Iran J Ped Hematol Oncol. 2026, Vol. 16, No. 4, 1006-1020

Original Article

Association of Serum Vitamin A and Vitamin D Levels with Oral Mucositis Severity in Pediatric Cancer Patients: A Single-Center Retrospective Observational Study

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Received: July 20, 2025
Accepted: September 15, 2026

Abstract

Background: Mucositis is a frequent complication of chemotherapy (CT) and radiotherapy (RT), causing painful oral lesions that may disrupt treatment continuity. Vitamins A and D are important for mucosal integrity. This study aimed to evaluate whether serum vitamin A and vitamin D levels are associated with mucositis severity in pediatric cancer patients with established mucositis.

Materials and Methods: This single-center retrospective observational study included 30 pediatric cancer patients (20 males, 10 females; mean age 8.1 years, range 1–18 years) treated at Dr. Sami Ulus Hospital between September 2016 and June 2017 and diagnosed with mucositis. Serum vitamin A and vitamin D levels were measured at mucositis diagnosis. Two control groups of 40 healthy children each were used for comparison. Associations between vitamin levels and mucositis stage, frequency, and duration were analyzed.

Results: Serum vitamin A levels were significantly lower in the mucositis group than controls. Vitamin A deficiency was more frequent among patients with severe mucositis (stages 3–4) than those with mild mucositis (stages 1–2) ($p = 0.02$). No significant association was observed between serum vitamin D levels and mucositis severity ($p = 0.31$). However, longer vitamin D supplementation during infancy was associated with lower mucositis severity ($p = 0.025$).

Conclusion: Lower serum vitamin A levels were associated with greater mucositis severity among pediatric oncology patients. No significant association was observed between serum vitamin D levels and mucositis severity. The relationship between vitamin D supplementation duration in infancy and mucositis severity should be considered exploratory and hypothesis-generating. Given the retrospective design, cross-sectional vitamin assessment, heterogeneous malignancies and treatment regimens, and absence of an oncology control group without mucositis, these findings should be interpreted cautiously. Prospective multicenter studies are needed to clarify the relationship between vitamin status and mucositis severity in pediatric oncology patients.

Keywords: Child, Mucositis, Neoplasms, Vitamin A, Vitamin D

[DOI: 10.18502/ijpho.v16i4.22764]

Introduction

Vitamins A and D are essential micronutrients with important roles in growth, development, cellular metabolism, and immune regulation (1-3). Their active metabolites modulate various physiological processes, including tissue differentiation, epithelial barrier maintenance, bone and calcium homeostasis, and inflammatory responses (1-4). Among these, vitamins A and D are particularly important for immune function and mucosal integrity. Retinoic acid, the active metabolite of vitamin A, enhances epithelial barrier function and regulates T-cell-mediated immunity, whereas 1,25-dihydroxyvitamin D_3, the active form of vitamin D, modulates innate and adaptive immune responses and supports epithelial barrier function (1,2,5). In addition, vitamin D stimulates antimicrobial peptide production and contributes to epithelial regeneration, while vitamin A plays a central role in maintaining mucosal homeostasis and limiting epithelial injury. Because chemotherapy-induced oral mucositis develops through complex interactions among epithelial damage, inflammatory activation, oxidative stress, and impaired tissue repair, alterations in vitamin A and vitamin D status have been proposed as potential modifiers of mucosal toxicity. Nevertheless, current evidence remains insufficient to establish a causal relationship between vitamin status and mucositis severity (6).

Mucositis is a common and debilitating inflammatory complication of chemotherapy (CT) and radiotherapy (RT), characterized by erythema, ulceration, and mucosal breakdown in the gastrointestinal tract (6). It leads to severe pain, nutritional deficits, increased infection risk, and may necessitate treatment delays or dose reductions, ultimately affecting oncologic outcomes (6). Pediatric patients represent an important high-risk population, with oral mucositis occurring frequently during chemotherapy and other intensive cancer treatments (7). Recent clinical practice guidelines published by the Multinational Association of Supportive Care in Cancer and the International Society of Oral Oncology (MASCC/ISOO) recognize oral mucositis as one of the most clinically important treatment-related toxicities in pediatric oncology because of its substantial impact on nutritional status, quality of life, hospitalization, and continuity of anticancer therapy (6).

Emerging clinical evidence suggests that vitamin deficiencies may be associated with more severe mucosal injury. Observational studies have reported associations between lower vitamin A levels and greater severity of chemotherapy-induced mucositis, while early-life vitamin D supplementation has been linked to improved mucosal and immune health outcomes (8-11). For example, Oosterom et al. reported that children with acute lymphoblastic leukemia who experienced significant declines in 25-hydroxyvitamin D_3 during methotrexate therapy had higher rates of severe oral mucositis (8). Similarly, Pattanakitsakul et al. demonstrated that vitamin A supplementation helped maintain intestinal mucosal integrity in pediatric patients undergoing hematopoietic stem cell transplantation (9).

However, interpretation of these findings requires caution. Serum vitamin A and vitamin D concentrations in pediatric oncology patients may be influenced by numerous factors unrelated to mucositis itself, including the underlying malignancy, chemotherapy regimen, systemic inflammation, impaired nutritional status, reduced oral intake, hepatic metabolism, and treatment-related toxicity. Therefore, lower serum vitamin concentrations may represent markers of overall disease burden or treatment effects rather than direct determinants of mucositis severity. Consequently, currently available observational studies, including the present study, demonstrate associations but do not establish causality between vitamin status and oral mucositis (6,12).

