BMJ Open

Vitamin D levels in an Australian patient population - a large retrospective case series focusing on vitamin D levels and its association with patient demographics, medical specialty, seasonal trends and possible effect of supplementation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032567
Article Type:	Research
Date Submitted by the Author:	30-Jun-2019
Complete List of Authors:	Voo, Veronica Tsin Fong; The University of Melbourne Stankovich, Jim Butkueven, Helmut O'Brien , Terence; Monash University Monif, Mastura; Monash University,
Keywords:	Vitamin D, EPIDEMIOLOGY, Patient population, Australian cohort study

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Manuscript title:

Vitamin D levels in an Australian patient population - a large retrospective case series focusing on vitamin D levels and its association with patient demographics, medical specialty, seasonal trends and possible effect of supplementation

Authors:

Veronica Tsin Fong Voo[1,2,3], Jim Stankovich[2], Helmut Butzkueven[2,3], Terence O'Brien[2,3], Mastura Monif[1,2,3]

[1]Department of Physiology, University of Melbourne, Parkville VIC 3000 Australia
[2]Department of Neuroscience, Alfred Center, Melbourne VIC 3004 Australia
[3]Department of Neurology, Royal Melbourne Hospital, Parkville 3050 Australia

Correspondence to: mastura.monif@monash.edu

Declarations

Ethics approval

The ethics of this research was approved by Melbourne Health ethics committee (number/ID of approval QA2018084) and is done in accordance to the World Medical Association Declaration of Helsinki. Patient data and medical records extracted was de-identified and coded without identifiers.

Transparency declaration

The lead author, Veronica Voo affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

No funding and competing interests

This research received no specific grants from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activates that could appear to have influenced the submitted work.

Structured abstract

Objectives: To investigate whether gender, age, medical specialty, monthly, and seasonal variations in serum concentration of 25(OH)D are evident among an Australian patient population.

Design: Retrospective study analyzing the results of serum 25(OH)D lab tests and vitamin D supplementation from Royal Melbourne Hospital (RMH) between 2013 to 2017.

Setting: Tertiary health care center in Victoria, Australia.

Participants: 38,385 patients (inpatient and outpatient) who had their vitamin D levels measured at RMH between 2013 to 2017.

Main outcome measures: Variations in vitamin D levels according to each patient's gender, age, and medical specialty admitted to, as well as month, season and year (2013 to 2017) vitamin D level was measured.

Results: Mean vitamin D level of study population was 67.39 nmol/L (67.1 to 67.7) with P<0.0001. Only 36.8% patients in this cohort were sufficient in vitamin D (>75 nmol/L). On average, vitamin D levels in male patients were 5.68 units (5.02 to 6.33) lower than in females (P<0.0001). Linear regression analysis found that vitamin D levels increased by 0.15 unit (0.14 to 0.17) for every year increase in age (P<0.0001). One-way ANOVA showed patients from Neurology had the highest average vitamin D level, 73.34 nmol/L (71.2 to 75.5) compared to other medical specialties (P<0.0001). Mean vitamin D level during winter, 62.44 nmol/L (61.8 to 63.1) was significantly lower compared to other seasons despite supplementation (P<0.0001). Average vitamin D level measured in 2013, 60.53 nmol/L (59.7 to 61.2) was significantly lower than levels measured between 2014 to 2017 (P<0.0001).

Conclusions: There is a gender, age, medical specialty, seasonal, monthly, and yearly variation in vitamin D levels in an Australian patient population. The association between low vitamin D status and winter despite supplementation suggests other interventions are required to boast vitamin D levels.

Strengths and limitations of this study

- This is the largest study of vitamin D status in patients from a tertiary health center in Australia (n = 38,385).
- Our findings have contributed to the gap in literature concerning vitamin D status in large Australian cohorts.
- Our findings highlighted the contribution of individual characteristics, seasonal and geographic locations to vitamin D status, suggesting targeted approaches in interventions to improve vitamin D levels.
- Due to being a retrospective study, our study did not include factors such as dietary habits of patients or direct measure of sun exposure.
- The actual quantity of vitamin D supplementation was not recorded.

Introduction

Interest in the health risks of vitamin D deficiency has skyrocketed over the last 10 years. In Australia, this is reflected in the 128-fold increase in serum vitamin D testing from 2000 to 2014, and has raised the cost to Medicare from $1.02 million in 2000 to over $140 million in 2012. [1] Despite the increased interest in vitamin D, the optimal level that should be targeted remains variable. The consensus from scientific research appears to support serum 25(OH)D level sufficiency between 75-100nmol/L (with >250nmol/L as toxicity) and vitamin D deficiency is generally defined as <25nmol/L. [2-4] It is now widely accepted that vitamin D deficiency is a global health issue that afflicts more than one billion children and adults. [5] Indeed, there are good reasons why adequate vitamin D levels should be maintained in all life stages, from fetal development to old age. Low vitamin D status has been associated with an increased risk of rickets, osteoporosis, diabetes mellitus, cardiovascular disease, depression, autoimmunity, and even pregnancy complications. [6-13] Low prenatal and early life vitamin D levels may also increase susceptibility to schizophrenia and multiple sclerosis (MS) in later life. [14-16] In the elderly, higher risk of vitamin D deficiency and lower vitamin D status have been reported to be associated with increased fall risk. [17-19]

Sunlight exposure remains as the major source of vitamin D synthesis. Good dietary sources of vitamin D include fish, egg yolk, liver, and fortified foods. [20] Exposure of the skin to solar ultraviolet B (UVB; 290-315nm) radiation converts cutaneous 7-dehydrocholesterol to pre-vitamin D_3, which in turn becomes vitamin D_3. Vitamin D_3 is then metabolized to 25(OH)D in the liver and is subsequently converted to the biologically active form, $1,25(OH)_2D_3$ in the kidneys. Vitamin $1,25(OH)_2D_3$ plays a major role in calcium and phosphorus regulation, and as a result influences many metabolic pathways and skeletal health. [21] Since human vitamin D is largely synthesized in the skin, serum concentration of 25(OH)D is influenced by seasonal variation of UVB exposure, as well as time spent outdoors, the use of sunscreen, and clothing. Numerous studies from different countries have shown seasonal variation in vitamin D concentrations in children, adults, and the elderly; the lowest level is found to be at either spring or winter, and highest at either autumn or summer. [22-27] A review on worldwide vitamin D status identified children (especially those with low birth weight), pregnant women, and the elderly being at risk of vitamin D deficiency. [28] However, regarding gender variation in vitamin D concentration, findings from different studies remain inconsistent; some studies have shown women have higher serum 25(OH)D levels while others demonstrated to the contrary. [24, 27, 29]

Although variation in vitamin D levels have been addressed in several studies across different countries and geographical latitude, studies of vitamin D status in Victoria, Australia are lacking. Therefore, this study sought to investigate whether (1) gender, age, monthly, and seasonal variations in serum concentration of 25(OH)D are evident among the Victorian tertiary hospital population; (2) certain subgroups of patients are more at risk of vitamin D deficiency; and (3) patients with neurological conditions including MS have a lower vitamin D status.

Methods

Study population

This study retrospectively analyzed the results of serum 25(OH)D lab tests of 38,385 patients (inpatient and outpatient) at Royal Melbourne Hospital (RMH), Victoria, Australia between 2013 to 2017. The results were obtained from the pathology department of RMH after receiving approval from the Human Research Ethics Committee, RMH. Further, dispensing records of vitamin D supplementation (cholecalciferol 1,000 IU or 50,000 IU) were derived from pharmacy records of the same hospital.

Study design

Currently, there is no complete agreement on the definition of vitamin D deficiency or the optimal level, we therefore categorized the patients based on the recommendations from the Australian Prescriber. [30] The patients were classified into four diagnostic categories according to their serum 25(OH)D levels: deficiency (levels <25nmol/L), insufficiency (levels 25-50nmol/L), sub-optimal (levels 50-75nmol/L), and sufficiency (levels >75nmol/L). In addition, patients were also stratified according to gender, age, and the medical specialty that each patient was admitted to at the time that the vitamin D level was measured. Seasons were determined according to meteorological seasons in Australia: summer (December, January, February), autumn (March, April, May), winter (June, July, August), and spring (September, October, November).

Patient and public involvement

Due to being a retrospective study, patients were not involved in this research. Patients were not consulted to comment on the study design or interpret the study outcomes. It was difficult to directly involve patients due to data protection restrictions and patient confidentiality. Patients were not invited to contribute to the writing or editing of this document.

Statistical analyses

The statistical analysis was performed with R programming (package: dplyr) and statistical software. For those that had multiple testing, the first vitamin D test for each patient between 2013 to 2017 was used, while subsequent ones were excluded during analysis. For patients who were prescribed with multiple vitamin D prescriptions, only the ones issued >14 days before serum 25(OH)D was measured were included. This is to ensure that the vitamin D supplements have had enough time to affect serum 25(OH)D levels. Chi-square test was used to compare different vitamin D diagnostic categories, i.e. vitamin D deficient, insufficient, sub-optimal, and sufficient groups. Student's t-test was used to compare the vitamin D status between genders: female vs. male. For comparison of vitamin D levels within multiple groups, analysis of variance or one-way ANOVA with post-hoc test was utilized, i.e. age, medical specialty, seasonal, monthly, and yearly variabilities in vitamin D levels. Regression analysis was performed to analyze the relationship between age and vitamin D status. In addition, multivariable models were built to analyze the relationship between vitamin D concentration and Neurology patients, as well as the relationship between vitamin D levels and the year vitamin D tests were taken. Data is presented as mean±SEM (standard error of mean) unless specified otherwise.

Results

Characteristics of the study population are described in Table 1. Mean vitamin D serum level was 67.39 nmol/L (95% confidence interval 67.1-67.7, P<0.0001), with average age of 59.6 years (95% confidence interval 59.4-59.8, P<0.0001) (Figure 1). The study population comprised of 38,385 patients, of whom 61.6% were females and 38.3% males. Patients with undefined gender in their medical records were excluded from analysis. Female (P<0.0001), older age (P<0.0001), and summer (P<0.0001) were associated with higher vitamin D status. Male patients had significantly lower vitamin D levels compared to females. On average, vitamin D levels in male patients were 5.68 units lower than in females (95% confidence interval 5.02-6.33, P<0.0001). Patients <20 years (n=379) had the lowest mean vitamin D level (59.7 nmol/L), while patients >70 years (n=14,605) had the highest (70.8 nmol/L). Regardless, one-way ANOVA showed significant difference in average vitamin D concentrations between all age groups (P<0.0001). Linear regression analysis also found that vitamin D levels increased by 0.15 unit (95% confidence interval 0.14-0.17, P<0.0001) for every year increase in age. MS can occur at any age, but most commonly affects people between 20-40 years with a peak incidence occurring at 30 years of age. [31] Therefore, age group 20-29 was used as a reference group. Mean vitamin D level of patients >40 years was significantly higher than patients in the 20-29 year age category (Figure 2). However, mean vitamin D concentration of Neurology patients (47.7% have MS) was significantly higher than patients from all other medical specialties (Figure 3). Patients admitted to Neurology (73.34 nmol/L, 95% confidence interval 71.2-75.5) had the highest average vitamin D level, while patients from Infectious Disease (54.61 nmol/L, 95% confidence interval 49.6-59.6) had the lowest (P<0.0001). Regardless, average vitamin D levels between all medical specialties were found to be significantly different (P<0.0001). The variations in vitamin D levels correlated to the respective month and season. The lowest mean vitamin D level was found in winter (62.44 nmol/L, 95% CI 61.8-63.1), while the highest level was found in summer (72.39 nmol/L, 95% CI 70.8-73.9) (P<0.0001). Average vitamin D level at winter (Jun-Aug) was significantly lower compared to levels from all other seasons (Figure 4). The difference between the average vitamin D level between July and August and other months was 7.4 units (95% confidence interval 6.6-8.3, P<0.0001) (Figure 5). The average vitamin D concentration from tests measured in 2013 (60.53 nmol/L, 95% confidence interval 59.7-61.2) were significantly lower than levels measured in 2014-2017 (P<0.0001) (Figure 6).

Multivariable analyses

Results of the multivariable regression analyses on vitamin D levels are described in Table 2. Patients from Neurology were found to have the highest average vitamin D level compared to patients from other specialties. The average vitamin D level of patients from Neurology remained the highest after accounting for age and gender, the month and year that the vitamin D tests were measured, and vitamin D supplementation intake. The multivariable model also showed the average vitamin D level measured in 2013 remained the lowest compared to 2014 to 2017 after accounting for age, gender, medical specialty, month that the vitamin D tests were measured, and vitamin D supplementation.

Vitamin D diagnostic groups

Characteristics of the study population stratified according to their vitamin D levels are described in Table 3. Only 36.8% patients in this cohort were sufficient in vitamin D, and 55.9% of patients who were prescribed vitamin D supplementation had sufficient vitamin D levels. The proportions of patients on vitamin D supplementation varied significantly between various levels of vitamin D concentrations (P<0.0001, chi-square test). Although female patients had a higher mean vitamin D concentration than males, only 39.5% had sufficient vitamin D levels compared to 32.5% of male patients. The age of patients increased with increasing vitamin D levels; the average age (mean ± standard deviation) of patients who had vitamin D levels >75nmol/L was 60.3±21.2, while patients who were vitamin D deficient (<25nmol/L) had an average age of 57.0±21.6. Although patients from Neurology had the highest average vitamin D concentration, only 40.6% were sufficient in vitamin D levels. As expected, proportions of patients who were vitamin D sufficient were the lowest in winter (30.8%) compared to 43.0% of patients who had their vitamin D levels measured in summer. Interestingly, the proportions of patients who had their vitamin D levels measured in 2013 were significantly higher (P<0.0001) in the vitamin D deficient group, and significantly lower (P<0.0001) in the vitamin D sufficient group compared to patients between 2014 to 2017 (chi-square test). Chi-square tests also yielded significant differences between the proportions of patients with various levels of vitamin D insufficiency in the following categories: gender (P<0.0001), age (P<0.0001), medical specialty (P<0.0001), season (P<0.0001), and year (P<0.0001).

Vitamin D supplementation

Characteristics of the study population on vitamin D supplementation are shown in Table 4. A higher proportion of male patients were prescribed with supplementation compared to females. Prescription of vitamin D supplements increased with increasing age. Patients from Acute Medical Unit (AMU) and Nephrology (NEPH) were found to have the highest proportion of patients on supplementation. Unsurprisingly, higher proportion of patients were prescribed with vitamin D supplements during winter and spring. Interestingly, proportion of patients on vitamin D supplementation increased from 2013 to 2017 annually.

Discussion

This is the largest study on serum 25(OH)D levels in a tertiary hospital population in Victoria, Australia (n=38,385). Our study found age, gender, monthly, and seasonal variations in vitamin D levels. Patients who were not on vitamin D supplementation were found to have a higher risk of vitamin D deficiency, especially children and during winter. Moreover, our findings have shown that patients from Neurology had a higher mean vitamin D level compared to patients admitted to other medical specialties.

The mean serum vitamin D level for all 38,385 patients in our study was sub-optimal (67.39 nmol/L). Both female and male patients across all age groups and medical specialties showed sub-optimal serum vitamin D status. This finding is consistent to the serum vitamin D levels measured at all seasons between 2013 to 2017. Vitamin D sufficiency was only shown in patients who were on supplementation; mean vitamin D level was 80.40nmol/L (>75nnom/L). In the present study, we found that 6.3% of patients were vitamin D deficient and only 36.8% were sufficient in vitamin D. Pharmacy records on vitamin D supplementation correlated to serum 25(OH)D measured.

In the vitamin D sufficient group, 55.9% of patients were prescribed vitamin D supplements >14 days before their serum 25(OH)D levels were measured, while only 2.5% in the vitamin D deficient group were prescribed with supplements. However, this correlation was not reflected in the gender variation found in serum 25(OH)D levels measured. Females had a higher vitamin D status compared to males; 39.5% of females were sufficient in vitamin D while only 32.5% of male patients were vitamin D sufficient. According to pharmacy records, 6.7% of male patients were prescribed with vitamin D supplements, compared to 5.5% of females. Therefore, females had higher vitamin D levels despite less patients being prescribed with supplements. Our findings are in contrary with several previous studies on determinants of vitamin D status in Australia. [32, 33] Cross-sectional studies across Australia showed that overall women had lower vitamin D levels than men (southeast Queensland, P=0.06; Tasmania, P<0.01) [33] and another population-based study demonstrated vitamin D deficiency was more prevalent in women. [32] The inconsistency of our results with other studies could be due to female patients acquiring vitamin D supplements from other sources, or be a particular characteristic of patients attending a tertiary health care center.

Contrary to previous studies, [34-36] we found that vitamin D levels increased with increasing age. Vitamin D supplementation could have also contributed to this finding. Vitamin D levels were shown to increase with increasing age, but older patients were found to be more likely to be prescribed with vitamin D supplementation (Table 4), likely resulting in higher serum 25(OH)D measured. This could explain the contradiction of our findings to data demonstrating higher prevalence of vitamin D deficiency in both healthy and hospitalized elderly, [34-36] which did not take into account supplementation. However, similar to our results, users of vitamin D supplements were found to have higher serum 25(OH)D than non-users. [35, 36] Contrary to previous studies that demonstrated the association of vitamin D deficiency and neurological disorders, [37-39] our data showed that patients admitted to the Neurology specialty had the highest mean vitamin D level compared to patients from all other specialties. Pharmacy records showed that only 3.7% of patients from Neurology were prescribed with vitamin D supplementation, which was on the lower end compared to other specialties (Table 4). 47.7% of Neurology patients from our data were diagnosed with MS. Given the numerous literature on the role of vitamin D in MS, [40-43] it is conceivable that some MS patients could have acquired vitamin D supplementations from other sources, and total vitamin D intake was not recorded. This could help explain the contradiction of our results to other findings regarding vitamin D levels of patients with neurological disorders, in particular MS.

Seasonal variation in serum 25(OH)D concentration has been addressed in several prior studies. [44-46] Our results are in agreement with findings of previous studies that demonstrated lower vitamin D status during winter and higher levels during summer months. In a population-based study of 27,203 women (≥55 years) in Geelong (southeastern Australia), serum 25(OH)D was found to peak in summer and dip in winter (p<0.001), which is consistent with our results. [45] Our findings also showed monthly variation in vitamin D levels, which correlated with the seasonal variation found. Interestingly, despite the fact that more patients were prescribed vitamin D supplementation during winter (6.1%) compared to summer (5.8%), serum 25(OH)D levels remained

lower during winter, emphasizing the role of seasonal variation of UVB exposure on vitamin D status regardless of supplementation.

Notably, serum 25(OH)D status was significantly lower in 2013 compared to subsequent years (2014-2017). According to the records, the vitamin D assay used at RMH was changed from DiaSorin Liaison to Abbott Architect in Feb 2014. However comparison of the new assay to the previous one demonstrated a slope = 1 and intercept = 1nmol/L, i.e. inter-assay variability is likely to be minimal, hence the change in assay is unlikely to cause a shift in the vitamin D level measured. In fact, pharmacy records also showed a rise in prescription of vitamin D supplements every year from 2013 to 2017 (Table 4), particularly between 2013 and 2014. In 2013, only 0.2% of patients were prescribed vitamin D supplements compared to 4.1% in 2014. Thus, the rise in both serum 25(OH)D levels measured and prescription of vitamin D supplements from 2014 onwards are likely due to an increasing awareness on the health benefits of vitamin D.

