Review Article - Clinical

Vitamin D and eye: Current evidence and practice guidelines

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Vitamin D is a steroid hormone that has widespread role in human physiology, not only in the maintenance of calcium homeostasis but also in immunomodulation, cellular differentiation, and proliferation. The immunomodulatory effects of vitamin D are well known and are applicable to the ocular surface immune cells and structural cells. The role of vitamin D in ocular surface conditions such as dry eye disease (DED), keratoconus (KC), and post-surgical outcomes has received widespread and well-deserved attention. Vitamin D supplementation is shown to improve DED clinically as well as in experimental models. The anti-inflammatory properties may be crucial in the treatment of ocular surface conditions such as DED and KC. Vitamin D plays a multifaceted role in corneal wound healing with its anti-inflammatory and extracellular matrix remodeling properties. In this review, we discuss how to approach patients with DED and those undergoing refractive surgery with the available basic and clinical knowledge on the role of vitamin D in these conditions. We aim to highlight the importance of clinically harnessing vitamin D-mediated natural immuno-inflammatory modulation in combination with currently available standard of care strategies to reduce the morbidity and disease duration associated with ocular surface diseases.

Access this article online

Website: www.ijo.in

DOI: 10.4103/IJO.IJO_3174_22

Quick Response Code:

Key words: Dry eye disease, keratoconus, vitamin D, vitamin D deficiency

The initial interest in vitamin D was purely for its role in calcium and mineral homeostasis in skeletal tissues. At the time of its discovery, vitamin D was considered as a vitamin sourced from food to treat rickets.[1] However, evidence started pouring in from multiple specialties of medicine on its role in immune and inflammation modulation, sustenance of epithelial barrier integrity, and cellular homeostasis. Hence, vitamin D is now categorized as an endocrine mediator. In this article, we outline the existing basic and clinical knowledge on the role of vitamin D in the pathophysiology of ocular surface conditions such as dry eye disease (DED), keratoconus (KC), etc., and the treatment suggestions for vitamin D deficiency (VDD) in these patients.

Source and Metabolism of Vitamin D

There are two sources of vitamin D available to humans: dietary sources and local synthesis in skin. The dietary sources include foods that naturally contain vitamin D such as fish, egg yolk, and liver.[2] Many nations have arranged for the fortification of commonly used foods like milk, breakfast cereals, and orange juice.[3] The metabolites used for vitamin D fortification are either ergocalciferol (D2) or cholecalciferol (D3).[2] Endogenously, vitamin D3 is synthesized from 7-dehydrocholesterol in the skin through photo-conversion under the influence of ultraviolet B radiation. It is then transported to the liver where it is converted to 25 hydroxyvitamin D3 (25-OH-D3) by the enzyme 25-hydroxylase.[4] It then undergoes further modification in the proximal convoluted tubules of kidneys to 1,25 – $(OH)_2$D3 (calcitriol, active form of vitamin D) by the enzyme 25-OH-1 alpha hydroxylase.[5] This enzyme is encoded by the gene CYP27B1, whose expression is stimulated by the parathyroid hormone, depending on the serum concentrations of calcium and phosphorous.[6]

Metabolism of vitamin D in ocular surface and other peripheral tissues

Extrarenal synthesis of 1,25-$(OH)_2$D3 has been demonstrated in many tissues including the skin, respiratory system, breast, colon, endometrium, and prostate.[7-10] Similarly, the eye has many cell types including corneal epithelial cells, endothelial cells, scleral fibroblasts, and retinal pigment epithelial cells which express both 1α–hydroxylase and 25α–hydroxylase, the enzymes responsible for the activation of vitamin D3.[10,11] These observations establish that many ocular structures have machinery for metabolizing vitamin D3 into its active metabolites.

Mechanisms of Action of Vitamin D

The cellular effects of vitamin D are predominantly mediated by its interaction with the vitamin D receptor (VDR) and subsequent transcriptional regulation in the nucleus.[12] Nevertheless, there are certain cellular events that are too rapid

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Received: 04-Dec-2022 Revision: 17-Jan-2023
Accepted: 10-Feb-2023 Published: 05-Apr-2023

Cite this article as: Gorimanipalli B, Shetty R, Sethu S, Khamar P. Vitamin D and eye: Current evidence and practice guidelines. Indian J Ophthalmol 2023;71:1127-34.

in response to vitamin D, such as induction of ion channel activity, intracellular cell signaling, and related transcriptional activity while the VDR is still localized in the cell periphery, hence, considered to be the non-genomic effects of vitamin D.[13]

