Vitamin D and Oral Mucositis in Autologous Hematopoietic Stem Cell Transplantation: A Cross-Sectional Observation

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A B S T R A C T

Background: One of the most frequent complications of high-dose chemotherapy regimen before hematopoietic stem cell transplantation (HSCT) is oral mucositis (OM). Vitamin D (VD) has well-known immunoregulatory, anti-inflammatory, and antioxidant properties. This study aimed to evaluate the association of pre-HSCT VD level with OM as well as neutrophil and platelet engraftments in patients with multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma after autologous HSCT.

Methods: A sample of 71 patients was enrolled after obtaining informed consent. Serum samples were collected in the morning prior to the administration of conditioning regimen to measure the 25-hydroxy vitamin D (25-OH-D) level. OM was examined daily during hospital stay. The World Health Organization scale was used for scoring the OM. Absolute neutrophil count and platelet count were determined daily from transplantation until engraftment.

Results: Patients were 18 to 65 years old. Mean length of hospital stay was 15.8±5.7 days. OM was detected in 44/71 (62.0%) of patients. Mean time to the engraftment of neutrophils and platelets was 11.8±4.0 and 17.2±7.3 days, respectively. Mean level of 25-OH-D was 17.5±14.0 ng/ml. VD deficiency (<20 ng/ml) was diagnosed in 51/71 (71.8%) of patients. No association between the 25-OH-D level and incidence of OM (P=0.69) or OM grade 3-4 (P=0.46) was found. No significant correlations were detected between the 25-OH-D level and engraftment time of neutrophils (P=0.46) or platelets (P=0.17).

Conclusions: The prevalence of VD deficiency was high among the adult HSCT patients at the time of transplantation. However, no association was found between the pre-HSCT VD level and OM or engraftment time.

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Introduction

Oral mucositis (OM) is one of the most frequent adverse effects of conditioning regimen prior to hematopoietic stem cell transplantation (HSCT) in about 75% of patients (1). OM is associated with severe pain, dysphasia, and additional narcotic analgesics use. It can also increase the risk of bleeding and life-threatening infections, prolong

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the hospital stay, and raise the treatment costs and mortality (2). Chemotherapy agents form free radicals and damage the DNA, resulting in an inflammatory cascade in the first phase of OM. Then pro-inflammatory cytokines such as TNF-α, IL-1b, and IL-6 are generated. They damage the mucosal tissue and subsequently some lesions are formed (3).

Vitamin D (VD) plays main immunoregulatory roles through its receptors in various myeloid and lymphoid cells. VD can reduce the release of TNF-α and increase the synthesis of IL-10 (anti-inflammatory cytokine) (4). Moreover, VD induces the synthesis of antimicrobial peptides, such as defensin and cathelicidin, in immune cells (5). Animal studies show the anti-inflammatory effects of VD through modification of some cytokines, such as IL-1b, IL-10, and IL-17 (6).

Despite all the known benefits, VD level is suboptimal in approximately 70% to 80% of patients undergoing HSCT. The prevalence of low VD even rises after transplantation despite supplementation (7-12).

The optimal serum level of VD has not been precisely defined due to uncertainties in the relationship between VD levels and various clinical endpoints. Circulating 25-hydroxy vitamin D (25-OH-D) is the most reliable marker of the VD status reflecting the overall VD taken from the sun exposure and diet (13). The 25-OH-D level ≥20 ng/ml is a sufficient level of VD to cover the needs of 97.5% of the population (14).

Previous data suggest that VD might prevent graft-versus-host disease by immature dendritic cell populations that bias toward tolerizing rather than stimulatory T-cell populations (15). A relationship between low VD level and development of OM has been previously reported (16-18). However, the role of VD in the context of mucositis after HSCT remains unclear. Therefore, the objective of this study was to evaluate whether VD level at the time of HSCT was associated with OM and engraftment time of neutrophils and platelets.

Methods

A cross-sectional study was conducted from May to December 2015 at the adult bone marrow transplant wards of Shariati Hospital. Protocol and consent form of the study were approved by the ethics committee of Tehran University of Medical Sciences and the ethics code of IR.TUMS.REC.1394.1368 was assigned. Multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma patients waiting for autologous peripheral blood HSCT were included if declared consent.