Limitations

Our study had a number of limitations. First, this study was carried out retrospectively and factors such as dietary habits of patients or direct measure of sun exposure were not included. Second, there is no consensus on the desirable levels of serum 25(OH)D, we applied the recommendations from Australian Prescriber. [30] Third, since vitamin D supplementation data was derived from pharmacy dispensing records at RMH, it cannot be excluded that some patients might elect to purchase vitamin D supplements at other sources such as the supermarket, health food stores, or online. It is also important to note that because vitamin D supplementation does not require a prescription, vitamin D intake of some patients might not be included in the dispensing records. Fourth, in the supplementation data, the actual quantity of supplement (i.e. units of vitamin D dispensed) was not recorded. Fifth, the patient population presented here although quite large, may not be reflective of the general population (general community) as our patients were all from a tertiary hospital setting and likely with medical co-morbidities and potentially also with increased rates of vitamin D prescription.

Conclusion

This is the largest study of vitamin D status in patients from a tertiary health center in Australia. The findings reveal that there are gender, age, medical specialty, monthly, and seasonal variations in serum concentration of 25(OH)D. Based on our findings, patients who were not on vitamin D supplementation were at risk of vitamin D deficiency, especially children and during winter. Our findings showing older age, female gender, and Neurology patients having higher vitamin D levels are contrary to those previous reports globally. Our study reveals that despite the role of supplementation, the pharmacology and biology of vitamin D is more complex than previously thought. Further research is warranted to establish the role of vitamin D in health and disease state, as well as the variations in individual characteristics, seasonal, and geographic locations that contribute to the total vitamin D levels. Specifically given the association between vitamin D and immune-mediated diseases such as MS, understanding the role of this key vitamin would help delineate potential therapeutic approaches in combatting these diseases.

Reference

1. Medicare Australia. Medicare Australia Statistics. Available at www.medicareaustralia.gov.au [Accessed 11 February 2019].
2. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. The American journal of clinical nutrition. 2006;84(1):18-28.
3. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. Alternative medicine review : a journal of clinical therapeutic. 2005;10(2):94-111.
4. Heaney RP. The Vitamin D requirement in health and disease. The Journal of steroid biochemistry and molecular biology. 2005;97(1-2):13-9.
5. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. Reviews in endocrine & metabolic disorders. 2017;18(2):153-65.
6. Issa CM. Vitamin D and Type 2 Diabetes Mellitus. Advances in experimental medicine and biology. 2017;996:193-205.
7. Berridge MJ. Vitamin D deficiency and diabetes. The Biochemical journal. 2017;474(8):1321-32.
8. Podzolkov VI, Pokrovskaya AE, Panasenko OI. Vitamin D deficiency and cardiovascular pathology. Terapevticheskii arkhiv. 2018;90(9):144-50.
9. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. The British journal of psychiatry : the journal of mental science. 2013;202:100-7.
10. Woo YS, Kim S, Jeong JH, Jung YE, Kim MD, Bahk WM. Vitamin D Deficiency/Insufficiency among Inpatients with Depressive Symptoms. Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology. 2019;17(1):121-4.
11. Komisarenko YI, Bobryk MI. Vitamin D Deficiency and Immune Disorders in Combined Endocrine Pathology. Frontiers in endocrinology. 2018;9:600.
12. Sharif K, Sharif Y, Watad A, Yavne Y, Lichtbroun B, Bragazzi NL, et al. Vitamin D, autoimmunity and recurrent pregnancy loss: More than an association. American journal of reproductive immunology (New York, NY : 1989). 2018;80(3):e12991.
13. Bozdag H, Akdeniz E. Does severe vitamin D deficiency impact obstetric outcomes in pregnant women with thyroid autoimmunity? The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2018:1-11.
14. Balbuena LD, Middleton RM, Tuite-Dalton K, Pouliou T, Williams KE, Noble GJ. Sunshine, Sea, and Season of Birth: MS Incidence in Wales. PloS one. 2016;11(5):e0155181.
15. Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. Journal of neurology, neurosurgery, and psychiatry. 2013;84(4):427-32.
16. Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, et al. The association between neonatal vitamin D status and risk of schizophrenia. Scientific reports. 2018;8(1):17692.
17. Boettger SF, Angersbach B, Klimek CN, Wanderley ALM, Shaibekov A, Sieske L, et al. Prevalence and predictors of vitamin D-deficiency in frail older hospitalized patients. BMC Geriatrics. 2018;18(1):219.
18. Uusi-Rasi K, Patil R, Karinkanta S, Tokola K, Kannus P, Lamberg-Allardt C, et al. Serum 25-hydroxyvitamin D levels and incident falls in older women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2019;30(1):93-101.
19. van der Wielen RP, Lowik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. Lancet (London, England). 1995;346(8969):207-10.
20. Lamberg-Allardt C. Vitamin D in foods and as supplements. Progress in Biophysics and Molecular Biology. 2006;92(1):33-8.
21. Holick MF. Sunlight and Vitamin D AU - Wacker, Matthias. Dermato-Endocrinology. 2013;5(1):51-108.
22. Bhattoa HP, Nagy E, More C, Kappelmayer J, Balogh A, Kalina E, et al. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in healthy Hungarian men over 50 years of age: the HunMen Study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(1):179-86.

23. Gu Y, Zhu Z, Luan X, He J. Vitamin D status and its association with season, depression in stroke. Neurosci Lett. 2019;690:99-105.
24. Hansen L, Tjonneland A, Koster B, Brot C, Andersen R, Cohen AS, et al. Vitamin D Status and Seasonal Variation among Danish Children and Adults: A Descriptive Study. Nutrients. 2018;10(11).
25. McKenna MJ. Differences in vitamin D status between countries in young adults and the elderly. The American journal of medicine. 1992;93(1):69-77.
26. Niculescu DA, Capatina CAM, Dusceac R, Caragheorgheopol A, Ghemigian A, Poiana C. Seasonal variation of serum vitamin D levels in Romania. Arch Osteoporos. 2017;12(1):113.
27. Touvier M, Deschasaux M, Montourcy M, Sutton A, Charnaux N, Kesse-Guyot E, et al. Determinants of vitamin D status in Caucasian adults: influence of sun exposure, dietary intake, sociodemographic, lifestyle, anthropometric, and genetic factors. The Journal of investigative dermatology. 2015;135(2):378-88.
28. van Schoor NM, Lips P. Worldwide vitamin D status. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):671-80.
29. Lips P. Worldwide status of vitamin D nutrition. The Journal of steroid biochemistry and molecular biology. 2010;121(1-2):297-300.
30. Joshi D, Center JR, Eisman JA. Vitamin D deficiency in adults. Australian Prescriber. 2010;33:103-6.
31. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmunity reviews. 2010;9(5):A387-94.
32. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clinical Endocrinology. 2012;77(1):26-35.
33. van der Mei Ingrid AF, Ponsonby A-L, Engelsen O, Pasco Julie A, McGrath John J, Eyles Daryl W, et al. The High Prevalence of Vitamin D Insufficiency across Australian Populations Is Only Partly Explained by Season and Latitude. Environmental Health Perspectives. 2007;115(8):1132-9.
34. Goldray D, Mizrahi-Sasson E, Merdler C, Edelstein-Singer M, Algoetti A, Eisenberg Z, et al. Vitamin D Deficiency in Elderly Patients in a General Hospital. Journal of the American Geriatrics Society. 1989;37(7):589-92.
35. Omdahl JL, Garry PJ, Hunsaker LA, Hunt WC, Goodwin JS. Nutritional status in a healthy elderly population: vitamin D. The American journal of clinical nutrition. 1982;36(6):1225-33.
36. van der Wielen RPJ, de Groot LCPGM, van Staveren WA, Löwik MRH, van den Berg H, Haller J, et al. Serum vitamin D concentrations among elderly people in Europe. The Lancet. 1995;346(8969):207-10.
37. Annweiler C, Schott AM, Berrut G, Chauviré V, Le Gall D, Inzitari M, et al. Vitamin D and Ageing: Neurological Issues. Neuropsychobiology. 2010;62(3):139-50.
38. Littlejohns TJ, Henley WE, Lang IA, Annweiler C, Beauchet O, Chaves PHM, et al. Vitamin D and the risk of dementia and Alzheimer disease. Neurology. 2014;83(10):920.
39. Knekt P, Kilkkinen A, Rissanen H, Marniemi J, Sääksjärvi K, Heliövaara M. Serum Vitamin D and the Risk of Parkinson Disease. Archives of Neurology. 2010;67(7):808-11.
40. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS genetics. 2009;5(2):e1000369.
41. van der Mei IAF, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. Journal of Neurology. 2007;254(5):581.
42. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60.
43. Goldberg P, Fleming MC, Picard EH. Multiple sclerosis: Decreased relapse rate through dietary supplementation with calcium, magnesium and vitamin D. Medical Hypotheses. 1986;21(2):193-200.
44. Ma F, Levis S, Roos BA, Lai S, Gomez A, Jimenez C, et al. Vitamin D Deficiency and Seasonal Variation in an Adult South Florida Population. The Journal of Clinical Endocrinology & Metabolism. 2005;90(3):1557-62.
45. Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR, et al. Seasonal Periodicity of Serum Vitamin D and Parathyroid Hormone, Bone Resorption, and Fractures: The Geelong Osteoporosis Study. Journal of Bone and Mineral Research. 2004;19(5):752-8.
46. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. Canadian Medical Association Journal. 2002;166(12):1517.

Figure 1. Histogram of vitamin D distribution in study population (N=38,385)

Figure 1. Histogram showing distribution of vitamin D levels (nmol/L) among 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. The vertical solid line (in red) is the mean value, 67.39±0.16 nmol/L.

Figure 2. Bar graph of age variation in vitamin D levels in study population (N=38,385)

Figure 2. Bar graph showing age variation in vitamin D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Vitamin D levels increased with age, and patients >40 years had significantly higher mean vitamin D concentrations compared to age 20-29 years. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 3. Bar graph of variations in vitamin D level of patients admitted to various medical specialties (N=38,385)

Figure 3. Bar graph showing variation in vitamin D levels (nmol/L) of 38,385 patients admitted to all medical specialties at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis. Analysis showed the average vitamin D levels of patients from all other specialties were significantly lower compared to NEUR. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 4. Line graph of seasonal variation in vitamin D levels in study population (N=38,385)

Figure 4. Line graph showing seasonal variation in vitamin D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Analysis showed the average vitamin D levels of all other seasons were significantly higher compared to winter. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 5. Line graph of monthly variation in vitamin D levels in study population (N=38,385)

Figure 5. Line graph showing monthly variation in vitamin D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 6. Line graph of yearly variation (2013-2017) in vitamin D levels in study population (N=38,385)

Figure 6. Line graph showing variation in vitamin D levels (nmol/L) of 38,385 patients measured between 2013 to 2017 at Royal Melbourne Hospital, Victoria, Australia. Compared to 2014 to 2017, the mean vitamin D concentration measured in 2013 was significantly lower. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Table 1. Characteristics of the study population (N=38,385) and average vitamin D levels.

	N (%)	Mean vitamin D (SEM)
Total population	38385 (100%)	67.39 (0.16)
Gender		
Female	23626 (61.6%)	69.56 (0.21)
Male	14711 (38.3%)	63.88 (0.26)
Age group		
<20	379 (1.0%)	59.7 (1.79)
20-29	3605 (9.4%)	62.2 (0.51)
30-39	4844 (12.6%)	63.4 (0.48)
40-49	4518 (11.8%)	64.4 (0.49)
50-59	5128 (13.4%)	66.6 (0.46)
60-69	5306 (13.8%)	69.1 (0.42)
>70	14605 (38.1%)	70.8 (0.26)
Medical specialty		
NEUR	820 (2.1%)	73.34 (1.42)
AMU	1618 (4.2%)	68.92 (0.83)
BOE	1282 (3.3%)	69.09 (0.82)
EMER	2076 (5.4%)	68.54 (0.75)
ENDO	1479 (3.9%)	70.84 (0.82)
GAST	1885 (4.9%)	64.38 (0.70)
NEPH	1544 (4.0%)	62.06 (0.72)
ORTH	1334 (3.5%)	66.80 (0.80)
OTHER	17310 (45.1%)	66.65 (0.24)
PRIV	7737 (20.2%)	70.91 (0.39)
VIDS	1300 (3.4%)	54.61 (0.81)
Season of measurement		
Summer (Dec-Feb)	8711 (22.7%)	72.39 (0.52)
Autumn (Mar-May)	10030 (26.1%)	71.52 (0.31)
Winter (Jun-Aug)	9979 (26.0%)	62.44 (0.32)
Spring (Sep-Nov)	9665 (25.2%)	63.73 (0.33)
Year of measurement		
2013	8708 (22.7%)	60.53 (0.30)
2014	8631 (22.5%)	70.88 (0.35)
2015	7135 (18.6%)	70.64 (0.39)
2016	7120 (18.5%)	68.19 (0.39)
2017	6791 (17.7%)	67.53 (0.40)
Vitamin D supplementation		
Yes	2285 (6.0%)	80.40 (0.66)
No information	36100 (94.0%)	66.60 (0.17)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 2: Results of multivariable linear regression analyses to model vitamin D levels.

	Adjusted difference in mean vitamin D	95% confidence interval	P-value
Medical specialty			
NEUR	Reference	-	-
AMU	-10.9	-13.5, -8.2	<0.0001
BOE	-8.1	-10.9, -5.4	<0.0001
EMER	-9.3	-11.8, -6.8	<0.0001
ENDO	-4.1	-6.7, -1.4	0.003
GAST	-9.1	-11.6, -6.5	<0.0001
NEPH	-12.4	-15.0, -9.7	<0.0001
ORTH	-12.1	-14.9, -9.4	<0.0001
OTHER	-8.8	-11.0, -6.7	<0.0001
PRIV	-2.5	-4.8, -0.3	0.026
VIDS	-16.2	-18.9, -13.5	<0.0001
Year of measurement			
2013	Reference	-	-
2014	10.1	9.2, 11.0	<0.0001
2015	9.4	8.5, 10.4	<0.0001
2016	7.3	6.3, 8.2	<0.0001
2017	6.8	5.8, 7.8	<0.0001

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 3. Characteristics of the study population stratified according to vitamin D levels.

	Total N	Deficiency (<25nmol/L)	Insufficiency (25-50nmol/L)	Sub-optimal (50-75nmol/L)	Sufficiency (>75nmol/L)
Total population	38385	2437 (6.3%)	9233 (24.1%)	12577 (32.8%)	14138 (36.8%)
Gender					
Female	23626	1308 (5.5%)	5238 (22.2%)	7748 (32.8%)	9332 (39.5%)
Male	14711	1128 (7.7%)	3987 (27.1%)	4816 (32.7%)	4780 (32.5%)
Medical specialty					
AMU	1618	150 (9.3%)	341 (21.1%)	451 (27.9%)	676 (41.8%)
BOE	1282	54 (4.2%)	278 (21.7%)	453 (35.3%)	497 (38.8%)
EMER	2076	165 (7.9%)	493 (23.7%)	582 (28.1%)	836 (40.3%)
ENDO	1479	62 (4.2%)	312 (21.2%)	507 (34.3%)	598 (40.4%)
GAST	1885	114 (6.1%)	538 (28.5%)	618 (32.8%)	615 (32.6%)
NEPH	1544	124 (8.0%)	433 (28.1%)	514 (33.3%)	473 (30.6%)
NEUR	820	47 (5.7%)	167 (20.4%)	273 (33.2%)	333 (40.6%)
ORTH	1334	91 (6.8%)	286 (21.4%)	469 (35.2%)	488 (36.6%)
OTHER	17310	1103 (6.4%)	4227 (24.4%)	5703 (32.9%)	6277 (36.3%)
PRIV	7737	357 (4.6%)	1702 (22.0%)	2609 (33.7%)	3069 (39.7%)
VIDS	1300	170 (13.1%)	456 (35.1%)	398 (30.6%)	276 (21.2%)
Season of measurement					
Summer (Dec-Feb)	8711	329 (3.8%)	1643 (18.9%)	2993 (34.4%)	3746 (43.0%)
Autumn (Mar-May)	10030	384 (3.8%)	2037 (20.3%)	3424 (34.1%)	4185 (41.7%)
Winter (Jun-Aug)	9979	869 (8.7%)	2910 (29.2%)	3123 (31.3%)	3077 (30.8%)
Spring (Sep-Nov)	9665	855 (8.9%)	2643 (27.4%)	3037 (31.4%)	3130 (32.4%)
Year of measurement					
2013	8708	771 (8.9%)	2390 (27.4%)	3146 (36.1%)	2401 (27.6%)
2014	8631	315 (3.7%)	1943 (22.5%)	2900 (33.6%)	3473 (40.2%)
2015	7135	301 (4.2%)	1604 (22.5%)	2364 (33.1%)	2866 (40.2%)
2016	7120	512 (7.2%)	1663 (23.4%)	2183 (30.7%)	2762 (38.8%)
2017	6791	538 (7.9%)	1633 (24.1%)	1984 (29.2%)	2636 (38.8%)
Vitamin D supplementation					
Yes	2285	58 (2.5%)	265 (11.6%)	684 (29.9%)	1278 (55.9%)
No information	36100	2379 (6.6%)	8968 (24.8%)	11893 (32.9%)	12680 (35.6%)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 4. Characteristics of the study population on vitamin D supplementation

	Vitamin D supplementation +	No information (%)
Gender		
Female	1300 (5.5%)	22326 (94.5%)
Male	985 (6.7%)	13726 (93.3%)
Age group		
<20	2 (0.5%)	377 (99.5%)
20-29	89 (2.5%)	3516 (97.5%)
30-39	106 (2.2%)	4738 (97.8%)
40-49	125 (2.8%)	4393 (97.2%)
50-59	189 (3.7%)	4939 (96.3%)
60-69	293 (5.5%)	5013 (94.5%)
>70	1481 (10.1%)	13124 (89.9%)
Medical specialty		
NEUR	30 (3.7%)	790 (96.3%)
AMU	288 (17.8%)	1330 (82.2%)
BOE	20 (1.6%)	1262 (98.4%)
EMER	187 (9.0%)	1889 (91.0%)
ENDO	110 (7.4%)	1369 (92.6%)
GAST	98 (5.2%)	1787 (94.8%)
NEPH	157 (10.2%)	1387 (89.8%)
ORTH	90 (6.7%)	1244 (93.3%)
OTHER	1188 (6.9%)	16122 (93.1%)
PRIV	43 (0.6%)	7694 (99.4%)
VIDS	74 (5.7%)	1226 (94.3%)
Season of measurement		
Summer (Dec-Feb)	502 (5.8%)	8209 (94.2%)
Autumn (Mar-May)	563 (5.6%)	9467 (94.4%)
Winter (Jun-Aug)	605 (6.1%)	9374 (93.9%)
Spring (Sep-Nov)	615 (6.4%)	9050 (93.6%)
Year of measurement		
2013	19 (0.2%)	8689 (99.8%)
2014	352 (4.1%)	8279 (95.9%)
2015	551 (7.7%)	6584 (92.3%)
2016	657 (9.3%)	6463 (90.7%)
2017	706 (10.4%)	6085 (89.6%)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Authorship statement

Manuscript title:

Vitamin D levels in an Australian patient population - a large retrospective case series focusing on vitamin D levels and its association with patient demographics, medical specialty, seasonal trends and possible effect of supplementation

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *British Medical Journal (BMJ)*.

