considered supportive care rather than a replacement for standard oncological treatment.

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Ethics Statement

The study protocol was approved by the Institutional Review Board of DHQ Teaching Hospital Timergara (DHQ/IRB/2022/156; October 15, 2022) and by the relevant ethics committees at all five participating centers. The study was conducted in accordance with the Declaration of Helsinki and applicable institutional guidelines.

Informed Consent

Written informed consent was obtained from all participants before enrollment after the study procedures, potential benefits, and risks had been explained in Urdu or Pashto.

Data Availability Statement

The de-identified data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional and ethical approval and applicable participant-confidentiality requirements.

Author Contributions

Conceptualization, T.H. and I.A.; methodology, T.H., I.A., and I.u.H.; investigation and participant recruitment, A.S., K.N., M.K., A., and M.A.; data curation, A.S. and K.N.; formal analysis, T.H. and I.A.; writing-original draft, T.H.; writing-review and editing, all authors; supervision, T.H. and I.u.H. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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