Genomic actions of vitamin D - Vitamin D receptor

The vitamin D receptor (VDR) is a nuclear receptor that heterodimerizes with retinoid- X receptor, after binding with vitamin D.[14] The ligand-bound heterodimer then interacts with the vitamin D responsive element (VDRE) genes. After the interaction with the transcription factors, together with co-activators or co-repressors, the ligand-bound VDR-RXR complex either increases or decreases the transcription of a multitude of target genes.[12,15]

Non-genomic actions of vitamin D

Based on the cell type, vitamin D interacts with and activates ion channels (calcium, chloride), various intracellular signaling molecules such as phospholipase C (PLC), phospholipase A_2 (PLA_2), phosphatidylinositol-3 kinase (PI3K), and protein kinases such as MAP kinases among others.[13] These activated molecules further interact with transcription factors to regulate the expression of a variety of genes. Another non-genomic action of calcitriol (active form of vitamin D) is to influence the gene expression mediated by factors such as IFNα and TNFα by regulating VDR binding with signaling factors such as STAT1 and IKKβ that is critical in mediating the effects of IFNα and TNFα.[16]

Status of Vitamin D in Ocular Surface

Tear fluid vitamin D

Tear fluid contains higher levels of 25-OH-D3 than the serum in healthy human subjects.[17] The ocular surface including corneal and conjunctival epithelium is capable of metabolizing vitamin D. Since the serum concentrations of vitamin D are different from its tear levels, it is prudent to measure the tear fluid vitamin D levels, while assessing the association between vitamin D and ocular diseases.

Vitamin D receptor expression on ocular surface

As mentioned earlier, the ocular surface epithelial cells including corneal epithelium express VDR,[11] and vitamin D influences many cellular functions of these epithelial cells including the barrier function,[11,18] response to inflammation and infections.[19]

Vitamin D and Ocular Surface Physiology

Regulation of inflammation

Vitamin D is an established endogenous modulator of immune response and is known for its anti-inflammatory effects.[20] Calcitriol ($1,25\text{-}(OH)_2D3$) is shown to inhibit the hyperosmotic stress-induced cellular inflammation in human corneal epithelial cells (HCECs).[21] Vitamin D is also demonstrated to modify the toll-like receptor (TLR)-mediated inflammation and reduce the release of pro-inflammatory cytokines in HCECs.[19,22] Our group previously demonstrated that $1,25\text{-}(OH)_2D3$ in addition to the reduction of hyperosmotic stress-induced inflammatory gene expression, calcitriol (with genistein) was able to prevent hyperosmotic stress-induced VDR degradation in HCECs.[23] Topical application of $1,25\text{-}(OH)_2D3$ in animal models with dry eye reduced the corneal inflammation and improved clinical signs.[24] $1,25\text{-}(OH)_2D3$ complex inhibits maturation of dendritic cells (DC) and improves the tolerance of these cells by modulating the cytokine and chemokine production.[25] Serum vitamin D levels were shown to be inversely correlated to corneal dendritic cell density (DCD), potentiating the immunoregulatory role of this vitamin.[26]

Epithelial barrier function

1,25(OH)2D3 plays a favorable role in maintaining epithelial gap junction integrity. Lu *et al*.[18] demonstrated that 1,25(OH) 2D3 and 24R,25(OH) 2D3 enhanced gap junction connectivity in HCECs and cultured mouse primary epithelial cells. Activated VDR suppresses the activity of beta-catenin and epithelial cell proliferation.[27] 25(OH) D3- and 1,25(OH) 2D3-treated HCECs and rabbit corneal epithelial cells showed enhanced corneal epithelial barrier function.

Tear Fluid Homeostasis and Hyperosmolarity

Effect of Vitamin D on corneal epithelial cells under hyperosmotic stress

Tear hyperosmolarity plays a critical role in the pathogenesis of DED. The ocular surface of patients with DED showed increased expression of TonEBP (tonicity-responsive enhancer-binding protein, an osmoresponsive factor), inflammatory factors and reduced expression of VDR, suggestive of hyperosmotic stress-induced degradation of VDR, and increased inflammation. Our team has demonstrated that 1,25(OH)2D3 along with genistein reduced the TonEBP, inflammatory gene expression, and mitigated the VDR degradation.[23] Genistein is an isoflavone (present in soybeans) that has diverse biological activities including reduction of inflammation, ion channel modulation, and it also prevents stress-induced vitamin D receptor degradation. 1,25(OH)2D3 was shown to induce autophagy, suppress inflammation, reduce the oxidative stress, and protect HCECs from hyperosmotic stress-induced effects. Vitamin D supplementation through intramuscular injection reduced the tear film osmolarity in patients with VDD.[28]