Authorship contributions

Conception and design of study: Monif M, Butzkueven H, Voo VTF, Stankovich J, O'Brien T

Acquisition of data: Monif M, Voo VTF

Analysis and/or interpretation of data: Stankovich J, Voo VTF, Monif M, Butzkueven H

Drafting the manuscript: Voo VTF

Revising the manuscript critically: Voo VTF, Monif M, Stankovich J, O'Brien T

All authors contributed to refinement of the study protocol and approved the final manuscript.

Acknowledgements

We would like to acknowledge Maria Bisignano, research support coordinator at Melbourne Health Shared Pathology Service for extracting the results of serum 25(OH)D lab tests from Royal Melbourne Hospital pathology services.

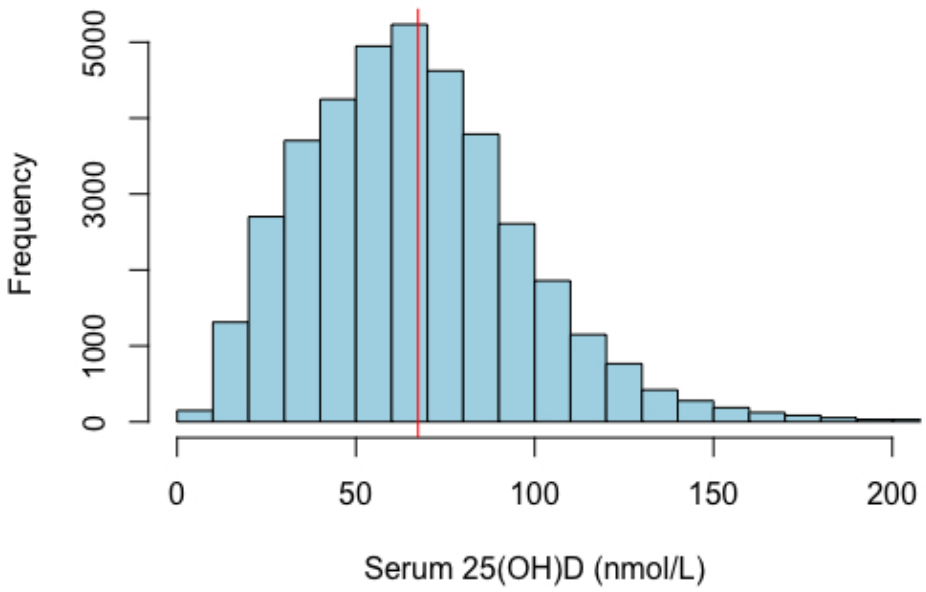
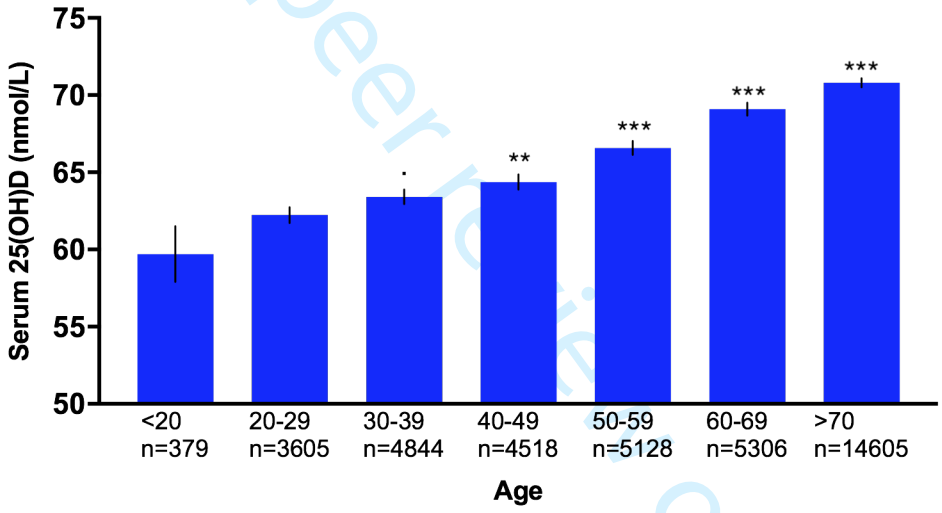
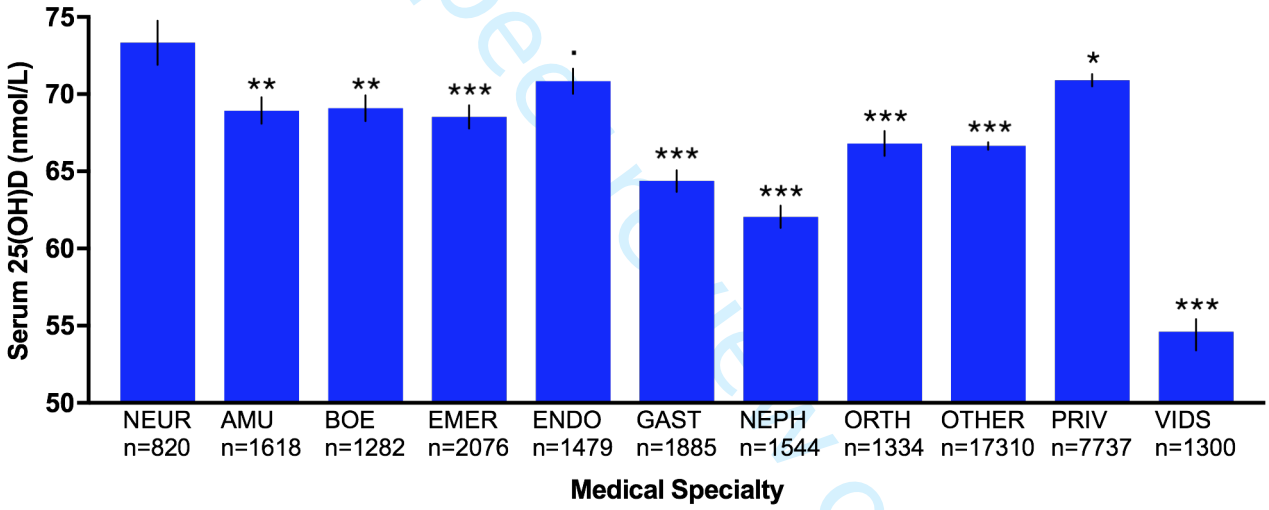
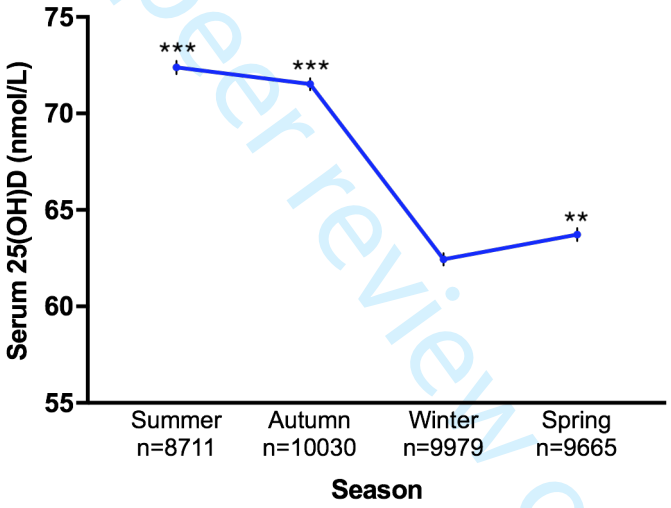


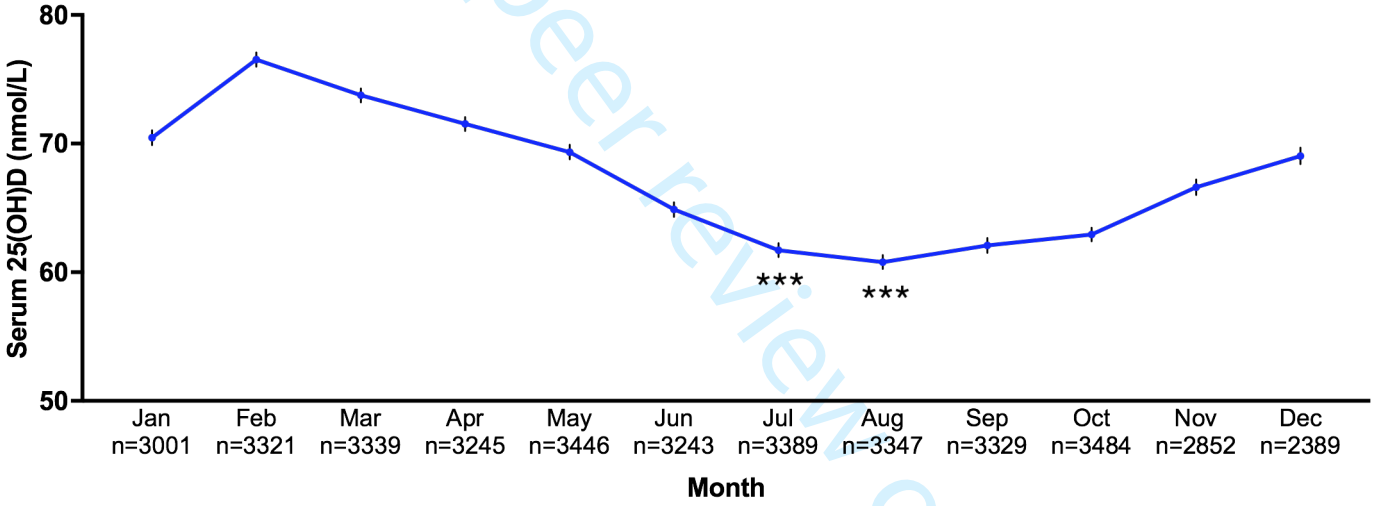
Figure 1. Histogram of vitamin D distribution in study population (N=38,385)
Figure 1. Histogram showing distribution of vitamin D levels (nmol/L) among 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. The vertical solid line (in red) is the mean value, 67.39□0.16 nmol/L.

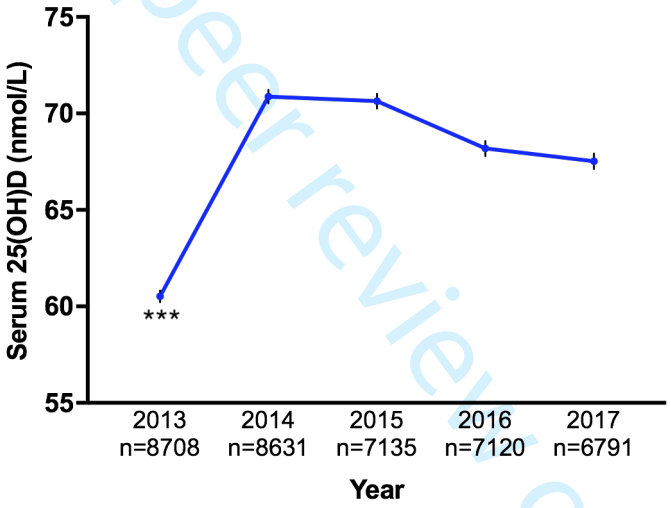
184x141mm (72 x 72 DPI)











BMJ Open

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors that influence variations in serum 25(OH)D.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032567.R1
Article Type:	Original research
Date Submitted by the Author:	17-Sep-2019
Complete List of Authors:	Voo, Veronica Tsin Fong; The University of Melbourne Stankovich, Jim ; Monash University Faculty of Medicine Nursing and Health Sciences Butzkueven , Helmut ; Monash University Faculty of Medicine Nursing and Health Sciences, Neuroscience O'Brien , Terence; Monash University Monif, Mastura; Monash University,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Nutrition and metabolism, Public health
Keywords:	Vitamin D, EPIDEMIOLOGY, Patient population, Australian cohort study

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Manuscript title:

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors that influence variations in serum 25(OH)D

Authors:

Veronica Tsin Fong Voo[1,2,3], Jim Stankovich[2], Helmut Butzkueven[2,3], Terence O'Brien[2,3], Mastura Monif[1,2,3]

[1]Department of Physiology, University of Melbourne, Grattan Street, Parkville VIC 3000 Australia
[2]Department of Neuroscience, Alfred Centre, 99 Commercial Road, Melbourne VIC 3004 Australia
[3]Department of Neurology, Royal Melbourne Hospital, Grattan Street, Parkville 3050 Australia

Correspondence to: mastura.monif@monash.edu

Declarations

Ethics approval

The ethics of this research was approved by Melbourne Health ethics committee (number/ID of approval QA2018084) and is done in accordance to the World Medical Association Declaration of Helsinki. Patient data and medical records extracted was de-identified and coded without identifiers.

Transparency declaration

The lead author, Veronica Voo affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

No funding and competing interests

This research received no specific grants from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activates that could appear to have influenced the submitted work.

Structured abstract

Objectives: To investigate whether gender, age, medical specialty, monthly, and seasonal variations in serum concentration of 25-hydroxy vitamin D (25(OH)D) are evident among an Australian patient population.

Design: Retrospective study analyzing the results of serum 25(OH)D lab tests and vitamin D supplementation from Royal Melbourne Hospital (RMH) between 2013 to 2017.

Setting: Tertiary health care center in Victoria, Australia.

Participants: 38,385 patients (inpatient and outpatient) who had their serum 25(OH)D levels measured at RMH between 2013 to 2017.

Main outcome measures: Serum 25(OH)D levels stratified according to patients' age, gender and medical specialty admitted to, as well as the month, season and year (2013 to 2017) 25(OH)D level was measured

Results: Mean serum 25(OH)D level of study population was 67.39 nmol/L (67.1 to 67.7). Only 36.8% patients in this cohort were sufficient in vitamin D (>75 nmol/L). On average, 25(OH)D levels in male patients were 5.68 units (5.02 to 6.33) lower than in females (P<0.0001). Linear regression analysis found that 25(OH)D levels increased by 0.15 unit (0.14 to 0.17) for every year increase in age (P<0.0001). One-way ANOVA showed patients from Neurology had the highest average 25(OH)D level, 73.34 nmol/L (71.2 to 75.5) compared to other medical specialties (P<0.0001). Mean 25(OH)D level during winter, 62.44 nmol/L (61.8 to 63.1) was significantly lower compared to other seasons despite supplementation (P<0.0001). Average 25(OH)D level measured in 2013, 60.53 nmol/L (59.7 to 61.2) was significantly lower than levels measured between 2014 to 2017 (P<0.0001).

Conclusions: There is a gender, age, medical specialty, seasonal, monthly, and yearly variation in vitamin D status in an Australian patient population. The association between low vitamin D status and winter despite supplementation suggests other interventions are required to boost serum 25(OH)D levels.

Strengths and limitations of this study

- This is the largest study of vitamin D status in patients from a tertiary health center in Australia (n = 38,385).
- Our findings have contributed to the gap in literature concerning vitamin D status in large Australian cohorts.
- Our findings highlighted the contribution of individual characteristics and seasonal variation of UVB exposure to vitamin D status, suggesting targeted approaches in interventions to improve vitamin D status.
- Due to being a retrospective study, our study did not include factors such as dietary habits of patients or direct measure of sun exposure.
- The actual quantity of vitamin D supplementation was not recorded.

Introduction

Interest in the health risks of vitamin D deficiency has skyrocketed over the last 10 years. In Australia, this is reflected in the 128-fold increase in serum vitamin D testing from 2000 to 2014, and has raised the cost to Medicare from $1.02 million in 2000 to over $140 million in 2012. [1] Despite the increased interest in vitamin D, the optimal level that should be targeted remains variable. The consensus from scientific research appears to support serum 25-hydroxy vitamin D (25(OH)D) level sufficiency >50nmol/L and vitamin D deficiency is generally defined as <25nmol/L. [2-4] It is now widely accepted that adequate vitamin D levels should be maintained in all life stages, from fetal development to old age. Low vitamin D status has been associated with an increased risk of rickets, osteoporosis, diabetes mellitus, cardiovascular disease, depression, autoimmunity, and even pregnancy complications. [5-12] Low prenatal and early life vitamin D levels may also increase susceptibility to schizophrenia and multiple sclerosis (MS) in later life. [13-15] In the elderly, higher risk of vitamin D deficiency and lower vitamin D status have been reported to be associated with increased fall risk. [16, 17]

Sunlight exposure remains as the major source of vitamin D synthesis. Exposure of the skin to solar ultraviolet B (UVB; 290-315nm) radiation converts cutaneous 7-dehydrocholesterol to pre-vitamin D_3, which in turn becomes vitamin D_3. Vitamin D_3 is then metabolized to 25(OH)D in the liver and is subsequently converted to the biologically active form, $1,25(OH)_2D_3$ in the kidneys. Vitamin $1,25(OH)_2D_3$ plays a major role in calcium and phosphorus regulation, and as a result influences many metabolic pathways and skeletal health. [18] Since human vitamin D is largely synthesized in the skin, serum concentration of 25(OH)D is influenced by seasonal variation of UVB exposure, as well as time spent outdoors, the use of sunscreen, and clothing. Numerous studies from different countries have shown seasonal variation in vitamin D concentrations in children, adults, and the elderly; the lowest level is found to be at either spring or winter, and highest at either autumn or summer. [19-24] A review on worldwide vitamin D status identified children (especially those with low birth weight), pregnant women, and the elderly being at risk of vitamin D deficiency. [25] However, regarding gender variation in vitamin D concentration, findings from different studies remain inconsistent; some studies have shown women have higher serum 25(OH)D levels while others demonstrated to the contrary. [22, 24, 26]

Although variation in vitamin D levels have been addressed in several studies across different countries and geographical latitude, studies of vitamin D status in Victoria, Australia are lacking. Therefore, this study sought to investigate whether (1) gender, age, monthly, and seasonal variations in serum concentration of 25(OH)D are evident among the Victorian tertiary hospital population; (2) certain subgroups of patients are more at risk of vitamin D deficiency; and (3) patients with neurological conditions including MS have a lower vitamin D status.

Methods

Study population

This study retrospectively analyzed the results of serum 25(OH)D lab tests of 38,385 patients (inpatient and outpatient) at Royal Melbourne Hospital (RMH), Victoria, Australia between 2013 to 2017. The results were obtained from the pathology department of RMH after receiving approval from the Human Research Ethics Committee, RMH. Further, dispensing records of vitamin D supplementation (cholecalciferol 1,000 IU or 50,000 IU) were derived from pharmacy records of the same hospital.

Laboratory assays for serum 25(OH)D measurements

The vitamin D assay used in 2013 to February 2014 was the DiaSorin LIAISON immunoassay, and on February 2014 the assay was changed to the Abbott ARCHITECT immunoassay. Alternative (quantitative) method comparison between the two assays was conducted. Regression analysis found the slope to be 1.075 (95% confidence interval 0.858-1.192, standard error estimate 20.78) with an intercept of 0.99nmol/L (95% confidence interval -8.08-10.05) and a Pearson coefficient of 0.8836. Approximately 80 samples were tested using the two methods and the mean bias between the two assays was found to be 5.94 (with a percent bias of 8.57%). Hence, inter-assay variability is small and the change in assay is unlikely to cause a significant shift in the 25(OH)D level measured.

Study design

Currently, there is no complete agreement on the definition of vitamin D deficiency or the optimal level, we therefore categorized the patients based on the recommendations from the Australian Prescriber. [27] The patients were classified into four diagnostic categories according to their serum 25(OH)D levels: deficiency (levels <25nmol/L), insufficiency (levels 25-50nmol/L), sub-optimal (levels 50-75nmol/L), and sufficiency (levels >75nmol/L). In addition, patients were also stratified according to gender, age, and the medical specialty that each patient was admitted to at the time that their serum 25(OH)D concentration was measured. Seasons were determined according to meteorological seasons in Australia: summer (December, January, February), autumn (March, April, May), winter (June, July, August), and spring (September, October, November).