Despite these insights, the relationship between vitamin A and D status and mucositis severity in pediatric oncology patients remains incompletely defined. In particular, data regarding the association between serum vitamin levels and the severity of established mucositis in children receiving cancer therapy remain limited. Furthermore, most published studies have evaluated selected patient populations or specific

[DOI: 10.18502/ijpho.v16i4.22764]

treatment protocols, limiting the generalizability of their findings to heterogeneous pediatric oncology populations.

A better understanding of these associations may help generate hypotheses for future studies evaluating the potential role of nutritional status in the clinical course of mucositis. Therefore, this retrospective observational study aimed to evaluate the association between serum vitamin A and vitamin D levels and the severity of established oral mucositis in pediatric oncology patients. Because of the observational design, the objective was not to determine causality but rather to identify potential clinical associations that may guide future prospective studies incorporating pediatric oncology control groups without mucositis, comprehensive nutritional assessment, inflammatory biomarkers, and multivariable analyses to better address potential confounding factors. In addition, we explored potential associations between vitamin status and selected clinical characteristics of mucositis, including stage, frequency, and duration.

Material and Methods

Study Design and Population

This retrospective observational study was conducted at the Pediatric Oncology Department of Dr. Sami Ulus Maternity and Child Health and Diseases Training and Research Hospital, Ankara, Türkiye, between September 2016 and June 2017. The study protocol was approved by the Institutional Ethics Committee (Decision No. 1533, dated October 25, 2017), and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants.

A total of 30 consecutive pediatric oncology patients diagnosed with chemotherapy-associated oral mucositis (20 males and 10 females; mean age 8.10 ± 4.5 years, range 1–18 years) were included in the study. Serum vitamin A and vitamin D levels were measured at the time of mucositis diagnosis, before resolution of mucosal lesions. The control group consisted of 40 age-matched healthy children without chronic disease, malignancy, or oral mucositis. Control data were obtained from hospital records of routine vitamin assessments performed during the same study period.

Medical records were reviewed retrospectively using a structured data collection form. Demographic characteristics, clinical diagnoses, treatment protocols, supportive care practices, nutritional variables, breastfeeding history, oral care habits, and vitamin supplementation history were recorded. Because of the retrospective design, all clinical and laboratory data were obtained from existing medical records, and no additional investigations or interventions were performed specifically for research purposes.

The primary outcome of the study was oral mucositis severity according to the World Health Organization (WHO) Oral Toxicity Scale. Secondary outcomes included mucositis duration, recurrence, and their associations with serum vitamin A and vitamin D levels.

Mucositis Staging

Mucositis severity was graded according to the World Health Organization (WHO) Oral Toxicity Scale:

Grade 0: Normal mucosa

Grade 1: Erythema and mild soreness without ulceration; normal oral intake

Grade 2: Erythema with ulcers <2 mm; mild pain; able to consume solid food

Grade 3: Ulcers >2 mm or generalized erythema with significant pain; able to consume liquids only

Grade 4: Severe ulceration involving >50% of the mucosa, severe pain, inability to eat, and requirement for enteral or parenteral nutritional support.

The primary analysis evaluated mucositis severity across the original four WHO grades. Because only a limited number of patients were represented within individual WHO categories (particularly Grades 1 and 4), an additional exploratory analysis was performed using clinically meaningful grouped categories of mild mucositis (WHO Grades 1–2) and severe mucositis (WHO Grades 3–4). This grouped analysis was undertaken to improve clinical interpretability and reduce statistical instability related to sparse subgroup sizes. Accordingly, the grouped analysis

[DOI: 10.18502/ijpho.v16i4.22764]

should be interpreted as complementary to, rather than replacing, the primary analysis based on the original WHO grading system.

Mucositis Management Protocol

All patients received standard preventive oral care consisting of soft-bristled tooth brushing two to three times daily and isotonic saline rinses five to six times daily. Additional supportive treatments were administered according to clinical severity, including sodium bicarbonate rinses, chlorhexidine-based mouthwashes, topical analgesics, and nutritional support when necessary.

Laboratory Analysis

Serum samples were collected at the time of mucositis diagnosis. Vitamin A concentrations were measured using gas chromatography–mass spectrometry (GC–MS; Shimadzu, Japan), and vitamin D concentrations were measured using high-performance liquid chromatography (HPLC; Thermo Scientific, USA).

Vitamin A deficiency was defined as a serum concentration <400 ng/mL, and vitamin D deficiency as <25 ng/mL. These laboratory cut-off values were based on the reference ranges routinely used by the institutional clinical laboratory during the study period.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of histograms. Normally distributed variables are presented as mean ± standard deviation (SD), whereas non-normally distributed variables are expressed as median (minimum–maximum). Categorical variables are presented as frequencies and percentages.

Comparisons between independent groups were performed using the independent-samples t-test for normally distributed continuous variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using the Chi-square test, Fisher's exact test, or the Fisher–Freeman–Halton test, as appropriate. Exact tests were applied when expected cell counts were small.

All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant. Because multiple statistical comparisons were performed in a relatively small sample, statistically significant findings, particularly those with borderline p-values, should be interpreted with caution.

No adjustments for multiple comparisons were performed because of the exploratory nature of the study. In addition, multivariable regression analyses were not undertaken because the limited sample size and the small number of outcome events did not provide adequate statistical power for reliable model construction. Consequently, potential confounding factors, including cancer type, chemotherapy regimen, nutritional status, and inflammatory status, could not be adjusted for and should be considered when interpreting the findings. The results should therefore be regarded as hypothesis-generating rather than confirmatory.