Vitamin D and Pain Modulation

We have earlier showed that serum 25-OH-D3 levels were inversely correlated with the ocular surface disease index (OSDI) scores in patients with evaporative DED.[26] We have also reported that patients with DED showed lower tear fluid anti-nociceptive factors and low serum vitamin D.[29]

Vitamin D Deficiency – Hypovitaminosis D

The serum 25-OH-D3 levels are considered to assess vitamin D status since it is a longer lasting metabolite with higher levels than 1,25(OH)2D3. The threshold serum levels of 25-OH-D3 below which VDD to be considered have been changed over the years.[30,31] During earlier days of our knowledge on role vitamin D in the maintenance of bone health, presence or absence of rickets was considered as the sign of VDD.[30] The threshold levels of 25-OH-D3 to be considered as normal are derived based on the relationship between 25-OH-D3 and parathyroid hormone (PTH). PTH closely regulates the serum calcium and phosphorous levels. The serum levels of PTH are negatively correlated to that of 25-OH-D3 until the latter reaches the levels of 30-40 ng/milliliter (ml) (75–100 nano mol/liter). The

PTH levels reach their lowest beyond these concentrations of the 25-OH-D3. Hence, the serum 25-OH-D3 concentrations of 30 ng/ml are considered as normal.[31,32] The serum levels of 21–29 ng/ml are considered as relative insufficiency, and those less than 20 ng/ml are considered as deficiency.[33]

Prevalence of vitamin D deficiency

The prevalence of VDD is reported to be different in countries with different levels of exposure to sunlight. The reported prevalence of VDD in India at community level was between 50 and 90%.[34] The high prevalence of VDD in healthy individuals is of concern.[35] Shukla *et al*.[35] reported the prevalence of VDD as 93% among healthy individuals who enrolled under preventive health checkup in an urban hospital. Healthy subjects of all age groups and both genders ranging from newborns, adolescents to post-menopausal women are reported to have high prevalence of VDD.[36-39] Similarly, multiple studies showed high prevalence of VDD among patients with various illnesses, including glucose intolerance, thyroid dysfunction, and chronic kidney disease.[40-42]

Etiology and risk factors of vitamin D deficiency

The etiology and risk factors of VDD are mentioned in Table 1. These etiologies can be categorized into those resulting in reduced bio-synthesis of vitamin D, reduced oral intake, bio-availability, impaired metabolism, and increased loss of vitamin D from the body. The vitamin D supplementation should be given more emphasis in the groups of individuals who are at higher risk of VDD including those with malabsorption syndromes, high rates of catabolism, parathyroid hormone anomalies, etc., [Table 2].

Vitamin D deficiency and Ocular Surface Pathologies

Dry eye disease (DED)

Association between hypovitaminosis D and DED

Few questionnaire-based public health and nutrition surveys previously reported no significant association between serum 25-OH-D3 levels and the risk of DED.[46] The diagnosis of DED was made based on OSDI score, or a self-reported diagnosis of DED in the past. These surveys have limitations in diagnosing the subjects with DED based solely on the patient reported questionnaires without an evaluation of tear film status. A case–control study which tested the subjects' Schirmer-1 test scores, tear breakup time, and OSDI scores, however, reported a significant association between serum 25-OH-D3 and DED incidence.[48] A recent meta-analysis showed that patients with VDD had higher mean OSDI scores and lower Schirmer's test scores suggesting higher symptomatic disturbances and lower tear secretion.[49] Similarly, patients with DED had lower mean serum vitamin D levels than controls.[49]

Hypovitaminosis D and aqueous deficient DED

Vitamin D, being an endogenous immunoregulator, may have a protective role in the pathogenesis of Sjogren's syndrome.[50] Serum 25-OH-D3 levels may be associated with severity of DED in patients with Sjogren's syndrome.[47]

Hypovitaminosis D and evaporative dry eye

An inverse correlation was reported between serum 25-OH-D3 levels and OSDI scores in patients with evaporative DED, suggestive of a possible role of vitamin D in pain modulation and regulation of inflammation.[29] Patients with low serum vitamin D levels showed lower TBUT and Schirmer's test scores than controls.[51]