Patient and public involvement

Due to being a retrospective study, patients were not involved in this research. Patients were not consulted to comment on the study design or interpret the study outcomes. It was difficult to directly involve patients due to data protection restrictions and patient confidentiality. Patients were not invited to contribute to the writing or editing of this document.

Statistical analyses

The statistical analysis was performed with R programming (package: dplyr) and statistical software. For those that had multiple testing, the first vitamin D test for each patient between 2013 to 2017 was used, while subsequent ones were excluded during analysis. For patients who were prescribed with multiple vitamin D

prescriptions, only the ones issued >14 days before serum 25(OH)D was measured were included. This is to ensure that the vitamin D supplements have had enough time to affect serum 25(OH)D levels. Chi-square test was used to compare different vitamin D diagnostic categories, i.e. vitamin D deficient, insufficient, sub-optimal, and sufficient groups. Student's t-test was used to compare the vitamin D status between genders: female vs. male. For comparison of vitamin D levels within multiple groups, analysis of variance or one-way ANOVA with post-hoc test was utilized, i.e. age, medical specialty, seasonal, monthly, and yearly variabilities in 25(OH)D concentration. Regression analysis was performed to analyze the relationship between age and vitamin D status. In addition, multivariable models were built to analyze the relationship between 25(OH)D concentration and Neurology patients, as well as the relationship between vitamin D status and the year vitamin D tests were taken. Data is presented as mean±SEM (standard error of mean) unless specified otherwise.

Results

Characteristics of the study population are described in Table 1. Mean serum 25(OH)D concentration was 67.39nmol/L (95% confidence interval 67.1-67.7), with average age of 59.6 years (95% confidence interval 59.4-59.8) (Figure 1). The study population comprised of 38,385 patients, of whom 61.6% were females and 38.3% males. Patients with undefined gender in their medical records were excluded from analysis. Female (P<0.0001), older age (P<0.0001), and summer (P<0.0001) were associated with higher vitamin D status. Male patients had significantly lower 25(OH)D levels compared to females. On average, 25(OH)D levels in male patients were 5.68 units lower than in females (95% confidence interval 5.02-6.33, P<0.0001). Patients <20 years (n=379) had the lowest mean vitamin D status (59.7 nmol/L), while patients >70 years (n=14,605) had the highest (70.8 nmol/L). Regardless, one-way ANOVA showed significant difference in average 25(OH)D concentrations between all age groups (P<0.0001). Linear regression analysis also found that vitamin D status increased by 0.15 unit (95% confidence interval 0.14-0.17, P<0.0001) for every year increase in age. MS can occur at any age, but most commonly affects people between 20-40 years with a peak incidence occurring at 30 years of age. [28] Therefore, age group 20-29 was used as a reference group. Mean serum 25(OH)D level of patients >40 years was significantly higher than patients in the 20-29 year age category (Figure 2). However, mean 25(OH)D concentration of Neurology patients (47.7% have MS) was significantly higher than patients from all other medical specialties (Figure 3). Patients admitted to Neurology (73.34 nmol/L, 95% confidence interval 71.2-75.5) had the highest average 25(OH)D level, while patients from Infectious Disease (54.61 nmol/L, 95% confidence interval 49.6-59.6) had the lowest (P<0.0001). Regardless, average 25(OH)D levels between all medical specialties were found to be significantly different (P<0.0001). The variations in vitamin D status corresponded to the respective month and season. The lowest mean 25(OH)D level was found in winter (62.44 nmol/L, 95% confidence interval 61.8-63.1), while the highest level was found in summer (72.39 nmol/L, 95% confidence interval 70.8-73.9) (P<0.0001). Average 25(OH)D level at winter (Jun-Aug) was significantly lower compared to levels from all other seasons (Figure 4). The difference between the average 25(OH)D level between July and August and other months was 7.4 units (95% confidence interval 6.6-8.3, P<0.0001) (Figure 5). The average 25(OH)D concentration from tests measured in 2013 (60.53 nmol/L, 95% confidence interval 59.7-61.2) were significantly lower than levels measured in 2014-2017 (P<0.0001) (Figure 6).

Multivariable analyses

Results of the multivariable regression analyses on 25(OH)D levels are described in Table 2. Patients from Neurology were found to have the highest average 25(OH)D level compared to patients from other specialties. The average 25(OH)D level of patients from Neurology remained the highest after accounting for age and gender, the month and year that the vitamin D tests were measured, and vitamin D supplementation intake. The multivariable model also showed the average 25(OH)D level measured in 2013 remained the lowest compared to 2014 to 2017 after accounting for age, gender, medical specialty, month that the vitamin D tests were measured, and vitamin D supplementation.

Vitamin D diagnostic groups

Characteristics of the study population stratified according to their serum 25(OH)D levels are described in Table 3. Only 36.8% patients in this cohort were sufficient in vitamin D, and 55.9% of patients who were prescribed vitamin D supplementation had sufficient vitamin D levels. The proportions of patients on vitamin D supplementation varied significantly between various levels of 25(OH)D concentrations (P<0.0001, chi-square test). Although female patients had a higher mean 25(OH)D concentration than males, only 39.5% had sufficient 25(OH)D levels compared to 32.5% of male patients. The age of patients increased with increasing 25(OH)D levels; the average age (mean ± standard deviation) of patients who had 25(OH)D levels >75nmol/L was 60.3±21.2, while patients who were vitamin D deficient (<25nmol/L) had an average age of 57.0±21.6. Although patients from Neurology had the highest average 25(OH)D concentration, only 40.6% were sufficient in vitamin D levels. As expected, proportions of patients who were vitamin D sufficient were the lowest in winter (30.8%) compared to 43.0% of patients who had their 25(OH)D levels measured in summer. Interestingly, the proportions of patients who had their 25(OH)D levels measured in 2013 were significantly higher (P<0.0001) in the vitamin D deficient group, and significantly lower (P<0.0001) in the vitamin D sufficient group compared to patients between 2014 to 2017 (chi-square test). Chi-square tests also yielded significant differences between the proportions of patients with various levels of vitamin D insufficiency in the following categories: gender (P<0.0001), age (P<0.0001), medical specialty (P<0.0001), season (P<0.0001), and year (P<0.0001).

Vitamin D supplementation

Characteristics of the study population on vitamin D supplementation are shown in Table 4. A higher proportion of male patients were prescribed with supplementation compared to females. Prescription of vitamin D supplements increased with increasing age. Patients from Acute Medical Unit (AMU) and Nephrology (NEPH) were found to have the highest proportion of patients on supplementation. Unsurprisingly, higher proportion of patients were prescribed with vitamin D supplements during winter and spring. Interestingly, proportion of patients on vitamin D supplementation increased from 2013 to 2017 annually.

Discussion

This is the largest study on serum 25(OH)D levels in a tertiary hospital population in Victoria, Australia (n=38,385). Our study found age, gender, monthly, and seasonal variations in serum 25(OH)D levels. Patients who were not

on vitamin D supplementation were found to have a higher risk of vitamin D deficiency, especially those below the age of 20 years and during winter. Moreover, our findings have shown that patients from Neurology had a higher mean 25(OH)D level compared to patients admitted to other medical specialties.

The mean serum 25(OH)D level for all 38,385 patients in our study was sub-optimal (67.39 nmol/L). Both female and male patients across all age groups and medical specialties showed sub-optimal serum vitamin D status. This finding is consistent to the serum 25(OH)D levels measured at all seasons between 2013 to 2017. Vitamin D sufficiency was only shown in patients who were on supplementation; mean 25(OH)D level was 80.40nmol/L (>75nnom/L). In the present study, we found that 6.3% of patients were vitamin D deficient and only 36.8% were sufficient in vitamin D. Pharmacy records on vitamin D supplementation correlated to serum 25(OH)D measured. In the vitamin D sufficient group, 55.9% of patients were prescribed vitamin D supplements >14 days before their serum 25(OH)D levels were measured, while only 2.5% in the vitamin D deficient group were prescribed with supplements. However, this correlation was not reflected in the gender variation found in serum 25(OH)D levels measured. Females had a higher vitamin D status compared to males; 39.5% of females were sufficient in vitamin D while only 32.5% of male patients were vitamin D sufficient. According to pharmacy records, 6.7% of male patients were prescribed with vitamin D supplements, compared to 5.5% of females. Therefore, females had higher 25(OH)D levels despite less patients being prescribed with supplements. Our findings are in contrary with several previous studies on determinants of vitamin D status in Australia. [29, 30] Cross-sectional studies across Australia showed that overall women had lower serum 25(OH)D levels than men (southeast Queensland, P=0.06; Tasmania, P<0.01) [30] and another population-based study demonstrated vitamin D deficiency was more prevalent in women. [29] The inconsistency of our results with other studies could be due to female patients acquiring vitamin D supplements from other sources, or be a particular characteristic of patients attending a tertiary health care center.

Contrary to previous studies, [31-33] we found that 25(OH)D levels increased with increasing age. Vitamin D supplementation could have also contributed to this finding. Serum 25(OH)D levels were shown to increase with increasing age, but older patients were found to be more likely to be prescribed with vitamin D supplementation (Table 4), likely resulting in higher serum 25(OH)D measured. This could explain the contradiction of our findings to data demonstrating higher prevalence of vitamin D deficiency with increasing age in both men and women, [31-33] which did not take into account supplementation. Contrary to previous studies that demonstrated the association of vitamin D deficiency and neurological disorders such as Parkinson's disease, Alzheimer's disease, MS and epilepsy, [34-38] our data showed that patients admitted to the Neurology specialty had the highest mean 25(OH)D level compared to patients from all other specialties. Pharmacy records showed that only 3.7% of patients from Neurology were prescribed with vitamin D supplementation, which was on the lower end compared to other specialties (Table 4). 47.7% of Neurology patients from our data were diagnosed with MS. Given the numerous literature on the role of vitamin D in MS; low serum vitamin D status is associated with increased susceptibility to MS and worse disease progression [39-46] it is conceivable that some MS patients could have acquired vitamin D supplementation from other sources, and total vitamin D intake was not recorded.

Moreover, patients with other neurological conditions (beside MS) could have also acquired vitamin D supplementation that was not recorded in the prescription records, resulting in higher serum 25(OH)D measured. [47-50] This could help explain the contradiction of our results to other findings regarding vitamin D status of patients with neurological disorders. Given the growing literature on the association between serum 25(OH)D and musculoskeletal health outcomes, [51-53] it is interesting to note that patients from the Orthopedic department has a sub-optimal mean serum 25(OH)D (66.80nmol/L). The mean 25(OH)D level for patients from Infectious Disease was the lowest (54.61nmol/L). This finding could suggest that patients with low serum 25(OH)D are more susceptible to various infection due to the immunomodulatory effects of vitamin D. [54-59] This is however speculative, and requires more data collection and in-depth analysis.

Seasonal variation in serum 25(OH)D concentration has been addressed in several prior studies. [60-62] Our results are in agreement with findings of previous studies that demonstrated lower vitamin D status during winter and higher levels during summer months. In a population-based study of 27,203 women (≥55 years) in Geelong (southeastern Australia), serum 25(OH)D was found to peak in summer and dip in winter ($p<0.001$), which is consistent with our results. [61] Our findings also showed monthly variation in vitamin D status, which correlated with the seasonal variation found. Interestingly, despite the fact that more patients were prescribed vitamin D supplementation during winter (6.1%) compared to summer (5.8%), serum 25(OH)D levels remained lower during winter, emphasizing the role of seasonal variation of UVB exposure on vitamin D status regardless of supplementation.

Notably, serum 25(OH)D status was significantly lower in 2013 compared to subsequent years (2014-2017). According to the records, the vitamin D assay used at RMH was changed from DiaSorin Liaison to Abbott Architect in Feb 2014. However comparison of the new assay to the previous one demonstrated a slope = 1 and intercept = 1nmol/L, i.e. inter-assay variability is likely to be minimal, hence the change in assay is unlikely to cause a shift in the vitamin D level measured. In fact, pharmacy records also showed a rise in prescription of vitamin D supplements every year from 2013 to 2017 (Table 4), particularly between 2013 and 2014. In 2013, only 0.2% of patients were prescribed vitamin D supplements compared to 4.1% in 2014. Thus, the rise in both serum 25(OH)D levels measured and prescription of vitamin D supplements from 2014 onwards are likely due to an increasing awareness on the health benefits of vitamin D, which is shown by the rising trend in vitamin D status for the past two decades. [63]

Limitations

Our study had a number of limitations. First, this study was carried out retrospectively and factors such as dietary habits of patients or direct measure of sun exposure were not included. Second, there is no consensus on the definition of the clinical terms vitamin D 'deficiency', 'insufficiency', 'sub-optimal' and 'sufficiency', as well as the desirable levels of serum 25(OH)D. The primary confounder of the efforts to standardize vitamin D status assessment is the variability in the assays used to measure 25(OH)D. [64] This lack of standardization has made comparison between studies difficult and could explain why our findings are in contrary with some of the studies

published. Third, since vitamin D supplementation data was derived from pharmacy dispensing records at RMH, it cannot be excluded that some patients might elect to purchase vitamin D supplements at other sources such as the supermarket, health food stores, or online. It is also important to note that because vitamin D supplementation does not require a prescription, vitamin D intake of some patients might not be included in the dispensing records. Fourth, in the supplementation data, the actual quantity of supplement (i.e. units of vitamin D dispensed) was not recorded. Fifth, because the data collected does not differentiate between patients who received inpatient and outpatient care, a comparison of vitamin D status between these two populations cannot be made. Sixth, due to the lack of information on the race-ethnicity of the patients, serum 25(OH)D levels were not stratified according to race-ethnicity. Seventh, the patient population presented here although quite large, may not be reflective of the general population (general community) as our patients were all from a tertiary hospital setting and likely with medical co-morbidities and potentially also with increased rates of vitamin D prescription.

Conclusion

This is the largest study of vitamin D status in patients from a tertiary health center in Australia. The findings reveal that there are gender, age, medical specialty, monthly, and seasonal variations in serum concentration of 25(OH)D. Based on our findings, patients who were not on vitamin D supplementation were at risk of vitamin D deficiency, especially those below the age of 20 years and during winter. Our findings showing older age, female gender, and Neurology patients having higher 25(OH)D levels are contrary to those previous reports globally. Our study also reveals that despite the role of supplementation, serum 25(OH)D remained the lowest during winter, highlighting the need of other interventions to boost vitamin D status during winter. Further research is warranted to establish the role of vitamin D in health and disease state, as well as the variations in individual characteristics, seasonal, and geographic locations that contribute to the total vitamin D levels. Specifically given the association between vitamin D and immune-mediated diseases such as MS, understanding the role of this key vitamin would help delineate potential therapeutic approaches in combatting these diseases.

References

1. Medicare Australia. Medicare Australia Statistics. Available at www.medicareaustralia.gov.au [Accessed 11 February 2019].
2. Sahota O. Understanding vitamin D deficiency. Age and ageing. 2014;43(5):589-91.
3. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. The Journal of clinical endocrinology and metabolism. 2011;96(1):53-8.
4. Rizzoli R, Boonen S, Brandi ML, Bruyere O, Cooper C, Kanis JA, et al. Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Current medical research and opinion. 2013;29(4):305-13.
5. Issa CM. Vitamin D and Type 2 Diabetes Mellitus. Advances in experimental medicine and biology. 2017;996:193-205.
6. Berridge MJ. Vitamin D deficiency and diabetes. The Biochemical journal. 2017;474(8):1321-32.
7. Podzolkov VI, Pokrovskaya AE, Panasenko OI. Vitamin D deficiency and cardiovascular pathology. Terapevticheskii arkhiv. 2018;90(9):144-50.

8. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. The British journal of psychiatry : the journal of mental science. 2013;202:100-7.
9. Woo YS, Kim S, Jeong JH, Jung YE, Kim MD, Bahk WM. Vitamin D Deficiency/Insufficiency among Inpatients with Depressive Symptoms. Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology. 2019;17(1):121-4.
10. Komisarenko YI, Bobryk MI. Vitamin D Deficiency and Immune Disorders in Combined Endocrine Pathology. Frontiers in endocrinology. 2018;9:600.
11. Sharif K, Sharif Y, Watad A, Yavne Y, Lichtbroun B, Bragazzi NL, et al. Vitamin D, autoimmunity and recurrent pregnancy loss: More than an association. American journal of reproductive immunology (New York, NY : 1989). 2018;80(3):e12991.
12. Bozdag H, Akdeniz E. Does severe vitamin D deficiency impact obstetric outcomes in pregnant women with thyroid autoimmunity? The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2018:1-11.
13. Balbuena LD, Middleton RM, Tuite-Dalton K, Pouliou T, Williams KE, Noble GJ. Sunshine, Sea, and Season of Birth: MS Incidence in Wales. PloS one. 2016;11(5):e0155181.
14. Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. Journal of neurology, neurosurgery, and psychiatry. 2013;84(4):427-32.
15. Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, et al. The association between neonatal vitamin D status and risk of schizophrenia. Scientific reports. 2018;8(1):17692.
16. Boettger SF, Angersbach B, Klimek CN, Wanderley ALM, Shaibekov A, Sieske L, et al. Prevalence and predictors of vitamin D-deficiency in frail older hospitalized patients. BMC Geriatrics. 2018;18(1):219.
17. Uusi-Rasi K, Patil R, Karinkanta S, Tokola K, Kannus P, Lamberg-Allardt C, et al. Serum 25-hydroxyvitamin D levels and incident falls in older women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2019;30(1):93-101.
18. Holick MF. Sunlight and Vitamin D AU - Wacker, Matthias. Dermato-Endocrinology. 2013;5(1):51-108.
19. Al Zarooni AAR, Al Marzouqi FI, Al Darmaki SH, Prinsloo EAM, Nagelkerke N. Prevalence of vitamin D deficiency and associated comorbidities among Abu Dhabi Emirates population. BMC research notes. 2019;12(1):503.
20. Bhattoa HP, Nagy E, More C, Kappelmayer J, Balogh A, Kalina E, et al. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in healthy Hungarian men over 50 years of age: the HunMen Study. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(1):179-86.
21. Gu Y, Zhu Z, Luan X, He J. Vitamin D status and its association with season, depression in stroke. Neurosci Lett. 2019;690:99-105.
22. Hansen L, Tjonneland A, Koster B, Brot C, Andersen R, Cohen AS, et al. Vitamin D Status and Seasonal Variation among Danish Children and Adults: A Descriptive Study. Nutrients. 2018;10(11).
23. Niculescu DA, Capatina CAM, Dusceac R, Caragheorgheopol A, Ghemigian A, Poiana C. Seasonal variation of serum vitamin D levels in Romania. Arch Osteoporos. 2017;12(1):113.
24. Touvier M, Deschasaux M, Montourcy M, Sutton A, Charnaux N, Kesse-Guyot E, et al. Determinants of vitamin D status in Caucasian adults: influence of sun exposure, dietary intake, sociodemographic, lifestyle, anthropometric, and genetic factors. The Journal of investigative dermatology. 2015;135(2):378-88.
25. van Schoor NM, Lips P. Worldwide vitamin D status. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):671-80.
26. Lips P. Worldwide status of vitamin D nutrition. The Journal of steroid biochemistry and molecular biology. 2010;121(1-2):297-300.
27. Joshi D, Center JR, Eisman JA. Vitamin D deficiency in adults. Australian Prescriber. 2010;33:103-6.
28. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmunity reviews. 2010;9(5):A387-94.
29. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clinical Endocrinology. 2012;77(1):26-35.