Results

A total of 30 consecutive pediatric oncology patients with chemotherapy-associated oral mucositis were included in the study. The cohort consisted of 20 males (66.7%) and 10 females (33.3%), with a mean age of 8.10 ± 4.5 years (range, 1–18 years). The control group consisted of 40 healthy children and did not differ significantly from the patient group with respect to age or sex distribution (both $p > 0.05$).

The study population comprised patients with a variety of malignancies, including acute lymphoblastic leukemia, acute myeloid leukemia, neuroblastoma, Wilms tumor, Burkitt lymphoma, lymphoma, sarcoma, and germ cell tumors (Table I).

According to the WHO Oral Toxicity Scale, 2 patients (6.7%) had Grade 1 mucositis, 9 (30.0%) had Grade 2, 13 (43.3%) had Grade 3, and 6 (20.0%) had Grade 4 mucositis. Overall, 19 patients (63.3%) were classified as having severe mucositis (WHO Grades 3–4). Neither age nor sex was significantly associated with mucositis severity (both $p > 0.05$), although males accounted

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for 73.7% of patients with severe mucositis.

Sociodemographic characteristics, including family size and monthly household income, were evaluated for potential associations with mucositis severity. Patients from lower-income households were more frequently represented among severe mucositis cases; however, these differences did not reach statistical significance ($p > 0.05$).

Clinical characteristics according to mucositis severity are summarized in Table II. Oral care education, adherence to a neutropenic diet, preventive oral care measures, previous vitamin D supplementation during infancy, and current vitamin supplementation were not significantly associated with mucositis severity (all $p > 0.05$). However, patients with mild mucositis (WHO Grades 1–2) had received vitamin D supplementation during infancy for a significantly longer duration than those with severe mucositis (WHO Grades 3–4) (7.50 ± 3.53 vs. 4.60 ± 2.50 months, $p = 0.025$). In addition, severe mucositis was associated with a significantly longer duration of mucosal symptoms ($p = 0.002$).

Comparison of serum vitamin concentrations between patients and healthy controls revealed significantly lower mean serum vitamin A levels in patients with oral mucositis than in healthy controls (442.03 ± 188.01 ng/mL vs. 513.88 ± 112.93 ng/mL, $p = 0.049$). In contrast, serum vitamin D concentrations did not differ significantly between the two groups (22.86 ± 20.45 ng/mL vs. 18.77 ± 9.79 ng/mL, $p = 0.281$) (Table III, Figure 1).

Serum vitamin A concentrations were significantly higher in males than in females (489.75 ± 196.88 ng/mL vs. 346.60 ± 129.94 ng/mL, $p = 0.047$), whereas serum vitamin D concentrations showed no significant sex-related differences ($p = 0.468$) (Table IV).

Based on the predefined laboratory thresholds, vitamin A deficiency (<400 ng/mL) was identified in 14 of 30 patients (46.7%), whereas vitamin D deficiency (<25 ng/mL) was present in 22 patients (73.3%).

When vitamin deficiencies were analyzed according to the original four WHO mucositis grades, no statistically significant association was observed between vitamin A deficiency and mucositis grade ($p = 0.086$) or between vitamin D deficiency and mucositis grade ($p = 0.214$) (Table V).

Because only a limited number of patients were represented within individual WHO mucositis grades, an additional exploratory analysis was performed using grouped categories of mild (WHO Grades 1–2) and severe (WHO Grades 3–4) mucositis. In this secondary analysis, vitamin A deficiency was significantly more frequent among patients with severe mucositis than among those with mild mucositis (63.2% vs. 18.2%, $p = 0.020$) (Figure 2). Patients with vitamin A deficiency had significantly higher unadjusted odds of severe mucositis than those without vitamin A deficiency (OR = 7.71, 95% CI: 1.29–46.22). In contrast, vitamin D deficiency was not significantly associated with grouped mucositis severity ($p = 0.31$) (Table VI).

No significant associations were identified between vitamin A or vitamin D status and the number of mucositis episodes, breastfeeding duration, birth weight, family size, monthly household income, or geographic region (all $p > 0.05$).

Overall, lower serum vitamin A levels and vitamin A deficiency were associated with greater oral mucositis severity in this cohort. However, the principal positive finding of the study was derived from the secondary grouped analysis and should therefore be interpreted within that context. Furthermore, these findings were based on unadjusted analyses from a relatively small retrospective cohort. Because multivariable analyses could not be performed, the potential influence of confounding factors—including cancer type, chemotherapy regimen, nutritional status, and inflammatory burden—cannot be excluded. Accordingly, the observed associations should be regarded as exploratory and hypothesis-generating rather than evidence of an independent or causal relationship between vitamin A status and oral mucositis severity.