Vitamin D and Keratoconus

Ectasia is a manifestation of dysregulated extracellular matrix remodeling (ECM). Even though initially considered as a non-inflammatory disease, tear fluid of patients with keratoconus has demonstrated elevated inflammatory mediators.[52-54] Multiple inflammatory cytokines, including IL-1β,[55] TNFα,[56] IL-6,[57,58] IL-17A,[59] IFNγ,[60] and MMPs,[58] that are capable of influencing ECM remodeling are elevated in the ocular surface tissues or tear fluid of keratoconus patients. Vitamin D, an established endogenous modulator of immune and inflammatory processes, positively regulates the ECM metabolism. Patients with keratoconus, particularly those with progressive disease, are shown to have lower serum levels of vitamin D than the normal population.[61-63] Our group has shown decreased expression of VDR in epithelium over the ectatic zones of cornea in keratoconus.[64] We further demonstrated that vitamin D addition helps induce VDR in epithelial cells under oxidative stress.[64] Clinically, Knapp in 1939 showed that vitamin D supplementation caused flattening of the cone in six patients with keratoconus.[65] Future studies should explore the benefits of vitamin D supplementation in treatment of keratoconus.

Table 1: Vitamin D deficiency: Risk factors and etiopathogenetic mechanisms

Pathophysiology	Etiology	Conditions
Reduced synthesis of vitamin D3 in skin	Reduced exposure to ultraviolet B (UV B) rays Increased absorption of UV B rays	Areas with low sun exposure[43] Sunscreen use[44,45] Increased melanin pigment in the skin
Reduced oral intake and bio-availability of vitamin D from food sources	Low vitamin D3 in the foods Reduced bio-availability due to high amount of phytates and phosphates Malabsorption syndromes	Vegetarian food sources, unfortified milk High-fiber foods Cystic fibrosis, celiac disease
Impaired metabolism of vitamin D	Decreased synthesis of 25-OH-D3 Decreased synthesis of 1,25(OH) 2D3 Increased de-activation of 25-OH-D3 and 1,25(OH) 2D3 and conversion to calcitroic acid	Liver disorders Chronic renal disorders Drug-induced HAART, glucocorticoids
Increased loss of vitamin D	Increased urinary excretion of 25-OH-D3	Nephrotic syndrome

D3 - calcitriol; HAART - Highly active antiretroviral therapy

Vitamin D and Post-photorefractive Keratectomy (PRK) Haze

Corneal wound healing is an orchestrated response to any kind of injury that involves cytokine and growth factor-mediated interaction between epithelium, stroma, immune cells, and nerves.[66] Vitamin D has a complex integrated role on the corneal wound healing response. It is shown to delay the re-epithelization after acute injuries,[67,68] but such effect is shown to be concentration and the micro-environment dependent.[67] We have previously shown a significant association and higher risk of developing haze after PRK with VDD.[69] Further research should be able to throw light on the clinical applications of vitamin D in the management of post-refractive surgery deviant outcomes.

Clinical Relevance and Management

Vitamin D replacement therapy and dry eye syndrome

Patients with DED should be pro-actively screened for vitamin D status. Those with VDD should be treated as per the standard guidelines. The daily recommended dose of vitamin D supplementation depends on age of the individual, presence of risk factors for VDD, and the status of the vitamin D sufficiency. Table 3 shows the recommended daily intakes of vitamin D for healthy individuals of different ages and those at risk of deficiency. The Institute of Medicine (IOM), USA, and the US Endocrine Society (ES) gave slightly differing definitions of VDD, 20 ng is considered as deficiency as per IOM, whereas ES considers serum vitamin D less than 30 ng as deficiency. This dissimilarity in the considerations of deficiency explains the differences in the recommended dietary intakes between the two committees. The Indian Council of Medical Research (ICMR) suggests recommended daily intake of 400 IU/day.[70] But, this amount of intake may be inadequate due to changes in lifestyle and increased time spent indoors by the population. The upper tolerable limit of supplement dose (oral 4000 IU per day in healthy individuals) should also be kept under consideration to avoid the risk of toxicity.[33] The daily supplement dose in healthy individuals is lower than the doses recommended for the deficient individuals. This difference in the doses should be considered before suggesting the supplementation since high doses of supplementation in healthy individuals with normal serum levels of vitamin D can cause vitamin toxicity. Similarly, one should strictly adhere to the duration of high dose supplementation in vitamin D-deficient individuals, since prolonged high dose supplementation could lead to vitamin D toxicity and hypercalcemia.[71,72]

Table 2: High-risk groups of individuals for vitamin D deficiency

Celiac disease, malabsorption syndromes
Hypo and hyperparathyroidism
Chronic liver failure, chronic renal failure
Obese individuals, post-bariatric surgery
Pregnancy and lactating women