30. van der Mei Ingrid AF, Ponsonby A-L, Engelsen O, Pasco Julie A, McGrath John J, Eyles Daryl W, et al. The High Prevalence of Vitamin D Insufficiency across Australian Populations Is Only Partly Explained by Season and Latitude. Environmental Health Perspectives. 2007;115(8):1132-9.
31. Ardawi MS, Qari MH, Rouzi AA, Maimani AA, Raddadi RM. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre- and postmenopausal women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2011;22(2):463-75.
32. Chen J, Yun C, He Y, Piao J, Yang L, Yang X. Vitamin D status among the elderly Chinese population: a cross-sectional analysis of the 2010-2013 China national nutrition and health survey (CNNHS). Nutrition journal. 2017;16(1):3.
33. Sadat-Ali M, Al Elq AH, Al-Turki HA, Al-Mulhim FA, Al-Ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Annals of Saudi medicine. 2011;31(6):602-8.
34. Annweiler C, Schott AM, Berrut G, Chauviré V, Le Gall D, Inzitari M, et al. Vitamin D and Ageing: Neurological Issues. Neuropsychobiology. 2010;62(3):139-50.
35. Littlejohns TJ, Henley WE, Lang IA, Annweiler C, Beauchet O, Chaves PHM, et al. Vitamin D and the risk of dementia and Alzheimer disease. Neurology. 2014;83(10):920.
36. Knekt P, Kilkkinen A, Rissanen H, Marniemi J, Sääksjärvi K, Heliövaara M. Serum Vitamin D and the Risk of Parkinson Disease. Archives of Neurology. 2010;67(7):808-11.
37. Hollo A, Clemens Z, Lakatos P. Epilepsy and vitamin D. The International journal of neuroscience. 2014;124(6):387-93.
38. Mazdeh M, Zamani M, Eftekharian MM, Komaki A, Arsang-Jang S, Taheri M, et al. Expression analysis of vitamin D receptor-associated lncRNAs in epileptic patients. Metabolic brain disease. 2019.
39. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60.
40. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS genetics. 2009;5(2):e1000369.
41. van der Mei IAF, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. Journal of Neurology. 2007;254(5):581.
42. Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, et al. Sun exposure and vitamin D are independent risk factors for CNS demyelination. Neurology. 2011;76(6):540-8.
43. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. PLoS medicine. 2015;12(8):e1001866.
44. Rhead B, Baarnhielm M, Gianfrancesco M, Mok A, Shao X, Quach H, et al. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurology Genetics. 2016;2(5):e97.
45. Zamzam D, Foad M, Swelam M, AbdelHafez M, AbdelNasser A, Mahmoud R, et al. Vitamin D and body mass index in Egyptian multiple sclerosis patients. Multiple sclerosis and related disorders. 2019;28:313-6.
46. Voo VTF, O'Brien T, Butzkueven H, Monif M. The role of vitamin D and P2X7R in multiple sclerosis. Journal of neuroimmunology. 2019;330:159-69.
47. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Noya M, Takahashi D, et al. Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease. The American journal of clinical nutrition. 2013;97(5):1004-13.
48. Taghizadeh M, Talaei SA, Djazayeri A, Salami M. Vitamin D supplementation restores suppressed synaptic plasticity in Alzheimer's disease. Nutritional neuroscience. 2014;17(4):172-7.
49. Gangwar AK, Rawat A, Tiwari S, Tiwari SC, Narayan J, Tiwari S. Role of Vitamin-D in the prevention and treatment of Alzheimer's disease. Indian journal of physiology and pharmacology. 2015;59(1):94-9.
50. Al-Nimer MS. The Benefit of Prescribing Vitamin D as Add on Therapy on the Electrocardiographic Changes in Epileptic Patients. Current clinical pharmacology. 2018;13(1):40-4.
51. Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, et al. Effectiveness and safety of vitamin D in relation to bone health. Evidence report/technology assessment. 2007(158):1-235.
52. Antoniak AE, Greig CA. The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: a systematic review and meta-analysis. BMJ open. 2017;7(7):e014619.

53. Hwang J, Zmuda JM, Kuipers AL, Bunker CH, Santanasto AJ, Wheeler VW, et al. Serum Vitamin D and Age-Related Muscle Loss in Afro-Caribbean Men: The Importance of Age and Diabetic Status. The Journal of frailty & aging. 2019;8(3):131-7.
54. Vanherwegen AS, Gysemans C, Mathieu C. Regulation of Immune Function by Vitamin D and Its Use in Diseases of Immunity. Endocrinology and metabolism clinics of North America. 2017;46(4):1061-94.
55. Wei R, Christakos S. Mechanisms Underlying the Regulation of Innate and Adaptive Immunity by Vitamin D. Nutrients. 2015;7(10):8251-60.
56. Zhou Q, Li L, Chen Y, Zhang J, Zhong L, Peng Z, et al. Vitamin D supplementation could reduce the risk of acute cellular rejection and infection in vitamin D deficient liver allograft recipients. International immunopharmacology. 2019;75:105811.
57. Goncalves-Mendes N, Talvas J, Duale C, Guttmann A, Corbin V, Marceau G, et al. Impact of Vitamin D Supplementation on Influenza Vaccine Response and Immune Functions in Deficient Elderly Persons: A Randomized Placebo-Controlled Trial. Frontiers in immunology. 2019;10:65.
58. Arihiro S, Nakashima A, Matsuoka M, Suto S, Uchiyama K, Kato T, et al. Randomized Trial of Vitamin D Supplementation to Prevent Seasonal Influenza and Upper Respiratory Infection in Patients With Inflammatory Bowel Disease. Inflammatory bowel diseases. 2019;25(6):1088-95.
59. Loeb M, Dang AD, Thiem VD, Thanabalan V, Wang B, Nguyen NB, et al. Effect of Vitamin D supplementation to reduce respiratory infections in children and adolescents in Vietnam: A randomized controlled trial. Influenza and other respiratory viruses. 2019;13(2):176-83.
60. Ma F, Levis S, Roos BA, Lai S, Gomez A, Jimenez C, et al. Vitamin D Deficiency and Seasonal Variation in an Adult South Florida Population. The Journal of Clinical Endocrinology & Metabolism. 2005;90(3):1557-62.
61. Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR, et al. Seasonal Periodicity of Serum Vitamin D and Parathyroid Hormone, Bone Resorption, and Fractures: The Geelong Osteoporosis Study. Journal of Bone and Mineral Research. 2004;19(5):752-8.
62. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. Canadian Medical Association Journal. 2002;166(12):1517.
63. McKenna MJ, Murray BF, O'Keane M, Kilbane MT. Rising trend in vitamin D status from 1993 to 2013: dual concerns for the future. Endocrine connections. 2015;4(3):163-71.
64. Binkley N, Carter GD. Toward Clarity in Clinical Vitamin D Status Assessment: 25(OH)D Assay Standardization. Endocrinology and metabolism clinics of North America. 2017;46(4):885-99.

Figure 1. Histogram of serum 25(OH)D distribution in study population (N=38,385)

Figure 1. Histogram showing distribution of serum 25(OH)D levels (nmol/L) among 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. The vertical solid line (in red) is the mean value, 67.39±0.16 nmol/L.

Figure 2. Bar graph of age variation in serum 25(OH)D levels in study population (N=38,385)

Figure 2. Bar graph showing age variation in serum 25(OH)D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. 25(OH)D levels increased with age, and patients >40 years had significantly higher mean 25(OH)D concentrations compared to age 20-29 years. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 3. Bar graph of variations in serum 25(OH)D level of patients admitted to various medical specialties (N=38,385)

Figure 3. Bar graph showing variation in serum 25(OH)D levels (nmol/L) of 38,385 patients admitted to all medical specialties at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis. Analysis showed the average 25(OH)D levels of patients from all other specialties were significantly lower compared to NEUR. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 4. Line graph of seasonal variation in serum 25(OH)D levels in study population (N=38,385)

Figure 4. Line graph showing seasonal variation in serum 25(OH)D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Analysis showed the average 25(OH)D levels of all other seasons were significantly higher compared to winter. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 5. Line graph of monthly variation in serum 25(OH)D levels in study population (N=38,385)

Figure 5. Line graph showing monthly variation in serum 25(OH)D levels (nmol/L) of 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 6. Line graph of yearly variation (2013-2017) in serum 25(OH)D levels in study population (N=38,385)

Figure 6. Line graph showing variation in serum 25(OH)D levels (nmol/L) of 38,385 patients measured between 2013 to 2017 at Royal Melbourne Hospital, Victoria, Australia. Compared to 2014 to 2017, the mean 25(OH)D concentration measured in 2013 was significantly lower. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Table 1. Characteristics of the study population (N=38,385) and average serum 25(OH)D levels.

	N (%)	Mean 25(OH)D (SEM)
Total population	38385 (100%)	67.39 (0.16)
Gender		
Female	23626 (61.6%)	69.56 (0.21)
Male	14711 (38.3%)	63.88 (0.26)
Age group		
<20	379 (1.0%)	59.7 (1.79)
20-29	3605 (9.4%)	62.2 (0.51)
30-39	4844 (12.6%)	63.4 (0.48)
40-49	4518 (11.8%)	64.4 (0.49)
50-59	5128 (13.4%)	66.6 (0.46)
60-69	5306 (13.8%)	69.1 (0.42)
>70	14605 (38.1%)	70.8 (0.26)
Medical specialty		
NEUR	820 (2.1%)	73.34 (1.42)
AMU	1618 (4.2%)	68.92 (0.83)
BOE	1282 (3.3%)	69.09 (0.82)
EMER	2076 (5.4%)	68.54 (0.75)
ENDO	1479 (3.9%)	70.84 (0.82)
GAST	1885 (4.9%)	64.38 (0.70)
NEPH	1544 (4.0%)	62.06 (0.72)
ORTH	1334 (3.5%)	66.80 (0.80)
OTHER	17310 (45.1%)	66.65 (0.24)
PRIV	7737 (20.2%)	70.91 (0.39)
VIDS	1300 (3.4%)	54.61 (0.81)
Season of measurement		
Summer (Dec-Feb)	8711 (22.7%)	72.39 (0.52)
Autumn (Mar-May)	10030 (26.1%)	71.52 (0.31)
Winter (Jun-Aug)	9979 (26.0%)	62.44 (0.32)
Spring (Sep-Nov)	9665 (25.2%)	63.73 (0.33)
Year of measurement		
2013	8708 (22.7%)	60.53 (0.30)
2014	8631 (22.5%)	70.88 (0.35)
2015	7135 (18.6%)	70.64 (0.39)
2016	7120 (18.5%)	68.19 (0.39)
2017	6791 (17.7%)	67.53 (0.40)
Vitamin D supplementation		
Yes	2285 (6.0%)	80.40 (0.66)
No information	36100 (94.0%)	66.60 (0.17)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 2: Results of multivariable linear regression analyses to model serum 25(OH)D levels.

	Adjusted difference in mean 25(OH)D	95% confidence interval	P-value
Medical specialty			
NEUR	Reference	-	-
AMU	-10.9	-13.5, -8.2	<0.0001
BOE	-8.1	-10.9, -5.4	<0.0001
EMER	-9.3	-11.8, -6.8	<0.0001
ENDO	-4.1	-6.7, -1.4	0.003
GAST	-9.1	-11.6, -6.5	<0.0001
NEPH	-12.4	-15.0, -9.7	<0.0001
ORTH	-12.1	-14.9, -9.4	<0.0001
OTHER	-8.8	-11.0, -6.7	<0.0001
PRIV	-2.5	-4.8, -0.3	0.026
VIDS	-16.2	-18.9, -13.5	<0.0001
Year of measurement			
2013	Reference	-	-
2014	10.1	9.2, 11.0	<0.0001
2015	9.4	8.5, 10.4	<0.0001
2016	7.3	6.3, 8.2	<0.0001
2017	6.8	5.8, 7.8	<0.0001

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 3. Characteristics of the study population stratified according to serum 25(OH)D levels.

	Total N	Deficiency (<25nmol/L)	Insufficiency (25-50nmol/L)	Sub-optimal (50-75nmol/L)	Sufficiency (>75nmol/L)	P-value
Total population	38385	2437 (6.3%)	9233 (24.1%)	12577 (32.8%)	14138 (36.8%)	
Gender						
Female	23626	1308 (5.5%)	5238 (22.2%)	7748 (32.8%)	9332 (39.5%)	<0.0001
Male	14711	1128 (7.7%)	3987 (27.1%)	4816 (32.7%)	4780 (32.5%)	
Age						
<20	379	40 (10.5%)	116 (30.6%)	131 (34.6%)	92 (24.3%)	
20-29	3605	290 (8.0%)	1048 (29.1%)	1214 (33.7%)	1053 (29.2%)	
30-39	4844	376 (7.8%)	1382 (28.5%)	1626 (33.6%)	1460 (30.1%)	
40-49	4518	331 (7.3%)	1247 (27.6%)	1513 (33.5%)	1427 (31.6%)	<0.0001
50-59	5128	306 (6.0%)	1339 (26.1%)	1699 (33.1%)	1784 (34.8%)	
60-69	5306	283 (5.3%)	1151 (21.7%)	1760 (33.2%)	2112 (39.8%)	
>70	14605	811 (5.6%)	2950 (20.1%)	4634 (31.8%)	6210 (42.5%)	
Medical specialty						
AMU	1618	150 (9.3%)	341 (21.1%)	451 (27.9%)	676 (41.8%)	
BOE	1282	54 (4.2%)	278 (21.7%)	453 (35.3%)	497 (38.8%)	
EMER	2076	165 (7.9%)	493 (23.7%)	582 (28.1%)	836 (40.3%)	
ENDO	1479	62 (4.2%)	312 (21.2%)	507 (34.3%)	598 (40.4%)	
GAST	1885	114 (6.1%)	538 (28.5%)	618 (32.8%)	615 (32.6%)	
NEPH	1544	124 (8.0%)	433 (28.1%)	514 (33.3%)	473 (30.6%)	<0.0001
NEUR	820	47 (5.7%)	167 (20.4%)	273 (33.2%)	333 (40.6%)	
ORTH	1334	91 (6.8%)	286 (21.4%)	469 (35.2%)	488 (36.6%)	
OTHER	17310	1103 (6.4%)	4227 (24.4%)	5703 (32.9%)	6277 (36.3%)	
PRIV	7737	357 (4.6%)	1702 (22.0%)	2609 (33.7%)	3069 (39.7%)	
VIDS	1300	170 (13.1%)	456 (35.1%)	398 (30.6%)	276 (21.2%)	
Season of measurement						
Summer (Dec-Feb)	8711	329 (3.8%)	1643 (18.9%)	2993 (34.4%)	3746 (43.0%)	
Autumn (Mar-May)	10030	384 (3.8%)	2037 (20.3%)	3424 (34.1%)	4185 (41.7%)	<0.0001
Winter (Jun-Aug)	9979	869 (8.7%)	2910 (29.2%)	3123 (31.3%)	3077 (30.8%)	
Spring (Sep-Nov)	9665	855 (8.9%)	2643 (27.4%)	3037 (31.4%)	3130 (32.4%)	
Year of measurement						
2013	8708	771 (8.9%)	2390 (27.4%)	3146 (36.1%)	2401 (27.6%)	
2014	8631	315 (3.7%)	1943 (22.5%)	2900 (33.6%)	3473 (40.2%)	
2015	7135	301 (4.2%)	1604 (22.5%)	2364 (33.1%)	2866 (40.2%)	<0.0001
2016	7120	512 (7.2%)	1663 (23.4%)	2183 (30.7%)	2762 (38.8%)	
2017	6791	538 (7.9%)	1633 (24.1%)	1984 (29.2%)	2636 (38.8%)	
Vitamin D supplementation						
Yes	2285	58 (2.5%)	265 (11.6%)	684 (29.9%)	1278 (55.9%)	<0.0001
No information	36100	2379 (6.6%)	8968 (24.8%)	11893 (32.9%)	12680 (35.6%)	

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 4. Characteristics of the study population on vitamin D supplementation

	Vitamin D supplementation +	No information (%)
Gender		
Female	1300 (5.5%)	22326 (94.5%)
Male	985 (6.7%)	13726 (93.3%)
Age group		
<20	2 (0.5%)	377 (99.5%)
20-29	89 (2.5%)	3516 (97.5%)
30-39	106 (2.2%)	4738 (97.8%)
40-49	125 (2.8%)	4393 (97.2%)
50-59	189 (3.7%)	4939 (96.3%)
60-69	293 (5.5%)	5013 (94.5%)
>70	1481 (10.1%)	13124 (89.9%)
Medical specialty		
NEUR	30 (3.7%)	790 (96.3%)
AMU	288 (17.8%)	1330 (82.2%)
BOE	20 (1.6%)	1262 (98.4%)
EMER	187 (9.0%)	1889 (91.0%)
ENDO	110 (7.4%)	1369 (92.6%)
GAST	98 (5.2%)	1787 (94.8%)
NEPH	157 (10.2%)	1387 (89.8%)
ORTH	90 (6.7%)	1244 (93.3%)
OTHER	1188 (6.9%)	16122 (93.1%)
PRIV	43 (0.6%)	7694 (99.4%)
VIDS	74 (5.7%)	1226 (94.3%)
Season of measurement		
Summer (Dec-Feb)	502 (5.8%)	8209 (94.2%)
Autumn (Mar-May)	563 (5.6%)	9467 (94.4%)
Winter (Jun-Aug)	605 (6.1%)	9374 (93.9%)
Spring (Sep-Nov)	615 (6.4%)	9050 (93.6%)
Year of measurement		
2013	19 (0.2%)	8689 (99.8%)
2014	352 (4.1%)	8279 (95.9%)
2015	551 (7.7%)	6584 (92.3%)
2016	657 (9.3%)	6463 (90.7%)
2017	706 (10.4%)	6085 (89.6%)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private; VIDS, Victorian Infectious Disease Service. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Authorship statement

Manuscript title:

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors that influence variations in serum 25(OH)D

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *British Medical Journal (BMJ)*.

Authorship contributions

Conception and design of study: Monif M, Butzkueven H, Voo VTF, Stankovich J, O'Brien T

Acquisition of data: Monif M, Voo VTF

Analysis and/or interpretation of data: Stankovich J, Voo VTF, Monif M, Butzkueven H

Drafting the manuscript: Voo VTF

Revising the manuscript critically: Voo VTF, Monif M, Stankovich J, O'Brien T

All authors contributed to refinement of the study protocol and approved the final manuscript.

Data availability

All data relevant to the study are included in the article or uploaded as supplementary information.

Acknowledgements

We would like to acknowledge Maria Bisignano, research support coordinator at Melbourne Health Shared Pathology Service for extracting the results of serum 25(OH)D lab tests from Royal Melbourne Hospital pathology services. We would also like to thank Karen Honson, pharmacist at Royal Melbourne Hospital for extracting the supplementation data from the prescription records.