[DOI: 10.18502/ijpho.v16i4.22764]

Table I: Diagnosis Distribution of Cases, Treatment Protocols, and Chemotherapy Administered Before Mucositis (n = 30)

Patient No.	Diagnosis	Chemotherapy Protocol	Treatment Administered Before Mucositis (Dose / Unit)
1	Pre-B ALL	ALL IC-BFM 2009	Vincristine 1.6 mg; Doxorubicin 30 mg; Dexamethasone 3×2 mg
2	Pre-B ALL	ALL IC-BFM 2009	Methylprednisolone 3×12 mg
3	Myelodysplastic syndrome	None	None
4	T-cell ALL (thymic)	ALL IC-BFM 2009	Cyclophosphamide 1000 mg/m²; Cytarabine 75 mg/m²; 6-mercaptopurine 60 mg/m²
5	Wilms tumor stage IV + lung metastasis	TPOG Wilms Tumor Protocol	Vincristine 0.8 mg; Dactinomycin 165 µg
6	T-cell lymphoma	BFM-NHL non-B protocol	Cytarabine 100 mg; 6-mercaptopurine
7	Pre-B ALL	ALL IC-BFM 2009	Vincristine 0.9 mg; Daunorubicin 18 mg; L-asparaginase 3000 IU
8	Wilms tumor + lung metastasis	Preoperative chemotherapy	Vincristine; Actinomycin-D
9	Pre-B ALL	ALL IC-BFM 2009	Dexamethasone 3×10 mg; L-asparaginase 5000 IU; Cytarabine 70 mg; 6-mercaptopurine 55 mg
10	Mixed germ cell tumor	PEB Protocol	Cisplatin 33 mg; Etoposide 164 mg; Bleomycin 29 mg
11	AML	AML BFM 2004	Cytarabine 2×180 mg; Mitoxantrone 2.7 mg
12	Neuroblastoma high-risk	TPOG Neuroblastoma Protocol	Vincristine 1.5 mg/m²; Dacarbazine 200 mg/m²; Ifosfamide 1500 mg/m²; Adriamycin 30 mg/m²
13	Neuroblastoma stage III	TPOG Neuroblastoma Protocol	Vincristine 1.5 mg/m²; Dacarbazine 200 mg/m²; Ifosfamide 1500 mg/m²; Adriamycin 30 mg/m²
14	Spindle cell sarcoma + metastasis	EPSSG RMS 2005	Vincristine; Actinomycin-D; Cyclophosphamide
15	AML high-risk	AML BFM 2004	Cytarabine 2×800 mg; Mitoxantrone 8 mg
16	T-cell ALL	St. Jude XV	Vincristine 1.7 mg; Cytarabine 2×2 g; L-asparaginase 20000 IU
17	ALL (MRG)	ALL IC-BFM 2009	High-dose Methotrexate
18	Pre-B ALL	St. Jude XV	Vincristine 2 mg; Dexamethasone 6 mg
19	Pre-B ALL	ALL IC-BFM 2009	Methylprednisolone 2×20 mg
20	Pre-B ALL	ALL IC-BFM 2009	Methylprednisolone 3×6 mg
21	Burkitt lymphoma stage III	NHL-BFM Registry 2012	Dexamethasone 30 mg/m²; Vincristine 1.5 mg/m²; Methotrexate 5 mg/m²
22	Pre-B ALL	ALL IC-BFM 2009	Doxorubicin 40 mg; Vincristine 2 mg; L-asparaginase 10000 IU; Dexamethasone 3×4.4 mg

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23	Neuroblastoma intermediate-risk	TPOG Neuroblastoma Protocol	Ifosfamide 500 mg; Vincristine 0.5 mg; Dacarbazine 70 mg; Adriamycin 13.5 mg
24	High-risk B-cell lymphoma + CNS relapse	LMB 96	Dexamethasone 4×10 mg; Cyclophosphamide 2×375 mg; Doxorubicin 90 mg
25	Relapsed ALL	REZ-BFM	High-dose Methotrexate
26	Burkitt lymphoma stage IV	NHL-BFM Registry 2012	Cytarabine 2×225 mg; Etoposide 150 mg; Vincristine 2 mg; Methotrexate 7.5 g; Dexamethasone 4×4 mg
27	T-cell lymphoma + AML	BFM-NHL non-B	Vincristine 1.7 mg; Daunorubicin 40 mg
28	Relapsed AML	AML BFM 2004	ATRA 2×20 mg; Cytarabine 2×150 mg; Etoposide 250 mg; Idarubicin 18 mg
29	Burkitt lymphoma	NHL-BFM Registry 2012	Dexamethasone 10 mg/m²; Vincristine 1.5 mg/m²; Doxorubicin 25 mg/m²; Methotrexate 5 g/m²; Cyclophosphamide 200 mg/m²
30	Relapsed AML	REZ-BFM	Idarubicin 60 mg/m²; Fludarabine 30 mg/m²; Cytarabine 2000 mg/m²; G-CSF 200 µg/m²

Data source: Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital, Central Biochemistry and Oncology Units (2016–2017).
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CNS, central nervous system; IU, international units; m², body surface area.

Table II. Distribution of Clinical Features According to Mucositis Stages

Clinical Feature	Category	Stage 1–2 (n=11)	Stage 3–4 (n=19)	p -value
Oral care education received, n (%)	yes	8 (72.7)	11 (57.9)	0.411[a]
	no	3 (27.3)	8 (42.1)	
Neutropenic diet adherence, n (%)	Sometimes	1 (9.1)	3 (15.8)	0.851[a]
	Always	1 (9.1)	2 (10.5)	
	Frequently	9 (81.8)	14 (73.7)	
Preventive mucositis treatment, n (%)	Negative	8 (72.7)	17 (89.5)	0.244[a]
	Positive	3 (27.3)	2 (10.5)	
Vitamin D use during infancy, n (%)	no	1 (9.1)	4 (21.1)	0.775[a]
	yes	10 (90.9)	15 (78.9)	
Duration of vitamin D use in infancy (months)	Mean ± SD	7.50 ± 3.53	4.60 ± 2.50	0.025[b]*
	Min–Max (Median)	3–12 (6.0)	3–12 (3.0)	
Current vitamin D or multivitamin use, n (%)	yes	8 (72.7)	15 (78.9)	0.700[a]
	no	3 (27.3)	4 (21.1)	