Filgrastim 10 µg/kg/d was administered subcutaneously as the mobilizing agent from day -7 to -3. Peripheral blood progenitor cells were harvested between days -2 and -1 if the circulating CD34$^+$ cell count was >20 cell/µl. The minimum acceptable count of CD34$^+$ was 2×10^6 cell/kg of the patient body weight obtained by one or two apheresis collections.

Hodgkin's and non-Hodgkin's lymphoma patients received conditioning regimen of etoposide (600 mg/m^2), cytarabine (1200 mg/m^2), carboplatin (1500 mg/m^2), and melphalan (140 mg/m^2) on days -2 to -1. Multiple myeloma patients received the melphalan (200 mg/m^2) conditioning regimen on day -1. Autologous peripheral blood HSCT was performed on day zero.

All the post-HSCT patients received subcutaneous filgrastim (5-10 µg/kg/day based on the neutrophil response), oral fluconazole (100 mg q.12.h), oral acyclovir (200 mg q.8.h), and oral nystatin (20 drops q.3.h) during hospitalization. Transfusion of packed-cell was indicated if hemoglobin concentration was <7 g/dl. Platelet was transfused if its count was <10000/µl or higher values in the case of clinical bleeding.

In order to measure the 25-OH-D, blood specimens (5 ml) were obtained in the morning prior to the administration of conditioning regimen. They were centrifuged for five minutes to isolate serum. The serums were cryostored in microtubes at -80 ^{c} until analysis.

Enzyme immunoassay was performed for the quantitative determination of 25-OH-D by the kits of Immunodiagnostic Systems Ltd., Frankfurt am Main, Germany. Based on the level of 25-OH-D, VD status was categorized as deficiency (<20 ng/ml), insufficiency (20-29.9 ng/ml), and sufficiency (30-100 ng/ml) (19).

A thorough examination for OM was completed daily during hospital stay with the help of mouth mirrors and a high-power lamp. The WHO scale was used for scoring the OM from grade zero to four (20). The pre-HSCT 25-OH-D level association with OM incidence, duration, and severity was the primary outcome.

Absolute neutrophil count and platelet count were determined daily from day of transplantation until engraftment by the automated hematology analyzer (Sysmex KX-21N, Sysmex Corp., Kobe, Japan) and biological microscope (Olympus® CX31, Olympus Corp., Tokyo, Japan). First of three consecutive days from day of transplantation to reach the neutrophils ≥0.5×10^3/µl and platelets ≥20×10^3/µl (without platelet transfusion) were defined as the engraftment days of neutrophils and platelets, respectively. The pre-HSCT 25-OH-D level association with time to recovery of neutrophils and platelets was the secondary outcome.

A sample size of 71 was considered for a power of 90% (a=0.05) and to overcome the probable dropouts, using data from a previous relevant study (17). As the level of 25-OH-D was <30 ng/ml in the majority of patients, the 25-OH-D level ≥20 ng/ml was considered the sufficient level for grouping analyses (14).

Mean ± standard deviation (SD) or standard error (SE) was reported for continuous data. Frequency and proportions were reported for categorical data. Comparison of continuous and categorical data between

study groups were performed by Mann–Whitney U test and chi-square test, respectively. The Cox-regression analysis, Spearman's Rho test, and logistic regressions were used to evaluate the association of pre-HSCT 25-OH-D level with outcomes. All statistical analyses were performed using SPSS software (version 24 for Windows, IBM Corp., Armonk, NY, USA) and statistical significance was set at P<0.05.

Results

Flow diagram of the participants is demonstrated in Figure 1. Baseline characteristics of participants (n=71) are summarized in Table 1. Patients were between 18 and 65 years old with the mean ± SD of 46.8±13.4 years. Mean ± SD length of hospital stay was 15.8±5.7 days (from transplantation to discharge).