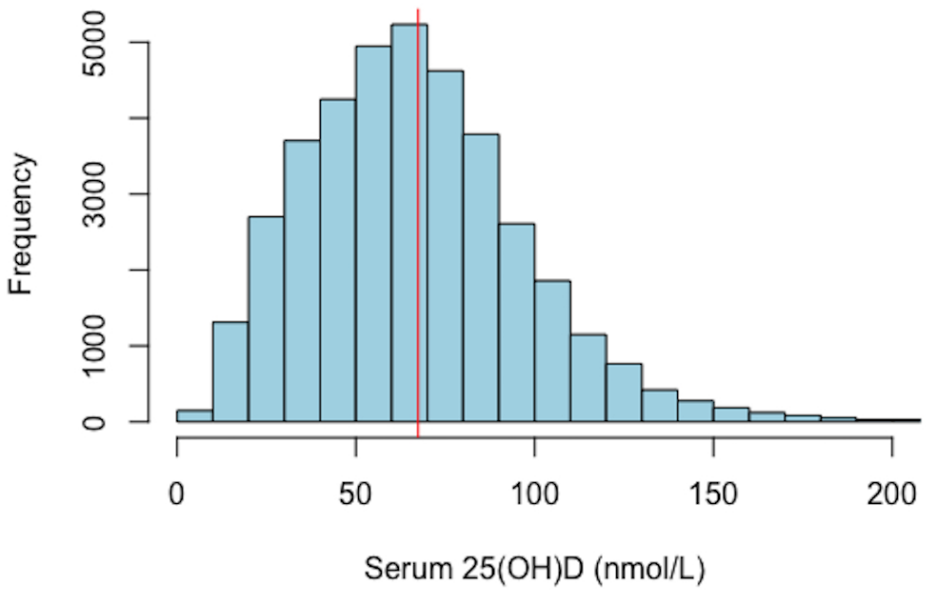
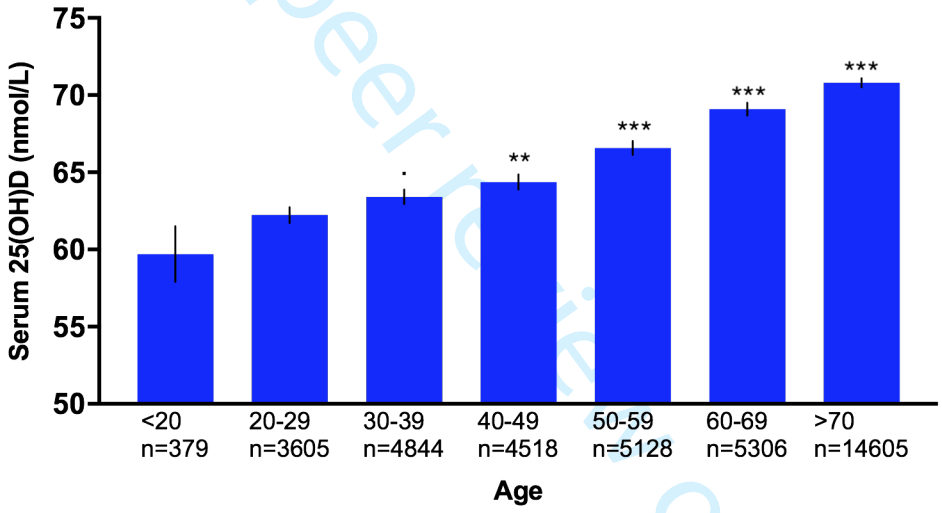
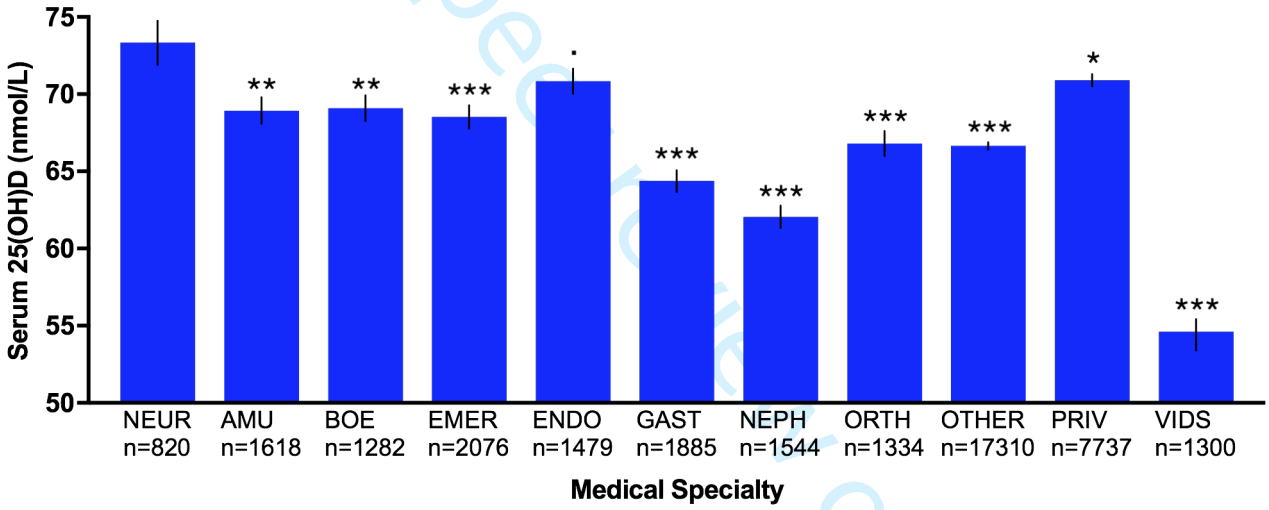
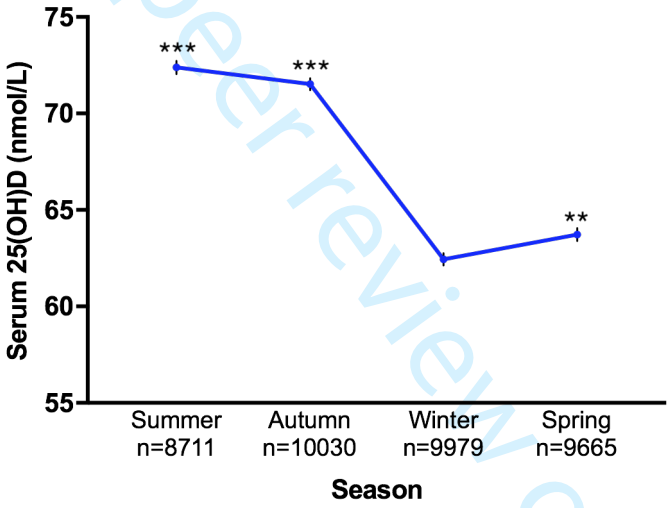
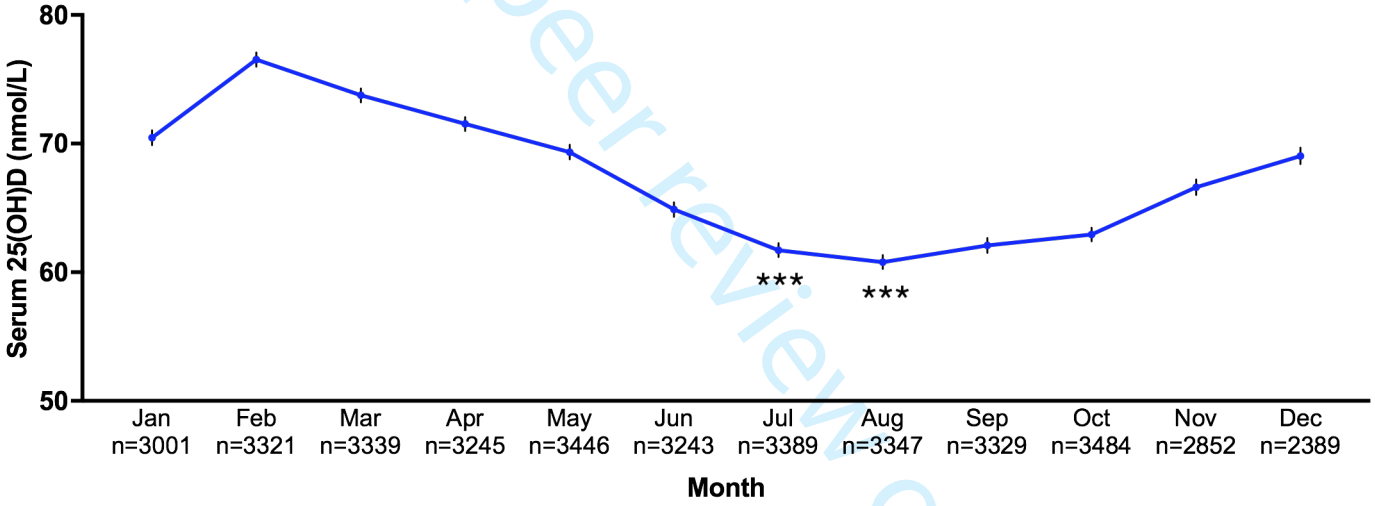


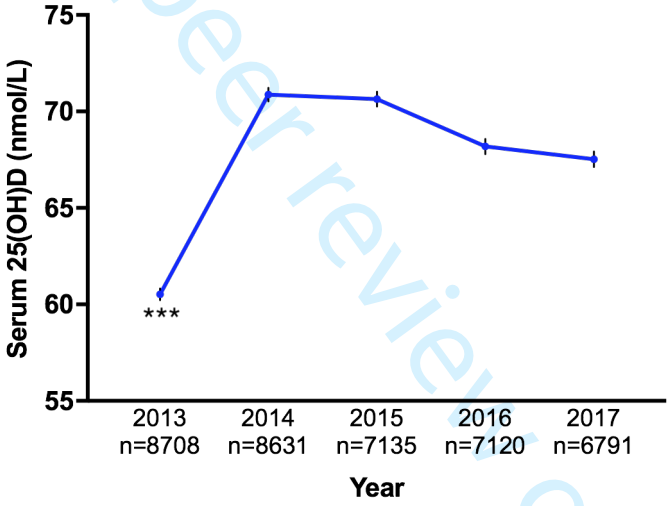
Figure 1. Histogram of serum 25(OH)D distribution in study population (N=38,385)
Figure 1. Histogram showing distribution of serum 25(OH)D levels (nmol/L) among 38,385 patients at Royal Melbourne Hospital, Victoria, Australia between 2013 to 2017. The vertical solid line (in red) is the mean value, 67.39□0.16 nmol/L.











BMJ Open

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors associated with variations in serum 25(OH)D

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032567.R2
Article Type:	Original research
Date Submitted by the Author:	20-Dec-2019
Complete List of Authors:	Voo, Veronica Tsin Fong; The University of Melbourne Stankovich, Jim ; Monash University Faculty of Medicine Nursing and Health Sciences O'Brien , Terence; Monash University Butzkueven , Helmut ; Monash University Faculty of Medicine Nursing and Health Sciences, Neuroscience Monif, Mastura; Monash University,
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Nutrition and metabolism, Public health
Keywords:	Vitamin D, EPIDEMIOLOGY, Patient population, Australian cohort study

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title page

Manuscript title:

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors associated with variations in serum 25(OH)D

Authors:

Veronica Tsin Fong Voo[1,2,3], Jim Stankovich[2], , Terence O'Brien[2,3], Helmut Butzkueven[2,3] Mastura Monif[1,2,3]

[1]Department of Physiology, University of Melbourne, Grattan Street, Parkville VIC 3000 Australia
[2]Department of Neuroscience, Alfred Centre, 99 Commercial Road, Melbourne VIC 3004 Australia
[3]Department of Neurology, Royal Melbourne Hospital, Grattan Street, Parkville 3050 Australia

Correspondence to: mastura.monif@monash.edu

Declarations

Ethics approval

The ethics of this research was approved by Melbourne Health ethics committee (number/ID of approval QA2018084) and is done in accordance to the World Medical Association Declaration of Helsinki. Patient data and medical records extracted was de-identified and coded without identifiers.

Transparency declaration

The lead author, Veronica Voo affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

No funding and competing interests

This research received no specific grants from any funding agency in the public, commercial or not-for-profit sectors. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activates that could appear to have influenced the submitted work.

Structured abstract

Objectives: To investigate whether sex, age, medical specialty, and seasonal variations in serum concentration of 25-hydroxy vitamin D (25(OH)D) are evident among an Australian patient population.

Design: Retrospective study analysing the results of serum 25(OH)D lab tests and vitamin D supplementation from Royal Melbourne Hospital (RMH) between 2014 to 2017.

Setting: Tertiary health care center in Victoria, Australia.

Participants: 30,023 patients (inpatient and outpatient) who had their serum 25(OH)D levels measured at RMH between 2014 to 2017.

Main outcome measures: Serum 25(OH)D levels stratified according to patients' sex, age, and medical specialty admitted to, as well as the season and year (2014 to 2017) 25(OH)D level was measured.

Results: Mean serum 25(OH)D level of study population was 69.9 nmol/L (95% CI: 69.5 to 70.2). Only 40.2% patients in this cohort were sufficient in vitamin D (>75 nmol/L). On average, 25(OH)D levels in male patients were 6.1 units (95% CI: 5.4 to 6.9) lower than in females. Linear regression analysis found that 25(OH)D levels increased by 0.16 unit (95% CI: 0.14 to 0.18) for every year increase in age. One-way ANOVA showed patients from Neurology had the highest average 25(OH)D level, 76.8 nmol/L (95% CI: 74.2 to 79.3) compared to other medical specialties. Mean 25(OH)D level during winter, 64.9 nmol/L (95% CI: 64.2 to 65.6) was significantly lower compared to other seasons despite supplementation. Average 25(OH)D level measured in 2014, 71.5 nmol/L (95 CI% 70.8 to 72.2) was significantly higher than levels measured in 2016 to 2017.

Conclusions: There is a sex, age, medical specialty, seasonal, and yearly variation in vitamin D status in an Australian patient population. The association between low vitamin D status and winter despite supplementation suggests other interventions are required to boost serum 25(OH)D levels.

Strengths and limitations of this study

- To date, this is the largest study of vitamin D status in patients from a tertiary health center in Australia (n = 30,023).
- Our findings have contributed to the gap in literature concerning vitamin D status in large Australian cohorts.
- Our findings highlighted the contribution of individual characteristics and seasonal variation of UVB exposure to vitamin D status, suggesting targeted approaches in interventions to improve vitamin D status.
- Due to being a retrospective study, our study did not include factors such as dietary habits of patients or direct measure of sun exposure.
- The actual quantity of vitamin D supplementation was not recorded, and information on total vitamin D intake of patients was not available.

Introduction

Interest in the health risks of vitamin D deficiency has skyrocketed over the last 10 years. In Australia, this is reflected in the 128-fold increase in serum vitamin D testing from 2000 to 2014, and has raised the cost to Medicare from $1.02 million in 2000 to over $140 million in 2012. [1] Despite the increased interest in vitamin D, the optimal level that should be targeted remains variable. The consensus from scientific research appears to support serum 25-hydroxy vitamin D (25(OH)D) level sufficiency >50nmol/L and vitamin D deficiency is generally defined as <25nmol/L. [2-4] It is now widely accepted that adequate vitamin D levels should be maintained in all life stages, from fetal development to old age. Low vitamin D status has been associated with an increased risk of rickets, osteoporosis, diabetes mellitus, cardiovascular disease, depression, autoimmunity, and even pregnancy complications. [5-12] Low prenatal and early life vitamin D levels may also increase susceptibility to schizophrenia and multiple sclerosis (MS) in later life. [13-15] In the elderly, higher risk of vitamin D deficiency and lower vitamin D status have been reported to be associated with increased fall risk. [16, 17]

Sunlight exposure remains as the major source of vitamin D synthesis. Exposure of the skin to solar ultraviolet B (UVB; 290-315nm) radiation converts cutaneous 7-dehydrocholesterol to pre-vitamin D_3, which in turn becomes vitamin D_3. Vitamin D_3 is then metabolised to 25(OH)D in the liver and is subsequently converted to the biologically active form, $1,25(OH)_2D_3$ in the kidneys. Vitamin $1,25(OH)_2D_3$ plays a major role in calcium and phosphorus regulation, and as a result influences many metabolic pathways and skeletal health. [18] Since human vitamin D is largely synthesised in the skin, serum concentration of 25(OH)D is influenced by seasonal variation of UVB exposure, as well as time spent outdoors, the use of sunscreen, and clothing. Numerous studies from different countries have shown seasonal variation in vitamin D concentrations in children, adults, and the elderly; the lowest level is found to be at either spring or winter, and highest at either autumn or summer. [19-24] A review on worldwide vitamin D status identified children (especially those with low birth weight), pregnant women, and the elderly being at risk of vitamin D deficiency. [25] However, regarding gender variation in vitamin D concentration, findings from different studies remain inconsistent; some studies have shown women have higher serum 25(OH)D levels while others demonstrated to the contrary. [22, 24, 26]

Although variation in vitamin D levels have been addressed in several studies across different countries and geographical latitude, studies of vitamin D status in Victoria, Australia are lacking. Therefore, this study sought to investigate whether (1) sex, age, and seasonal variations in serum concentration of 25(OH)D are evident among the Victorian tertiary hospital population; (2) certain subgroups of patients are more at risk of vitamin D deficiency; and (3) patients with neurological conditions including MS have a lower vitamin D status.

Methods

Study population

This study retrospectively analysed the results of serum 25(OH)D lab tests of 30,023 patients (inpatient and outpatient) at Royal Melbourne Hospital (RMH), Victoria, Australia between 2014 to 2017. The results were obtained from the pathology department of RMH after receiving approval from the Human Research Ethics Committee, RMH. Further, dispensing records of vitamin D supplementation (cholecalciferol 1,000 IU or 50,000 IU) were derived from pharmacy records of the same hospital.

Laboratory assay for serum 25(OH)D measurement

The vitamin D assay used between March 2014 to December 2017 was the Abbott ARCHITECT immunoassay. Assay performance review was conducted on a daily basis (internally) and monthly basis (externally), and if the assay CV% drifted >8%, the protocol was to adjust the issue. From 2014 to 2017, internal as well as external assay quality assurance and validity measures were performed at multiple levels of vitamin D. The coefficient of variation percentage (CV%) obtained during this time was <6% for the clinically relevant values of serum 25(OH)D. A different assay was used prior to 2014, and hence data prior to 2014 is not included.

Study design

Currently, there is no complete agreement on the definition of vitamin D deficiency or the optimal level, we therefore categorised the patients based on the recommendations from the government journal Australian Prescriber. [27] The patients were classified into four diagnostic categories according to their serum 25(OH)D levels: deficiency (levels <25nmol/L), insufficiency (levels 25-50nmol/L), sub-optimal (levels 50-75nmol/L), and sufficiency (levels >75nmol/L). In addition, patients were also stratified according to their sex, age, and the medical specialty that each patient was admitted to at the time that their serum 25(OH)D concentration was measured. Patients below the age of 20 years were excluded from analysis as they represent only 1% of the study population (n=379), but had the highest rate of vitamin D deficiency. Moreover, due to the lack of information on the race-ethnicity of the patients, serum 25(OH)D levels were not stratified according to race-ethnicity. Additionally, because the data collected does not differentiate between patients who received inpatient and outpatient care, a comparison of vitamin D status between these two populations cannot be made. It is also important to note that the word “admitted” used throughout the paper refers to both inpatients and outpatients. Seasons were determined according to meteorological seasons in Australia: summer (December, January, February), autumn (March, April, May), winter (June, July, August), and spring (September, October, November).

Patient and public involvement

Due to being a retrospective study, patients were not involved in this research. Patients were not consulted to comment on the study design or interpret the study outcomes. It was difficult to directly involve patients due

to data protection restrictions and patient confidentiality. Patients were not invited to contribute to the writing or editing of this document.