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Mucositis duration, n (%)	<10 days	8 (72.7)	2 (10.5)	0.002[c]**
	10–15 days	1 (9.1)	9 (47.4)	
	≥15 days	2 (18.2)	8 (42.1)	

[a] **Fisher's Exact Test;** [b] **Mann–Whitney U Test;** [c] **Fisher–Freeman–Halton Test**

- $p < 0.05$, ** $p < 0.01$
  Data source: Dr. Sami Ulus Maternity and Child Health and Diseases Training and Research Hospital, 2016–2017

Table III: Distribution of Average Vitamin Values by Groups

Parameter	Group	Mean	SD	Unit	Statistical Test	p-value
Vitamin A level	Control	513.88	112.93	ng/mL	Independent samples t-test	0.049*
Vitamin A level	Patient	442.03	188.01	ng/mL	Independent samples t-test	0.049*
Vitamin D level	Control	18.77	9.79	ng/mL	Mann–Whitney U test	0.281
Vitamin D level	Patient	22.86	20.45	ng/mL	Mann–Whitney U test	0.281

Footnotes:
Data are presented as mean ± standard deviation. Vitamin A deficiency was defined as <400 ng/mL and vitamin D deficiency as <25 ng/mL. Vitamin A levels were measured using gas chromatography–mass spectrometry (GC–MS) and vitamin D levels using high-performance liquid chromatography (HPLC). All data were obtained from the central biochemistry laboratory of Dr. Sami Ulus Hospital (2016–2017). *$p < 0.05$ was considered statistically significant. The borderline statistical significance of vitamin A levels ($p = 0.049$) should be interpreted cautiously in the context of multiple comparisons.

Table IV: Average Vitamin Levels According to Gender of Groups

Parameter	Gender	Mean± SD	Unit	Statistical Test	p-value	Interpretation
Vitamin A level	Male	489.75 ± 196.88	ng/mL	Independent samples t-test	0.047*	
Vitamin A level	Female	346.60 ± 129.94	ng/mL	Independent samples t-test	0.047*	
Vitamin D level	Male	20.90 ± 19.95	ng/mL	Mann–Whitney U test	0.468	**NS**
Vitamin D level	Female	26.78 ± 21.95	ng/mL	Mann–Whitney U test	0.468	**NS**

Footnote:
Data are presented as mean ± standard deviation. Vitamin A deficiency was defined as <400 ng/mL and vitamin D deficiency as <25 ng/mL. Vitamin A levels were measured using gas chromatography–mass spectrometry (GC–

[DOI: 10.18502/ijpho.v16i4.22764]

MS) and vitamin D levels using high-performance liquid chromatography (HPLC). *p < 0.05 was considered statistically significant; NS: not significant. Given the borderline statistical significance for vitamin A (p = 0.047), results should be interpreted cautiously, particularly in the context of multiple comparisons.

Table V: Vitamin A and D Levels According to Mucositis Stages of Cases

Parameter	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)	Stage 4 n (%)	Total n (%)	p value
Vitamin A <400 ng/mL	0 (0.0)	2 (22.2)	9 (69.2)	3 (50.0)	14 (46.7)	0.086
Vitamin A ≥400 ng/mL	2 (100.0)	7 (77.8)	4 (30.8)	3 (50.0)	16 (53.3)	
Vitamin D <25 ng/mL	2 (100.0)	5 (55.6)	9 (69.2)	6 (100.0)	22 (73.3)	0.214
Vitamin D ≥25 ng/mL	0 (0.0)	4 (44.4)	4 (30.8)	0 (0.0)	8 (26.7)	

Statistical analysis: Chi-square test
Units: Vitamin A (ng/mL), Vitamin D (ng/mL)
Data source: Dr. Sami Ulus Hospital Central Biochemistry Laboratory, 2016–2017

Table VI: Vitamin A and D Levels According to Mucositis severity

Parameter	Stage 1–2 n	Stage 1–2 %	Stage 3–4 n	Stage 3–4 %	Total n	Total %	p value
Vitamin A <400 ng/mL	2	18.2	12	63.2	14	46.7	0.02*
Vitamin A ≥400 ng/mL	9	81.8	7	36.8	16	53.3	
Vitamin D <25 ng/mL	7	63.6	15	78.9	22	73.3	0.31
Vitamin D ≥25 ng/mL	4	36.4	4	21.1	8	26.7	

Statistical analysis: Chi-square test
Units: Vitamin A (ng/mL), Vitamin D (ng/mL)
Data source: Dr. Sami Ulus Hospital Central Biochemistry Laboratory, 2016–2017
Patients with vitamin A deficiency had higher odds of severe mucositis compared with patients without vitamin A deficiency (unadjusted OR = 7.71, 95% CI: 1.29–46.22).