Figure 1. Flow diagram of the study.
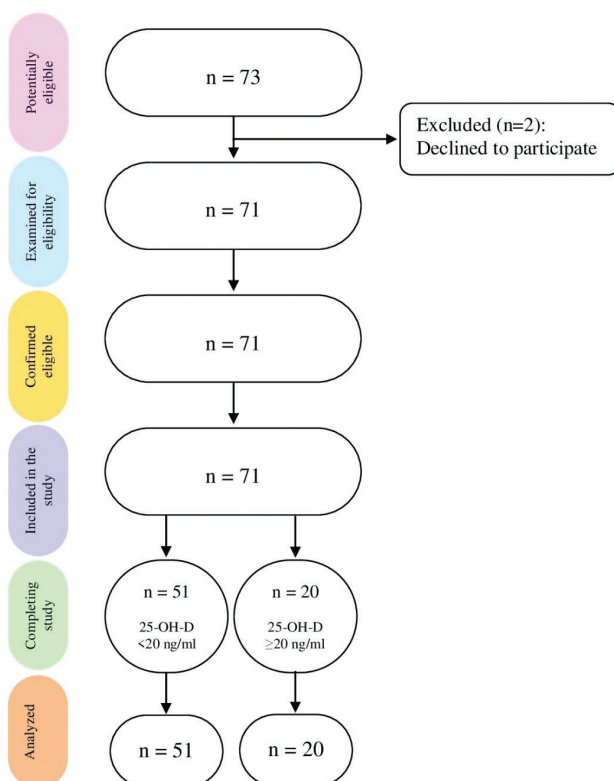
OM was detected in 44/71 (62.0%) of all patients. While 9/71 (12.7%) were diagnosed with OM grade 3-4. Mean ± SD time to the engraftment of neutrophils and platelets were 11.8±4.0 and 17.2±7.3 days, respectively.

Mean ± SD level of 25-OH-D was 17.5±14.0 ng/ml. VD deficiency in 51/71 (71.8%), insufficiency in 13/71 (18.3%), and sufficiency in 7/71 (9.9%) of all patients was diagnosed.

Logistic regressions showed no association between the pre-HSCT 25-OH-D levels and incidence of OM

(P=0.69) or OM grade 3-4 (P=0.46). Spearman's Rho test showed no significant correlations between the pre-HSCT 25-OH-D levels and duration of OM (P=0.54) or OM grade 3-4 (P=0.80). Cox-regression analysis showed no significant relations between the pre-HSCT levels of 25-OH-D and time to the beginning of OM grade 3-4 (P=0.52). No significant correlations were found between the pre-HSCT levels of 25-OH-D and engraftment time of neutrophils (P=0.46) or platelets (P=0.17) with Spearman's Rho test.

Dividing patients in two groups based on the level of pre-HSCT VD (<20 vs ≥20 ng/ml), no significant differences in terms of OM and engraftment were found (Table 2). Logistic regressions confirmed no association between the pre-HSCT 25-OH-D <20 or ≥20 ng/ml and incidence of OM (P=0.74) or OM grade 3-4 (P=0.67).

Table 1. Baseline characteristics of patients (n = 71).

Characteristics	Value
Age, mean ± SD (y)	46.8 ± 13.4
Gender, n (%)	
Male	48 (67.6)
Female	23 (32.4)
BMI, mean ± SD (kg/m²)	26.7 ± 4.3
Disease, n (%)	
Multiple myeloma	36 (50.7)
Hodgkin's lymphoma	17 (23.9)
Non-Hodgkin's lymphoma	18 (25.4)
Disease status, n (%)	
Complete remission 1	40 (56.3)
Complete remission 2	29 (40.8)
Complete remission 3	2 (2.8)
Conditioning regimen, n (%)	
MLP	36 (50.7)
VP16 + CYT + CBP + MLP	35 (49.3)

Abbreviations: BMI: body mass index; CBP: carboplatin; CYT: cytarabine; MLP: melphalan; SD: standard deviation; VP16: etoposide.

Table 2. Comparison of OM and engraftments between patients with the level of pre-HSCT 25-OH-D <20 and ≥20 ng/ml.

	<20 ng/ml (n = 51)	≥20 ng/ml (n = 20)	P value
Incidence of OM, n (%)	31 (60.8)	13 (65.0)	0.74[*]
Incidence of OM grade 3-4, n (%)	7 (13.7)	2 (10.0)	0.67[*]
Duration of OM, mean ± SE (day)	5.5 ± 0.7	5.9 ± 1.5	0.87[†]
Duration of OM grade 3-4, mean ± SE (day)	0.9 ± 0.4	0.9 ± 0.7	0.73[†]
Time to absolute neutrophil engraftment, mean ± SE (day)	12.2 ± 0.6	10.7 ± 0.4	0.49[†]
Time to platelet engraftment, mean ± SE (day)	17.9 ± 1.0	15.4 ± 1.5	0.12[†]

[*] Chi-square test
[†] Mann-Whitney U test
Abbreviations: HSCT: hematopoietic stem cell transplantation; OM: oral mucositis; SE: standard error.