Statistical analysis

The statistical analysis was performed with R programming (package: dplyr) and Prism 7. For those that had multiple tests available, only the first vitamin D test for each patient between 2014 to 2017 was used. For patients who were prescribed with multiple vitamin D prescriptions, only the ones issued >14 days before serum 25(OH)D was measured were included. This is to ensure that the vitamin D supplements have had enough time to affect serum 25(OH)D levels. Chi-square test was used to compare different vitamin D diagnostic categories, i.e. vitamin D deficient, insufficient, sub-optimal, and sufficient groups. Student's t-test was used to compare the vitamin D status between sex: female vs. male. For comparison of vitamin D levels within multiple groups, analysis of variance or one-way ANOVA with post-hoc test was utilised, i.e. age, medical specialty, seasonal, and yearly variabilities in 25(OH)D concentration. Regression analysis was performed to analyse the relationship between age and vitamin D status. In addition, multivariable model was used to analyse the relationship between 25(OH)D concentration and Neurology patients. Data is presented as mean±SEM (standard error of mean) unless specified otherwise.

Results

Characteristics of the study population are described in Table 1. Mean serum 25(OH)D concentration was 69.9 nmol/L (95% confidence interval 69.5-70.2), with average age of 59.6 years (95% confidence interval 59.4-59.8) (Figure 1). The study population comprised of 30,023 patients, of whom 62.3% were females and 37.6% males. Patients with undefined sex in their medical records were excluded from analysis. Female (P<0.0001), older age (P<0.0001), and summer (P<0.0001) were associated with higher vitamin D status. Male patients had significantly lower 25(OH)D levels compared to females (P<0.0001). On average, 25(OH)D levels in male patients were 6.1 units lower than in females (95% confidence interval 5.4-6.9). Patients 20-29 years (n=3,099) had the lowest mean vitamin D status (63.3 nmol/L), while patients >70 years (n=11,225) had the highest (73.2 nmol/L). Regardless, one-way ANOVA showed significant difference in average 25(OH)D concentrations between all age groups (P<0.0001). Linear regression analysis also found that vitamin D status increased by 0.16 unit (95% confidence interval 0.14-0.18) for every year increase in age. MS can occur at any age, but most commonly affects people between 20-40 years with a peak incidence occurring at 30 years of age. [28] Therefore, age group 20-29 was used as a reference group. Mean serum 25(OH)D level of patients ≥50 years was significantly higher than patients in the 20-29 year age category (P<0.0001) (Figure 2). However, mean 25(OH)D concentration of Neurology patients (41.7% have MS) was significantly higher than patients from all other medical specialties (Figure 3). Patients admitted to Neurology (76.8 nmol/L, 95% confidence interval 74.2-79.3) had the highest average 25(OH)D level, while patients from Nephrology (64.1 nmol/L, 95% confidence interval 58.5-69.7) had the lowest. Regardless, average 25(OH)D levels between all medical specialties were found to be significantly different (P<0.0001). The lowest mean 25(OH)D level was found in winter (64.9 nmol/L, 95% confidence interval 64.2-65.6), while the highest level was found in summer (76.1

nmol/L, 95% confidence interval 74.3-77.9). Average 25(OH)D level at winter (Jun-Aug) was significantly lower compared to levels from all other seasons (Figure 4). The average 25(OH)D concentration from tests measured in 2014 (71.5 nmol/L, 95% confidence interval 70.8-72.2) was significantly higher than levels measured in 2016-2017 (P<0.0001) (Figure 5).

Multivariable analysis

Results of the multivariable regression analysis on 25(OH)D levels by specialty are described in Table 2. Patients from Neurology were found to have the highest average 25(OH)D level compared to patients from other specialties, after accounting for sex and age, the season and year that the vitamin D tests were measured, as well as vitamin D supplementation intake.

Vitamin D diagnostic groups

Characteristics of the study population stratified according to their serum 25(OH)D levels are described in Table 3. Only 40.2% patients in this cohort were sufficient in vitamin D, and 55.8% of patients who were prescribed vitamin D supplementation had sufficient vitamin D levels. The proportion of patients on vitamin D supplementation varied significantly between various levels of 25(OH)D concentration (P<0.0001, chi-square test). Although female patients had a higher mean 25(OH)D concentration than males, only 43.1% had sufficient 25(OH)D levels compared to 35.5% of male patients. The age of patients increased with increasing 25(OH)D levels; the average age (mean ± standard deviation) of patients who had 25(OH)D levels >75nmol/L was 60.6±20.8, while patients who were vitamin D deficient (<25nmol/L) had an average age of 55.6±22.3. Although patients from Neurology had the highest average 25(OH)D concentration, only 44.6% were sufficient in vitamin D level. As expected, proportion of patients who were vitamin D sufficient were the lowest in winter (34.0%) compared to 47.8% of patients who had their 25(OH)D level measured in summer. Interestingly, the proportion of patients who had their 25(OH)D levels measured in 2014 were significantly higher (P<0.0001) in the vitamin D sufficient group, and significantly lower (P<0.0001) in the vitamin D deficient group compared to patients between 2015 to 2017 (chi-square test). Chi-square tests also yielded significant differences between the proportion of patients with various levels of vitamin D insufficiency in the following categories: sex (P<0.0001), age (P<0.0001), medical specialty (P<0.0001), season (P<0.0001), and year (P<0.0001).

Vitamin D supplementation

Characteristics of the study population on vitamin D supplementation are shown in Table 4. A higher proportion of male patients were prescribed with supplementation compared to females. Prescription of vitamin D supplements increased with increasing age. Patients from Acute Medical Unit (AMU) and Nephrology (NEPH) were found to have the highest proportion of patients on supplementation. Unsurprisingly, more patients were prescribed with vitamin D supplements during winter and spring. Interestingly, proportion of patients on vitamin D supplementation increased from 2014 to 2017 annually.

Discussion

This is the largest study on serum 25(OH)D levels in a tertiary hospital population in Victoria, Australia (n=30,023) to date. Our study found sex, age, and seasonal variations in serum 25(OH)D levels. Patients who were not on vitamin D supplementation were found to have a higher risk of vitamin D deficiency, especially during winter. Moreover, our findings have shown that patients from Neurology had a higher mean 25(OH)D level compared to patients admitted to other medical specialties.

The mean serum 25(OH)D level for all 30,023 patients in our study was sub-optimal (69.9 nmol/L). Both female and male patients across all age groups and medical specialties (except for patients admitted to Neurology and private hospitals) showed sub-optimal serum vitamin D status. Only patients who were on supplementation, and patients admitted to Neurology and private hospital were sufficient in vitamin D (>75 nmol/L). Moreover, only patients who had their vitamin D levels measured in summer had sufficient vitamin D level compared to other seasons. In the present study, we found that 5.5% of patients were vitamin D deficient and only 40.2% were sufficient in vitamin D. Pharmacy records on vitamin D supplementation correlated to serum 25(OH)D measured. In the vitamin D sufficient group, 55.8% of patients were prescribed vitamin D supplements >14 days before their serum 25(OH)D levels were measured, while only 2.4% in the vitamin D deficient group were prescribed with supplements. However, this correlation was not reflected in the gender variation found in serum 25(OH)D levels measured. Females had a higher vitamin D status compared to males; 43.1% of females were sufficient in vitamin D while only 35.5% of male patients were vitamin D sufficient. According to pharmacy records, 9.9% of male patients were prescribed with vitamin D supplements, compared to 7.9% of females. Therefore, females had higher 25(OH)D levels despite less patients being prescribed with supplements. Our findings are in contrary with several previous studies on determinants of vitamin D status in Australia. [29, 30] Cross-sectional studies across Australia showed that overall women had lower serum 25(OH)D levels than men (southeast Queensland, P=0.06; Tasmania, P<0.01) [30] and another population-based study demonstrated vitamin D deficiency was more prevalent in women. [29] The inconsistency of our results with other studies could be due to female patients acquiring vitamin D supplements from other sources, or be a particular characteristic of patients attending a tertiary health care center.

Contrary to previous studies, [31-33] we found that 25(OH)D level increased with increasing age. Vitamin D supplementation could have also contributed to this finding. Serum 25(OH)D levels were shown to increase with increasing age, but older patients were found to be more likely to be prescribed with vitamin D supplementation (Table 4), likely resulting in higher serum 25(OH)D measured. This could explain the contradiction of our findings to data demonstrating higher prevalence of vitamin D deficiency with increasing age in both men and women, [31-33] which did not take into account supplementation. Contrary to previous studies that demonstrated the association of vitamin D deficiency and neurological disorders such as Parkinson's disease, Alzheimer's disease, MS and epilepsy, [34-38] our data showed that patients admitted to the Neurology specialty had the highest mean 25(OH)D level compared to patients from all other specialties. Pharmacy records showed that only 5.5% of patients from Neurology were prescribed with vitamin D supplementation, which was on the lower end compared to other specialties (Table 4). 41.7% of Neurology patients from our data were diagnosed with MS. Given the numerous literature on the role of vitamin D in MS;

low serum vitamin D status is associated with increased susceptibility to MS and worse disease progression, [39-46] it is conceivable that some MS patients could have acquired vitamin D supplementation from other sources, and total vitamin D intake was not recorded. Moreover, patients with other neurological conditions (beside MS) could have also acquired vitamin D supplementation that was not recorded in the prescription records, resulting in higher serum 25(OH)D measured. [47-50] This could help explain the contradiction of our results to other findings regarding vitamin D status of patients with neurological disorders. Our findings also showed that patients admitted to private hospitals had the second highest mean 25(OH)D level. As patients from private hospitals are a heterogeneous group, and comprised of patients with a variety of medical conditions, it is difficult to elucidate specific factors that could have contributed to the higher 25(OH)D levels measured. One of the possible explanations for this finding could be that patients from private hospitals are presumably more health literate, and hence are more likely to use supplementations. Moreover, our results showed that patients admitted to Endocrinology had a higher mean 25(OH)D level compared to patients from other specialties (except Neurology and private hospitals). Most patients admitted to Endocrinology at RMH were treated for osteoporosis. Given the numerous literature on vitamin D and bone health, [51-53] these patients are commonly prescribed with vitamin D and calcium supplementation as part of their treatment. A study on 68 residential aged care facilities in Australia (n=9,094) in 2014-2017 found that 60% of the residents consistently use vitamin D supplementation. [54] Hence, it is unsurprising that the mean serum 25(OH)D level for Endocrinology was higher compared to other medical specialties in our study. Our findings also showed that patients admitted to Nephrology had the lowest mean serum 25(OH)D level compared to patients from other specialties. The majority of patients from Nephrology at RMH were treated for chronic kidney disease (CKD). Vitamin D levels are generally low in these patients, as CKD is associated with low levels of vitamin D, especially in patients with end-stage renal disease and kidney transplant recipient. [55-59] Our findings are consistent with results from previous studies that demonstrated the association between low vitamin D levels and CKD.

Seasonal variation in serum 25(OH)D concentration has been addressed in several prior studies. [60-62] Our results are in agreement with findings of previous studies that demonstrated lower vitamin D status during winter and higher levels during summer months. In a population-based study of 27,203 women (≥55 years) in Geelong (southeastern Australia), serum 25(OH)D was found to peak in summer and dip in winter ($p<0.001$), which is consistent with our results. [61] Interestingly, despite the fact that more patients were prescribed vitamin D supplementation during winter (n=697) compared to summer (n=529), serum 25(OH)D levels remained lower during winter, emphasising the role of seasonal variation of UVB exposure on vitamin D status regardless of supplementation. Notably, serum 25(OH)D status was significantly higher in 2014 compared to 2016-2017. This is contradictory to the rising trend in vitamin D status for the past two decades. [63] Moreover, pharmacy records showed a rise in prescription of vitamin D supplements every year from 2014 to 2017 (Table 4), particularly between 2014 and 2015. In 2014, only 5.6% of patients were prescribed vitamin D supplements compared to 8.6% in 2015. One of the possible explanations for this contradiction is the increasing prevalence of obesity in Australia. According to the Australian Bureau of Statistics, in 2017-2018, 67.0% of Australian

adults were overweight and obese, an increase from 63.4% in 2014-2015. [64] Current literature shows a consistent association between obesity and low vitamin D concentrations as well as lower vitamin D supplementation intake. [65, 66] Possible mechanisms for lower serum vitamin D status in obese individuals have been proposed, [67, 68] and given the growing literature on the association between obesity and low vitamin D concentrations, it would be interesting to include the body mass index (BMI) of patients to elucidate this possible association.

Limitations

Our study had a number of limitations. First, this study was carried out retrospectively and factors such as dietary habits of patients or direct measure of sun exposure were not included. Second, there is no consensus on the definition of the clinical terms vitamin D 'deficiency', 'insufficiency', 'sub-optimal' and 'sufficiency', as well as the desirable levels of serum 25(OH)D. Third, since vitamin D supplementation data was derived from pharmacy dispensing records at RMH, it cannot be excluded that some patients might elect to purchase vitamin D supplements at other sources such as the supermarket, health food stores, or online. It is also important to note that because vitamin D supplementation does not require a prescription, vitamin D intake of some patients might not be included in the dispensing records. Fourth, in the supplementation data, the actual quantity of supplement (i.e. units of vitamin D dispensed) was not recorded. Fifth, because the data collected does not differentiate between patients who received inpatient and outpatient care, a comparison of vitamin D status between these two populations cannot be made. Sixth, due to the lack of information on the race-ethnicity of the patients, serum 25(OH)D levels were not stratified according to race-ethnicity. Seventh, the patient population presented here although quite large, may not be reflective of the general population (general community) as our patients were all from a tertiary hospital setting and likely with medical co-morbidities and potentially also with increased rates of vitamin D prescription.

Conclusion

This is the largest study of vitamin D status in patients from a tertiary health center in Australia to date. The findings reveal that there are sex, age, medical specialty, and seasonal variations in serum concentration of 25(OH)D in an Australian tertiary hospital population. Based on our findings, patients who were not on vitamin D supplementation were at risk of vitamin D deficiency, especially during winter. Our findings showing female gender, older age, and Neurology patients having higher 25(OH)D levels are contrary to those previous reports globally. Our study also reveals that despite the role of supplementation, serum 25(OH)D remained the lowest during winter, highlighting the need of other interventions to boost vitamin D status during winter. Further research is warranted to establish the role of vitamin D in health and disease state, as well as the variations in individual characteristics, seasonal, and geographic locations that contribute to the total vitamin D levels. Specifically given the association between vitamin D and immune-mediated diseases such as MS, understanding the role of this key vitamin would help delineate potential therapeutic approaches in combatting these diseases.

References

1. Medicare Australia. Medicare Australia Statistics. Available at www.medicareaustralia.gov.au [Accessed 11 February 2019].
2. Sahota O. Understanding vitamin D deficiency. Age and ageing. 2014;43(5):589-91.
3. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. The Journal of clinical endocrinology and metabolism. 2011;96(1):53-8.
4. Rizzoli R, Boonen S, Brandi ML, Bruyere O, Cooper C, Kanis JA, et al. Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Current medical research and opinion. 2013;29(4):305-13.
5. Issa CM. Vitamin D and Type 2 Diabetes Mellitus. Advances in experimental medicine and biology. 2017;996:193-205.
6. Berridge MJ. Vitamin D deficiency and diabetes. The Biochemical journal. 2017;474(8):1321-32.
7. Podzolkov VI, Pokrovskaya AE, Panasenko OI. Vitamin D deficiency and cardiovascular pathology. Terapevticheskii arkhiv. 2018;90(9):144-50.
8. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. The British journal of psychiatry: the journal of mental science. 2013;202:100-7.
9. Woo YS, Kim S, Jeong JH, Jung YE, Kim MD, Bahk WM. Vitamin D Deficiency/Insufficiency among Inpatients with Depressive Symptoms. Clinical psychopharmacology and neuroscience: the official scientific journal of the Korean College of Neuropsychopharmacology. 2019;17(1):121-4.
10. Komisarenko YI, Bobryk MI. Vitamin D Deficiency and Immune Disorders in Combined Endocrine Pathology. Frontiers in endocrinology. 2018;9:600.
11. Sharif K, Sharif Y, Watad A, Yavne Y, Lichtbroun B, Bragazzi NL, et al. Vitamin D, autoimmunity and recurrent pregnancy loss: More than an association. American journal of reproductive immunology (New York, NY : 1989). 2018;80(3):e12991.
12. Bozdag H, Akdeniz E. Does severe vitamin D deficiency impact obstetric outcomes in pregnant women with thyroid autoimmunity? The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2018:1-11.
13. Balbuena LD, Middleton RM, Tuite-Dalton K, Pouliou T, Williams KE, Noble GJ. Sunshine, Sea, and Season of Birth: MS Incidence in Wales. PloS one. 2016;11(5):e0155181.
14. Dobson R, Giovannoni G, Ramagopalan S. The month of birth effect in multiple sclerosis: systematic review, meta-analysis and effect of latitude. Journal of neurology, neurosurgery, and psychiatry. 2013;84(4):427-32.
15. Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, et al. The association between neonatal vitamin D status and risk of schizophrenia. Scientific reports. 2018;8(1):17692.
16. Boettger SF, Angersbach B, Klimek CN, Wanderley ALM, Shaibekov A, Sieske L, et al. Prevalence and predictors of vitamin D-deficiency in frail older hospitalized patients. BMC Geriatrics. 2018;18(1):219.
17. Uusi-Rasi K, Patil R, Karinkanta S, Tokola K, Kannus P, Lamberg-Allardt C, et al. Serum 25-hydroxyvitamin D levels and incident falls in older women. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2019;30(1):93-101.
18. Holick MF. Sunlight and Vitamin D AU - Wacker, Matthias. Dermato-Endocrinology. 2013;5(1):51-108.
19. Al Zarooni AAR, Al Marzouqi FI, Al Darmaki SH, Prinsloo EAM, Nagelkerke N. Prevalence of vitamin D deficiency and associated comorbidities among Abu Dhabi Emirates population. BMC research notes. 2019;12(1):503.
20. Bhattoa HP, Nagy E, More C, Kappelmayer J, Balogh A, Kalina E, et al. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in healthy Hungarian men over 50 years of age: the HunMen Study. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013;24(1):179-86.
21. Gu Y, Zhu Z, Luan X, He J. Vitamin D status and its association with season, depression in stroke. Neurosci Lett. 2019;690:99-105.