[DOI: 10.18502/ijpho.v16i4.22764]

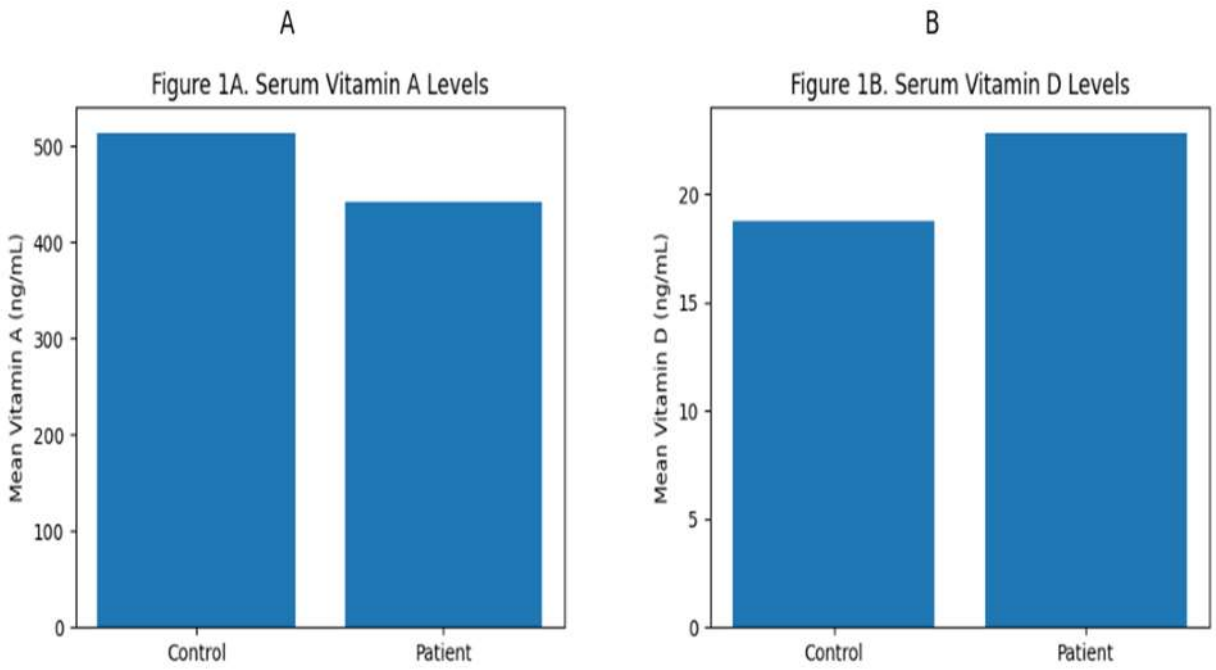

Figure 1. Comparison of serum vitamin levels between pediatric oncology patients with mucositis and healthy controls.
(A) Mean serum vitamin A concentrations in patients and controls.
(B) Mean serum vitamin D concentrations in patients and controls.

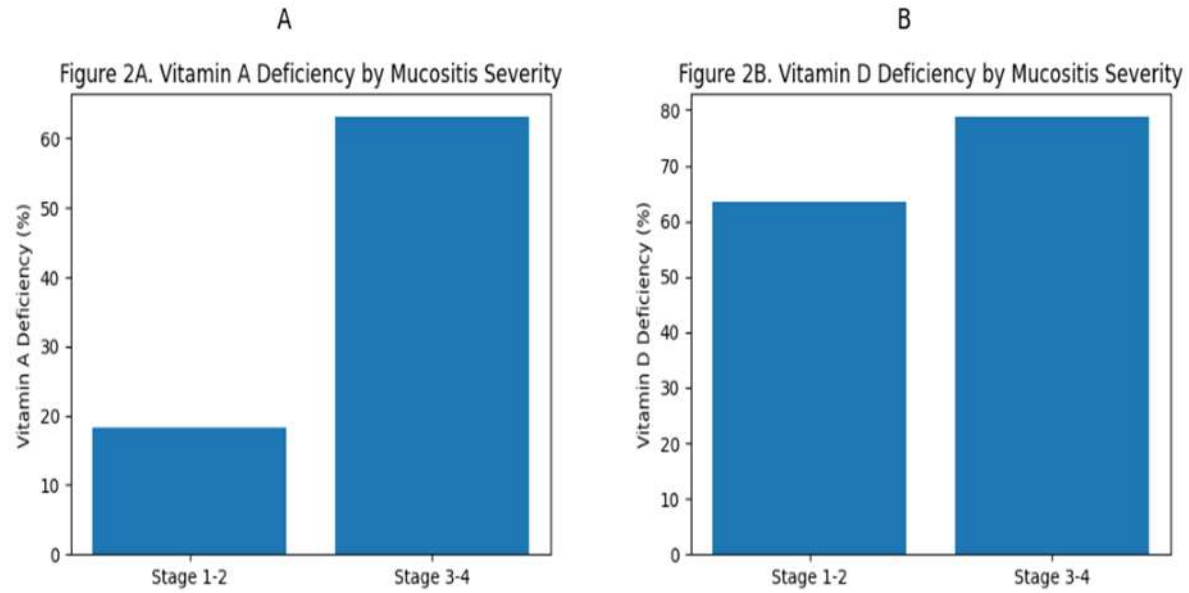

Figure 2. Vitamin deficiency according to mucositis severity.
(A) Frequency of vitamin A deficiency (<400 ng/mL) among patients with mild (WHO stages 1–2) and severe (WHO stages 3–4) mucositis.
(B) Frequency of vitamin D deficiency (<25 ng/mL) among patients with mild (WHO stages 1–2) and severe (WHO stages 3–4) mucositis.