Discussion

Humans attain VD mainly from exposure to sunlight and to a lesser extent from dietary sources. Changing views on optimal 25-OH-D levels complicate understanding the true prevalence of clinically significant VD deficiency. There is a broad agreement that VD levels <20 ng/ml indicate deficiency, and levels <30 ng/ml present insufficiency (19). Several risk factors following HSCT predispose patients to VD deficiency, such as low sun exposure, malnourishment, malabsorption, kidney or liver impairment, and reduction in sunlight hours during autumn and winter (21).

Despite its central roles as an important micronutrient in immunological processes, 71.8% (51/71) of our patients showed VD deficiency (<20 ng/ml) at the time of HSCT, in line with the analyses of others. In 32%, 33%, 37.3%, and 69% of children and adolescents in Campos *et al.* (9), Wallace *et al.* (11), Duncan *et al.* (7), and Hansson *et al.* (8), respectively, as well as 64% of adults in von Bahr *et al.* (10) studies <20 ng/ml levels of 25-OH-D were reported at the time of HSCT. In view of insufficiency (<30 ng/ml), the above statistics will even grow more to 90.1% (64/71) in our and 71% in Campos *et al.*, 70% in Wallace *et al.*, 80.6% in Duncan *et al.*, and 91% in Hansson *et al.* reports. This topic has been mainly studied in the allogeneic HSCT, thus data in the autologous population are scarce.

Several studies have implicated a relation between low VD level (or receptor expression) and the development of an increased inflammatory response in the mucosa, e.g. in inflammatory bowel disease, by modulating T-cell receptor responses and cytokine release (22,23). Furthermore, VD deficiency has been concerned to play a role in tissue barrier defects in the gastrointestinal system (24,25). Therefore, it could be very well justified that low VD level aggravates the course of OM.

In spite of previous reports (16-18), we did not find any association between the VD level and development of OM. In a prospective cohort study by Nejatinamini *et al.* (17), patients with head and neck cancer who developed mucositis during treatment had significantly lower plasma 25-OH-D levels. Oosterom *et al.* (18) observed a decrease in 25-OH-D levels during methotrexate therapy in children with acute lymphocytic leukemia developing severe OM compared to children without OM. Whereas VD levels before the start of methotrexate were not associated with the development of OM. Impaired intake and decreased VD uptake due to gastrointestinal mucositis might have been the cause. In Hamidieh *et al.* (16) study on Fanconi anemia, higher pre-HSCT VD level was significantly associated with higher recovery rates of OM in pediatrics. However, in another study by Kitchen *et al.* (26), no relationship was detected between the 25-OH-D level and chemotherapy-induced toxicities in a mixed group of cancer patients.

We did not find any differences between the patients with optimal and suboptimal pre-HSCT VD regarding OM and engraftments. However, interpretation of the data was limited by small sample size, especially in the normal group.

We believe that bias was minimized by analyzing the serum samples at the end of the study and hence blinding the researchers. Nevertheless, we did not determine the inflammatory markers and 25-OH-D level post-HSCT. These factors could be taken into account in future studies to get insight into the possible mechanisms. Further studies with larger sample size are required to confirm the actual relationship between VD and OM.

In conclusion, the prevalence of VD deficiency was high among adult HSCT patients at the time of transplantation. However, no association was found between the pre-HSCT level of VD and OM or engraftment time of neutrophils and platelets.

References

1. Stiff P. Mucositis associated with stem cell transplantation: current status

and innovative approaches to management. Bone Marrow Transplant 2001;27(S2):S3–S11.

2. Worthington HV, Clarkson JE, Bryan G, et al. Interventions for preventing oral mucositis for patients with cancer receiving treatment. Cochrane Database Syst Rev 2011;(4):CD000978.

3. Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004;4:277-9.84.

4. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. Immunology 2011;134(2):123-39.

5. Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: effect on haematopoiesis and immune system and clinical applications. Int J Mol Sci 2018;19(9):2663.

6. Hopkins MH, Owen J, Ahearn T, et al. Effects of supplemental vitamin D and calcium on biomarkers of inflammation in colorectal adenoma patients: a randomized, controlled clinical trial. Cancer Prev Res 2011;4(10):1645-54.

7. Duncan CN, Vrooman L, Apfelbaum EM, Whitley K, Bechard L, Lehmann LE. 25-Hydroxy vitamin D deficiency following pediatric hematopoietic stem cell transplant. Biol Blood Marrow Transplant 2011;17(5):749-53.

8. Hansson ME, Norlin AC, Omazic B, et al. Vitamin D levels affect outcome in pediatric hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2014;20(10):1537-43.

9. Campos DJ, Biagini GLK, Funke VAM, Bonfim CMS, Boguszewski CL, Borba VZC. Vitamin D deficiency in children and adolescents submitted to hematopoietic stem cell transplantation. Rev Bras Hematol Hemoter 2014;36(2):126-31.

10. von Bahr L, Blennow O, Alm J, et al. Increased incidence of chronic GvHD and CMV disease in patients with vitamin D deficiency before allogeneic stem cell transplantation. Bone Marrow Transplant.2015;50(9):1217-23.

11. Wallace G, Jodele S, Howell J, et al. Vitamin D deficiency and survival in children after hematopoietic stem cell transplant. Biol Blood Marrow Transplant 2015;21(9):1627-31.

12. Wallace G, Jodele S, Myers KC, et al. Vitamin D deficiency in pediatric hematopoietic stem cell transplantation patients despite both standard and aggressive supplementation. Biol Blood Marrow Transplant 2016;22(7):1271-4.

13. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357(3):266-81.

14. Ross AC, Manson JE, Abrams SA, et al. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J Clin Endocrinol Metab 2011;96(1):53-8.

15. Rosenblatt J, Bissonnette A, Ahmad R, et al. Immunomodulatory effects of vitamin D: implications for GvHD. Bone Marrow Transplant 2010;45(9):1463-8.

16. Hamidieh AA, Sherafatmand M, Mansouri A, et al. Calcitriol for oral mucositis prevention in patients with Fanconi anemia undergoing hematopoietic SCT: a double-blind, randomized, placebo-controlled trial. Am J Ther 2016;23(6):e1700-e8.

17. Nejatinamini S, Debenham BJ, Clugston RD, Mawani A, Parliament M, Wismer WV, et al. Poor Vitamin Status is Associated with Skeletal Muscle Loss and Mucositis in Head and Neck Cancer Patients. Nutrients 2018;10(9):1236.

18. Oosterom N, Dirks NF, Heil SG, et al. A decrease in vitamin D levels is associated with methotrexate-induced oral mucositis in children with acute lymphoblastic leukemia. Support Care Cancer 2019;27(1):183-90.

19. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab.2011;96(7):1911-30.

20. Bensinger W, Schubert M, Ang K, et al. NCCN task force report: prevention and management of mucositis in cancer care. J Natl Compr Canc Netw 2008;6:S1-21.

21. Ros-Soto J, Anthias C, Madrigal A, Snowden JA. Vitamin D: is it important in haematopoietic stem cell transplantation? a review. Bone Marrow Transplant 2019;54(6):810-820.

22. Sun J. Vitamin D and mucosal immune function. Curr Opin Gastroenterol. . 2010;26(6):591-5.

23. Lim WC, Hanauer SB, Li YC. Mechanisms of disease: vitamin D and inflammatory bowel disease. Nat Clin Pract Gastroenterol Hepatol 2005;2(7):308-15.

24. Zhang YG, Wu S, Sun J. Vitamin D, vitamin D receptor, and tissue barriers. Tissue Barriers 2013;1(1): pii: e23118.

25. Zhang YG, Wu S, Lu R, et al. Tight junction CLDN2 gene is a direct target of the vitamin D receptor. Sci Rep 2015;5:10642.

26. Kitchen D, Hughes B, Gill I, et al. The relationship between vitamin D and chemotherapy-induced toxicity - a pilot study. Br J Cancer 2012;107(1):158-60.