22. Hansen L, Tjonneland A, Koster B, Brot C, Andersen R, Cohen AS, et al. Vitamin D Status and Seasonal Variation among Danish Children and Adults: A Descriptive Study. Nutrients. 2018;10(11).
23. Niculescu DA, Capatina CAM, Dusceac R, Caragheorgheopol A, Ghemigian A, Poiana C. Seasonal variation of serum vitamin D levels in Romania. Arch Osteoporos. 2017;12(1):113.
24. Touvier M, Deschasaux M, Montourcy M, Sutton A, Charnaux N, Kesse-Guyot E, et al. Determinants of vitamin D status in Caucasian adults: influence of sun exposure, dietary intake, sociodemographic, lifestyle, anthropometric, and genetic factors. The Journal of investigative dermatology. 2015;135(2):378-88.
25. van Schoor NM, Lips P. Worldwide vitamin D status. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):671-80.
26. Lips P. Worldwide status of vitamin D nutrition. The Journal of steroid biochemistry and molecular biology. 2010;121(1-2):297-300.
27. Joshi D, Center JR, Eisman JA. Vitamin D deficiency in adults. Australian Prescriber. 2010;33:103-6.
28. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmunity reviews. 2010;9(5):A387-94.
29. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clinical Endocrinology. 2012;77(1):26-35.
30. van der Mei Ingrid AF, Ponsonby A-L, Engelsen O, Pasco Julie A, McGrath John J, Eyles Daryl W, et al. The High Prevalence of Vitamin D Insufficiency across Australian Populations Is Only Partly Explained by Season and Latitude. Environmental Health Perspectives. 2007;115(8):1132-9.
31. Ardawi MS, Qari MH, Rouzi AA, Maimani AA, Raddadi RM. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre- and postmenopausal women. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2011;22(2):463-75.
32. Chen J, Yun C, He Y, Piao J, Yang L, Yang X. Vitamin D status among the elderly Chinese population: a cross-sectional analysis of the 2010-2013 China national nutrition and health survey (CNNHS). Nutrition journal. 2017;16(1):3.
33. Sadat-Ali M, Al Elq AH, Al-Turki HA, Al-Mulhim FA, Al-Ali AK. Influence of vitamin D levels on bone mineral density and osteoporosis. Annals of Saudi medicine. 2011;31(6):602-8.
34. Annweiler C, Schott AM, Berrut G, Chauviré V, Le Gall D, Inzitari M, et al. Vitamin D and Ageing: Neurological Issues. Neuropsychobiology. 2010;62(3):139-50.
35. Littlejohns TJ, Henley WE, Lang IA, Annweiler C, Beauchet O, Chaves PHM, et al. Vitamin D and the risk of dementia and Alzheimer disease. Neurology. 2014;83(10):920.
36. Knekt P, Kilkkinen A, Rissanen H, Marniemi J, Sääksjärvi K, Heliövaara M. Serum Vitamin D and the Risk of Parkinson Disease. Archives of Neurology. 2010;67(7):808-11.
37. Hollo A, Clemens Z, Lakatos P. Epilepsy and vitamin D. The International journal of neuroscience. 2014;124(6):387-93.
38. Mazdeh M, Zamani M, Eftekharian MM, Komaki A, Arsang-Jang S, Taheri M, et al. Expression analysis of vitamin D receptor-associated lncRNAs in epileptic patients. Metabolic brain disease. 2019.
39. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60.
40. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS genetics. 2009;5(2):e1000369.
41. van der Mei IAF, Ponsonby AL, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. Journal of Neurology. 2007;254(5):581.
42. Lucas RM, Ponsonby AL, Dear K, Valery PC, Pender MP, Taylor BV, et al. Sun exposure and vitamin D are independent risk factors for CNS demyelination. Neurology. 2011;76(6):540-8.
43. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. PLoS medicine. 2015;12(8):e1001866.
44. Rhead B, Baarnhielm M, Gianfrancesco M, Mok A, Shao X, Quach H, et al. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurology Genetics. 2016;2(5):e97.
45. Zamzam D, Foad M, Swelam M, AbdelHafez M, AbdelNasser A, Mahmoud R, et al. Vitamin D and body mass index in Egyptian multiple sclerosis patients. Multiple sclerosis and related disorders. 2019;28:313-6.

46. Voo VTF, O'Brien T, Butzkueven H, Monif M. The role of vitamin D and P2X7R in multiple sclerosis. Journal of neuroimmunology. 2019;330:159-69.
47. Suzuki M, Yoshioka M, Hashimoto M, Murakami M, Noya M, Takahashi D, et al. Randomized, double-blind, placebo-controlled trial of vitamin D supplementation in Parkinson disease. The American journal of clinical nutrition. 2013;97(5):1004-13.
48. Taghizadeh M, Talaei SA, Djazayeri A, Salami M. Vitamin D supplementation restores suppressed synaptic plasticity in Alzheimer's disease. Nutritional neuroscience. 2014;17(4):172-7.
49. Gangwar AK, Rawat A, Tiwari S, Tiwari SC, Narayan J, Tiwari S. Role of Vitamin-D in the prevention and treatment of Alzheimer's disease. Indian journal of physiology and pharmacology. 2015;59(1):94-9.
50. Al-Nimer MS. The Benefit of Prescribing Vitamin D as Add on Therapy on the Electrocardiographic Changes in Epileptic Patients. Current clinical pharmacology. 2018;13(1):40-4.
51. Hill TR, Verlaan S, Biesheuvel E, Eastell R, Bauer JM, Bautmans I, et al. A Vitamin D, Calcium and Leucine-Enriched Whey Protein Nutritional Supplement Improves Measures of Bone Health in Sarcopenic Non-Malnourished Older Adults: The PROVIDE Study. Calcified tissue international. 2019;105(4):383-91.
52. Thiagarajan NR, Kumar CGD, Sahoo J, Krishnamurthy S. Effect of Vitamin D and Calcium Supplementation on Bone Mineral Content in Children with Thalassemia. Indian pediatrics. 2019;56(4):307-10.
53. Weaver CM, Alexander DD, Boushey CJ, Dawson-Hughes B, Lappe JM, LeBoff MS, et al. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016;27(1):367-76.
54. Lind KE, Jorgensen ML, Gray LC, Georgiou A, Westbrook JI. Anti-osteoporosis Medication Use in a High Fracture-Risk Population: Contemporary Trends in Australian Residential Aged Care Facilities. Health services insights. 2019;12:1178632919852111.
55. Capelli I, Cianciolo G, Gasperoni L, Galassi A, Ciceri P, Cozzolino M. Nutritional vitamin D in CKD: Should we measure? Should we treat? Clinica chimica acta; international journal of clinical chemistry. 2019.
56. Gonzalez EA, Sachdeva A, Oliver DA, Martin KJ. Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. American journal of nephrology. 2004;24(5):503-10.
57. Thorsen IS, Bleskestad IH, Asberg A, Hartmann A, Skadberg O, Brede C, et al. Vitamin D as a risk factor for patient survival after kidney transplantation: A prospective observational cohort study. Clinical transplantation. 2019;33(5):e13517.
58. Timalsina S, Sigdel MR, Baniya S, Subedee S. Status of vitamin D and parameters of calcium homeostasis in renal transplant recipients in Nepal: a cross sectional study. BMC nephrology. 2018;19(1):290.
59. Wu CC, Liao MT, Hsiao PJ, Lu CL, Hsu YJ, Lu KC, et al. Antiproteinuria Effect of Calcitriol in Patients With Chronic Kidney Disease and Vitamin D Deficiency: A Randomized Controlled Study. Journal of renal nutrition: the official journal of the Council on Renal Nutrition of the National Kidney Foundation. 2019.
60. Ma F, Levis S, Roos BA, Lai S, Gomez A, Jimenez C, et al. Vitamin D Deficiency and Seasonal Variation in an Adult South Florida Population. The Journal of Clinical Endocrinology & Metabolism. 2005;90(3):1557-62.
61. Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR, et al. Seasonal Periodicity of Serum Vitamin D and Parathyroid Hormone, Bone Resorption, and Fractures: The Geelong Osteoporosis Study. Journal of Bone and Mineral Research. 2004;19(5):752-8.
62. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. Canadian Medical Association Journal. 2002;166(12):1517.
63. McKenna MJ, Murray BF, O'Keane M, Kilbane MT. Rising trend in vitamin D status from 1993 to 2013: dual concerns for the future. Endocrine connections. 2015;4(3):163-71.
64. Australian Bureau of Statistics. 2018. National Health Survey: First Results, 2017-18. Available at https://www.abs.gov.au/ausstats/abs@.nsf/mf/4364.0.55.001 [Accessed 12 December 2019].
65. Beketova NA, Pavlovskaya EV, Kodentsova VM, Vrzhesinskaya OA, Kosheleva OA, Sokolnikov AA, et al. [Biomarkers of vitamin status in obese school children]. Voprosy pitaniia. 2019;88(4):66-74.
66. Orces C. The Association between Body Mass Index and Vitamin D Supplement Use among Adults in the United States. Cureus. 2019;11(9):e5721.
67. Li J, Gao Y, Yu T, Lange JK, LeBoff MS, Gorska A, et al. Obesity and Leptin Influence Vitamin D Metabolism and Action in Human Marrow Stromal Cells. The Journal of steroid biochemistry and molecular biology. 2019:105564.
68. Vanlint S. Vitamin D and obesity. Nutrients. 2013;5(3):949-56.

Figure 1. Histogram of serum 25(OH)D distribution in study population (N=30,023)
Histogram showing distribution of serum 25(OH)D levels (nmol/L) among 30,023 patients at Royal Melbourne Hospital, Victoria, Australia between 2014 to 2017. The vertical solid line (in red) is the mean value, 69.9±0.19 nmol/L.

Figure 2. Bar graph of age variation in serum 25(OH)D levels in study population (N=30,023)
Bar graph showing age variation in serum 25(OH)D levels (nmol/L) of 30,023 patients at Royal Melbourne Hospital, Victoria, Australia between 2014 to 2017. 25(OH)D levels increased with age, and patients ≥50 years had significantly higher mean 25(OH)D concentrations compared to patients aged 20-29 years. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 3. Bar graph of variations in serum 25(OH)D level of patients admitted to various medical specialties (N=30,023)
Bar graph showing variation in serum 25(OH)D levels (nmol/L) of 30,023 patients admitted to all medical specialties at Royal Melbourne Hospital, Victoria, Australia between 2014 to 2017. Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis. Analysis showed the average 25(OH)D levels of patients from all other specialties were significantly lower compared to NEUR except ENDO and PRIV. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 4. Line graph of seasonal variation in serum 25(OH)D levels in study population (N=30,023)
Line graph showing seasonal variation in serum 25(OH)D levels (nmol/L) of 30,023 patients at Royal Melbourne Hospital, Victoria, Australia between 2014 to 2017. Analysis showed the average 25(OH)D levels of all other seasons were significantly higher compared to winter. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Figure 5. Line graph of yearly variation (2014-2017) in serum 25(OH)D levels in study population (N=30,023)
Line graph showing variation in serum 25(OH)D levels (nmol/L) of 30,023 patients measured between 2014 to 2017 at Royal Melbourne Hospital, Victoria, Australia. The mean 25(OH)D level measured in 2014 was significantly higher than levels measured in 2016-2017. Error bars represent SEM. Statistical significance of P-values: ˙P<0.05; *P<0.01; **P<0.001; ***P<0.0001.

Table 1. Characteristics of the study population (N=30,023) and average serum 25(OH)D levels.

	N (%)	Mean 25(OH)D (SEM)
Total population	30023 (100%)	69.9 (0.19)
Sex		
Female	18688 (62.3%)	72.1 (0.25)
Male	11302 (37.6%)	66.0 (0.30)
Age group		
20-29	3099 (10.3%)	63.3 (0.55)
30-39	3721 (12.4%)	66.3 (0.56)
40-49	3570 (11.9%)	66.6 (0.57)
50-59	4131 (13.8%)	69.5 (0.53)
60-69	4277 (14.2%)	71.7 (0.49)
>70	11225 (37.4%)	73.2 (0.31)
Medical specialty		
AMU	1690 (5.6%)	69.0 (0.81)
BOE	1053 (3.5%)	71.2 (0.92)
EMER	1656 (5.5%)	69.6 (0.86)
ENDO	1229 (4.1%)	74.0 (0.90)
GAST	1574 (5.2%)	66.9 (0.79)
NEPH	1261 (4.2%)	64.1 (0.80)
NEUR	654 (2.2%)	76.8 (1.66)
ORTH	1080 (3.6%)	67.9 (0.89)
OTHER	14214 (47.3%)	68.0 (0.27)
PRIV	5612 (18.7%)	75.5 (0.47)
Season of measurement		
Summer (Dec-Feb)	5588 (18.6%)	76.1 (0.43)
Autumn (Mar-May)	8366 (27.9%)	74.0 (0.36)
Winter (Jun-Aug)	8320 (27.7%)	64.9 (0.36)
Spring (Sep-Nov)	7749 (25.8%)	66.2 (0.37)
Year of measurement		
2014	8063 (26.9%)	71.5 (0.37)
2015	7620 (25.3%)	71.3 (0.38)
2016	7401 (24.7%)	68.6 (0.39)
2017	6939 (23.1%)	67.7 (0.39)
Vitamin D supplementation		
Yes	2588 (8.6%)	80.4 (0.61)
No information	27435 (91.4%)	68.9 (0.20)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 2: Results of multivariable linear regression analyses to model serum 25(OH)D levels by specialty, adjusting for sex, age, season, year of measurement and vitamin D supplementation intake

	Adjusted difference in mean 25(OH)D	95% confidence interval	P-value
Medical specialty			
NEUR	Reference	-	-
AMU	-12.4	-15.3, -9.4	<0.0001
BOE	-9.1	-12.2, -5.9	<0.0001
EMER	-11.9	-14.9, -9.0	<0.0001
ENDO	-4.8	-7.9, -1.8	0.0017
GAST	-9.7	-12.6, -6.8	<0.0001
NEPH	-14.4	-17.4, -11.4	<0.0001
ORTH	-14.3	-17.4, -11.1	<0.0001
OTHER	-10.8	-13.3, -8.3	<0.0001
PRIV	-1.9	-4.5, -0.7	0.1565

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 3. Characteristics of the study population stratified according to serum 25(OH)D levels.

	Total N	Deficiency (<25nmol/L)	Insufficiency (25-50nmol/L)	Sub-optimal (50-75nmol/L)	Sufficiency (>75nmol/L)	P-value
Total population	30023	1655 (5.5%)	6824 (22.7%)	9467 (31.5%)	12077 (40.2%)	
Sex						
Female	18688	884 (4.7%)	3920 (21.0%)	5836 (31.2%)	8048 (43.1%)	<0.0001
Male	11302	770 (6.8%)	2899 (25.7%)	3622 (32.0%)	4011 (35.5%)	
Age						
20-29	3099	217 (7.0%)	875 (28.2%)	1054 (34.0%)	953 (30.8%)	
30-39	3721	236 (6.3%)	991 (26.6%)	1242 (33.4%)	1252 (33.7%)	
40-49	3570	227 (6.4%)	954 (26.7%)	1163 (32.6%)	1226 (34.3%)	<0.0001
50-59	4131	214 (5.2%)	995 (24.1%)	1320 (32.0%)	1602 (38.7%)	
60-69	4277	204 (4.8%)	862 (20.1%)	1354 (31.7%)	1857 (43.4%)	
>70	11225	557 (5.0%)	2147 (19.1%)	3334 (29.7%)	5187 (46.2%)	
Medical specialty						
AMU	1690	154 (9.1%)	352 (20.8%)	480 (28.4%)	704 (41.7%)	
BOE	1053	37 (3.5%)	212 (20.1%)	358 (34.0%)	446 (42.4%)	
EMER	1656	122 (7.4%)	392 (23.7%)	460 (27.8%)	682 (41.2%)	
ENDO	1229	42 (3.4%)	221 (18.0%)	417 (33.9%)	549 (44.7%)	
GAST	1574	76 (4.8%)	426 (27.1%)	502 (31.9%)	570 (36.2%)	<0.0001
NEPH	1261	86 (6.8%)	336 (26.6%)	405 (32.1%)	434 (34.4%)	
NEUR	654	33 (5.0%)	120 (18.3%)	209 (32.0%)	292 (44.6%)	
ORTH	1080	72 (6.7%)	215 (19.9%)	376 (34.8%)	417 (38.6%)	
OTHER	14214	859 (6.0%)	3456 (24.3%)	4462 (31.4%)	5437 (38.3%)	
PRIV	5612	174 (3.1%)	1094 (19.5%)	1798 (32.0%)	2546 (45.4%)	
Season of measurement						
Summer (Dec-Feb)	5588	168 (3.0%)	901 (16.1%)	1847 (33.1%)	2672 (47.8%)	
Autumn (Mar-May)	8366	266 (3.2%)	1570 (18.7%)	2732 (32.7%)	3798 (45.4%)	<0.0001
Winter (Jun-Aug)	8320	625 (7.5%)	2324 (27.9%)	2545 (30.6%)	2826 (34.0%)	
Spring (Sep-Nov)	7749	596 (7.7%)	2029 (26.2%)	2343 (30.2%)	2781 (35.9%)	
Year of measurement						
2014	8063	277 (3.4%)	1794 (22.2%)	2662 (33.0%)	3330 (41.3%)	
2015	7620	310 (4.1%)	1669 (21.9%)	2530 (33.2%)	3111 (40.8%)	<0.0001
2016	7401	523 (7.1%)	1700 (23.0%)	2267 (30.6%)	2911 (39.3%)	
2017	6939	545 (7.9%)	1661 (23.9%)	2008 (28.9%)	2725 (39.3%)	
Vitamin D supplementation						
Yes	2588	63 (2.4%)	294 (11.4%)	786 (30.4%)	1445 (55.8%)	<0.0001
No information	27435	1592 (5.8%)	6530 (23.8%)	8681 (31.6%)	10632 (38.8%)	

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Table 4. Characteristics of the study population on vitamin D supplementation

	Vitamin D supplementation +	No information (%)
Sex		
Female	1468 (7.9%)	17220 (92.1%)
Male	1120 (9.9%)	10182 (90.1%)
Age group		
20-29	93 (3.0%)	3006 (97.0%)
30-39	127 (3.4%)	3594 (96.6%)
40-49	148 (4.1%)	3422 (95.9%)
50-59	221 (5.3%)	3910 (94.7%)
60-69	327 (7.6%)	3950 (92.4%)
>70	1672 (14.9%)	9553 (85.1%)
Medical specialty		
AMU	326 (19.3%)	1364 (80.7%)
BOE	23 (2.2%)	1030 (97.8%)
EMER	211 (12.7%)	1445 (87.3%)
ENDO	129 (10.5%)	1100 (89.5%)
GAST	114 (7.2%)	1460 (92.8%)
NEPH	184 (14.6%)	1077 (85.4%)
NEUR	36 (5.5%)	618 (94.5%)
ORTH	91 (8.4%)	989 (91.6%)
OTHER	1422 (10.0%)	12792 (90.0%)
PRIV	52 (0.9%)	5560 (99.1%)
Season of measurement		
Summer (Dec-Feb)	529 (9.5%)	5059 (90.5%)
Autumn (Mar-May)	672 (8.0%)	7694 (92.0%)
Winter (Jun-Aug)	697 (8.4%)	7623 (91.6%)
Spring (Sep-Nov)	690 (8.9%)	7059 (91.1%)
Year of measurement		
2014	455 (5.6%)	7608 (94.4%)
2015	652 (8.6%)	6968 (91.4%)
2016	730 (9.9%)	6671 (90.1%)
2017	751 (10.8%)	6188 (89.2%)

Abbreviations: AMU, Acute Medical Unit; BOE, Breast/Oncology/Endocrine Surgery; EMER, Emergency; ENDO, Endocrinology; GAST, Gastroenterology; NEPH, Nephrology; NEUR, Neurology; ORTH, Orthopaedic; PRIV, Private. Specialties with <1000 patients (except for NEUR) were placed into OTHER for analysis.

Authorship statement

Manuscript title:

Vitamin D status in an Australian patient population – a large retrospective case series focusing on factors associated with variations in serum 25(OH)D

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *British Medical Journal (BMJ)*.

Authorship contributions

Conception and design of study: Monif M, Butzkueven H, Voo VTF, Stankovich J, O'Brien T

Acquisition of data: Monif M, Voo VTF

Analysis and/or interpretation of data: Stankovich J, Voo VTF, Monif M, Butzkueven H

Drafting the manuscript: Voo VTF

Revising the manuscript critically: Voo VTF, Monif M, Stankovich J, O'Brien T

All authors contributed to refinement of the study protocol and approved the final manuscript.

Acknowledgements

We would like to acknowledge Maria Bisignano, research support coordinator at Melbourne Health Shared Pathology Service for extracting the results of serum 25(OH)D lab tests from Royal Melbourne Hospital pathology services. We would also like to thank Karen Honson, pharmacist at Royal Melbourne Hospital for extracting the supplementation data from the prescription records.

Data Sharing statement: All data are presented in results. No additional data are available.

1