Discussion

Mucositis remains one of the most

[DOI: 10.18502/ijpho.v16i4.22764]

challenging treatment-related toxicities in pediatric oncology, frequently occurring as a painful and dose-limiting complication of chemotherapy (CT) and radiotherapy (RT). It substantially compromises nutritional status, quality of life, and adherence to anticancer treatment while increasing hospitalization, healthcare costs, and the risk of infectious complications (2-6). Among children receiving high-dose CT or hematopoietic stem cell transplantation (HSCT), oral and gastrointestinal mucositis develops in up to 80% of patients, whereas 22–66% of patients undergoing head and neck RT experience clinically significant mucosal injury. Severe mucositis often necessitates opioid analgesia, enteral or parenteral nutritional support, and intensive supportive care because of the increased risk of neutropenic sepsis (2-6). These clinical consequences emphasize the importance of identifying factors associated with mucositis severity and improving supportive care strategies in pediatric oncology (6).

The present study demonstrated an association between lower serum vitamin A levels and greater oral mucositis severity in pediatric oncology patients. However, these findings should be interpreted cautiously. Although vitamin A deficiency was significantly more frequent among patients with severe mucositis in the secondary grouped analysis (WHO Grades 1–2 versus WHO Grades 3–4), no statistically significant association was observed when the original four WHO mucositis grades were analyzed separately. Accordingly, the principal positive finding of this study was derived from the grouped exploratory analysis and should be interpreted within that context. Furthermore, because the analyses were unadjusted and based on a relatively small retrospective cohort, the observed associations should be regarded as hypothesis-generating rather than confirmatory.

Vitamin A plays a central role in maintaining epithelial integrity, regulating mucosal immunity, promoting epithelial regeneration, and limiting oxidative tissue injury (8-10). Experimental studies have demonstrated that vitamin A supplementation preserves intestinal villus architecture, reduces crypt loss, and attenuates chemotherapy-induced mucosal injury (8,9). Limited clinical evidence has also suggested beneficial effects of topical vitamin A in reducing the severity of oral mucositis (10,11). Collectively, these observations provide biological plausibility for a relationship between vitamin A status and mucosal injury (12). Our findings are broadly consistent with this hypothesis; however, because serum vitamin A concentrations were measured only after mucositis had developed, the present study cannot determine whether lower vitamin A levels contributed to severe mucositis or were simply a consequence of the inflammatory process.

An important consideration is that vitamin A is a negative acute-phase reactant. Consequently, decreased serum vitamin A concentrations may reflect systemic inflammation, reduced oral intake, treatment-related toxicity, impaired nutritional status, or other disease-related factors rather than a pre-existing susceptibility to severe mucosal injury. Likewise, measurement of serum vitamin concentrations at a single time point precluded evaluation of temporal relationships between vitamin status and mucositis development. Prospective longitudinal studies incorporating serial vitamin measurements together with inflammatory biomarkers are therefore required to determine whether vitamin A independently influences the development or severity of chemotherapy-associated oral mucositis.

Vitamin D, although traditionally recognized for its role in calcium homeostasis and bone metabolism, also exerts important immunomodulatory effects through regulation of T-cell differentiation, cytokine production, antimicrobial peptide expression, and epithelial repair (11-14). In the present study, serum vitamin D concentrations were not significantly associated with mucositis severity. Nevertheless, patients with mild mucositis had received vitamin D supplementation during infancy for a significantly longer duration than those with severe mucositis. Given the

[DOI: 10.18502/ijpho.v16i4.22764]

observational design, small sample size, and absence of adjustment for potential confounding variables, this finding should be considered exploratory and hypothesis-generating rather than evidence of a protective effect.

Several previous studies have suggested that vitamin D may contribute to maintenance of mucosal integrity and modulation of inflammatory responses. Ghorbanzadeh et al. reported that vitamin D deficiency aggravated radiation-induced proctitis (13), whereas Anand et al. demonstrated improvements in quality of life together with reductions in treatment-related mucosal toxicity following vitamin D supplementation during chemoradiotherapy (14). In pediatric oncology, Oosterom et al. observed a markedly higher frequency of severe oral mucositis among children with serum 25-hydroxyvitamin D_3 concentrations below 20 ng/mL during methotrexate therapy (8). Similarly, Bakr et al. demonstrated that topical $25(OH)D_3$ reduced both pain and severity of oral mucositis in patients with head and neck cancer (15). Furthermore, a recent systematic review concluded that vitamin D supplementation may reduce the incidence, severity, and duration of therapy-induced oral mucositis, although the overall quality of available evidence remains limited (16). Taken together, these studies support a potential biological role of vitamin D in mucosal homeostasis. Nevertheless, our results did not demonstrate a significant association between current serum vitamin D concentrations and oral mucositis severity, underscoring the need for larger prospective studies.

Clinical Implications and Significance

Several important limitations should be considered when interpreting the present findings. First, the study population was heterogeneous with respect to both underlying malignancies and chemotherapy regimens. Because treatment intensity and the mucotoxic potential of individual chemotherapeutic agents are well-established determinants of oral mucositis severity, treatment-related factors may have influenced the observed associations. Consequently, it cannot be determined whether vitamin A deficiency was independently associated with severe mucositis or whether the findings reflected differences in treatment exposure, disease characteristics, nutritional status, or other clinical variables.

Second, the study did not include a pediatric oncology control group without oral mucositis. Comparisons were therefore limited to healthy children, making it impossible to determine whether lower serum vitamin A concentrations were specifically associated with mucositis severity or reflected broader consequences of malignancy, chemotherapy, systemic inflammation, or nutritional compromise. Future studies including pediatric oncology patients without mucositis as disease controls would help clarify whether vitamin status is independently associated with the development and severity of oral mucositis.

Third, multivariable analyses could not be performed because of the limited sample size and the relatively small number of outcome events. Therefore, important potential confounders—including cancer type, chemotherapy regimen, cumulative treatment intensity, nutritional status, inflammatory burden, and socioeconomic factors—could not be adjusted for. Consequently, the observed associations should not be interpreted as independent effects but rather as preliminary observations requiring confirmation in adequately powered prospective studies.

An additional consideration is that serum vitamin concentrations were measured only once, at the time of mucositis diagnosis. This cross-sectional assessment precluded evaluation of temporal relationships and prevented determination of whether vitamin deficiencies preceded the development of mucositis or occurred as a consequence of inflammation, reduced oral intake, or treatment-related toxicity. Serial measurements obtained before, during, and after anticancer therapy would provide a more comprehensive understanding of the dynamic relationship between vitamin status and mucosal injury.

Other clinical and demographic

[DOI: 10.18502/ijpho.v16i4.22764]

characteristics—including age, sex, family size, socioeconomic status, adherence to a neutropenic diet, breastfeeding duration, birth weight, and number of siblings—were not significantly associated with mucositis severity. Although males represented a greater proportion of patients with severe mucositis despite having higher mean serum vitamin A concentrations, this observation was not statistically significant and should be interpreted cautiously given the limited sample size.

Consistent with previous reports, higher WHO mucositis grades were associated with longer symptom duration, emphasizing the substantial clinical burden of severe mucosal injury. Although oral care education and preventive oral care measures were not significantly associated with mucositis severity in the present study, standardized oral care remains a cornerstone of supportive pediatric oncology because of its well-established role in maintaining oral hygiene, reducing secondary infections, and improving patient comfort (6).

From a clinical perspective, the present findings should not be interpreted as supporting routine vitamin supplementation for the prevention or treatment of chemotherapy-associated oral mucositis. Rather, they emphasize the importance of comprehensive nutritional assessment as part of supportive care in pediatric oncology patients, who are particularly vulnerable to nutritional deficiencies, treatment-related toxicity, and impaired oral intake. The observed association between lower serum vitamin A levels and greater mucositis severity suggests that vitamin A status may serve as a potential marker of disease severity; however, this hypothesis requires validation in prospective studies before any clinical recommendations can be made.

Future multicenter prospective studies should include pediatric oncology control groups without mucositis, standardized chemotherapy protocols, comprehensive nutritional assessments, inflammatory biomarkers, serial vitamin measurements, and adequately powered multivariable statistical analyses. Such studies will be essential to determine whether vitamin A or vitamin D status independently contributes to the development and severity of chemotherapy-associated oral mucositis or simply reflects the systemic consequences of cancer and its treatment (12).

Conclusion

Lower serum vitamin A levels and vitamin A deficiency were associated with greater oral mucositis severity in this cohort of pediatric oncology patients. However, because of the retrospective study design and the cross-sectional assessment of serum vitamin concentrations at the time of mucositis diagnosis, these findings do not establish a causal relationship.

No significant association was observed between serum vitamin D concentrations and oral mucositis severity, although the observed association between longer duration of vitamin D supplementation during infancy and less severe mucositis should be considered exploratory and hypothesis-generating.

Given the small sample size, heterogeneous malignancies and treatment regimens, absence of a pediatric oncology control group without mucositis, and inability to adjust for potential confounding factors, these findings should be interpreted with caution. Prospective multicenter studies incorporating pediatric oncology control groups, comprehensive nutritional assessment, serial vitamin measurements, inflammatory biomarkers, and multivariable analyses are warranted to determine whether vitamin status is independently associated with chemotherapy-associated oral mucositis severity.

Availability of Data

The data supporting the findings of this study are available in the supplementary material of this article.

Acknowledgements

The authors declare that they did not use artificial intelligence (AI) tools in the

[DOI: 10.18502/ijpho.v16i4.22764]

preparation of this manuscript. The authors are solely responsible for the scientific content of this study.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This research received no external funding.

Ethical Considerations

This study was approved by the Ethics Committee of Ankara Keçiören Training and Research Hospital (Decision No. 1533, dated October 25, 2017). Additionally, ethical approval previously granted by the Ethics Committee of Dr. Sami Ulus Maternity, Child Health, and Diseases Training and Research Hospital (TUEK) was deemed sufficient for the conduct of this research. All procedures were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. İn addition, Informed consent was obtained from all participants and/or their legal guardians for inclusion in the study and for publication of anonymized data.amendments.

Authors' Contributions

- Muhammet Sancaktar, MD: Conceptualization and design of the study, data collection, literature review, drafting, critical revision, and final editing of the manuscript.
- Gülseren Şahin, MD: Study methodology, data collection and manuscript writing.
- Gürses Şahin, MD: Supervision, project administration, conceptualization, methodology, data collection and revising the manuscript for intellectual content.

All authors have reviewed and approved the final version of the manuscript and accept full responsibility for the integrity and accuracy of the work.

Artificial Intelligence Use Disclosure Statement

This is an original research study conducted by the authors. Artificial intelligence was not used to generate the study, data, results, or scientific content.

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[DOI: 10.18502/ijpho.v16i4.22764]