Route of administration

Oral formulations are shown to rapidly increase the serum 25(OH)D and 1,25 – $(OH)_2$D3 levels than similar doses of intramuscular injection.[75,76] The delayed bio-availability of intramuscular route could be due to the oily nature of the depot injection and the deposition at the injection site.[77] Guidelines addressing prevention and treatment of rickets and VDD preferred oral route to intramuscular injection.[78] Nevertheless, intramuscular injections are the treatment of choice in patients with malabsorption syndromes affecting alimentary tract and hepato-biliary system, including patients post-bariatric surgery.[79] In addition, intramuscular injections result in more sustained long-term increase in serum levels of vitamin D.[76] Hence, we believe that the vitamin D supplementation through intramuscular route in individuals with severe vitamin D deficiency (serum levels <10 ng/ml) leads to more sustained improvement in the serum vitamin D levels, with beneficial effects on ocular surface VDR expression and dampening of ocular surface inflammation in patients with DED and keratoconus.[23,64]

Dosage and duration of administration

Daily, weekly, or large single-dosage (Stoss therapy) schedules can be followed for the treatment of VDD. Weekly semi-large doses for a duration of 8–12 weeks are the most commonly suggested dosing schedule.[33,73,78] The efficacy, tolerability, and safety of single megadose (of variable proportions) have been established through multiple studies, and such dosing practice should be an informed, individualized decision by the physician and the patient.[80,81] The two available forms of vitamin D supplements, ergocalciferol (D2) and cholecalciferol (D3), are equally effective with daily dosing, but cholecalciferol is preferable in weekly or single megadosing schedules.[78]

Vitamin D Toxicity

Although rare, vitamin D toxicity presents with severe, dramatic cases of life-threatening symptoms. There is a "U"-shaped biological response to the serum levels of vitamin D with deleterious effects at very low and high serum concentrations, including mortality, cardiovascular effects, and certain

Table 3: Treatment regimen for vitamin D deficiency and supplementation protocol

Age	Balasubramanian *et al.*[73,74]		Holick *et al.*[33]		
	Daily regimen (12 weeks)	Weekly regimen (6 weeks)	Daily regimen (6 weeks)	Weekly regimen (6 weeks)	Daily intake in healthy individuals
1-18 years	3000-6,000IU	60,000 IU	2000-6000 IU	50,000 IU	400 IU
>18 years	6000 IU	60,000 IU	6000 IU	50,000 IU	400-600 IU
Patients with obesity, malabsorption syndromes, or on medications affecting vitamin D metabolism (>18 years)	600-10,000 IU		6000-10,000 IU		600-2000 IU

IU - International Units

cancers.[71] Institute of Medicine (IOM) advises caution against maintaining serum vitamin D levels more than 50 ng/ml.[82] Stoss therapy, which involves single large bolus doses oral or intramuscular vitamin D, has higher risk of resulting in dangerously high serum levels of vitamin D and symptoms of toxicity, than the weekly or daily supplementation regimen.[83,84] The clinicians treating VDD should be well aware of the dosing regimen and risks of toxicity of vitamin D supplementation. The maximum doses for daily, weekly, and Stoss therapy are 6000IU, 60,000 IU, and 6,00,000 IU, respectively.[33,73]

Approach to a patient with DED [Fig. 1]

Any patient with complaints of ocular discomfort, itching, dryness, and fatigue should be evaluated for possible DED, with the following clinical tests.

1. Schirmer's test with and without anesthesia
2. Tear stability – Tear breakup time and non-invasive tear breakup time.
3. Ocular surface (corneal and conjunctival) staining
4. Meibomian gland assessment
5. Ocular adnexal examination
6. Tear osmolarity (if available).

Along with the ocular examination, patients should be screened for VDD. Based on the tear film metrics, if aqueous-deficient DED is suspected, they should be screened for autoimmune etiology. The mode and dose of vitamin D supplementation depend on the status of the serum vitamin D. Table 2 shows the suggested vitamin D supplementation strategy for patients with DED and VDD. The algorithmic approach is explained in Figs. 1 and 3.

Considerations in prerefractive surgery patients [Fig 2]

An optimal ocular surface health should be ensured before proceeding with refractive surgery to achieve optimal post-surgical outcomes. If these patients show signs of DED, higher ocular surface inflammation, or meibomian gland dysfunction (MGD), they should be treated for the underlying pathology and screened for VDD. Ocular surface inflammation and tear fluid pro-nociceptive factors are shown to increase in individuals with low serum vitamin D levels. The sub-clinical ocular surface inflammation even without overt DED in individuals with VDD could potentially increase the risk of post-refractive surgery complications like regression, post-photorefractive keratectomy haze, pain, etc., Hence, these patients should undergo refractive surgery after ensuring vitamin D sufficiency and ocular surface stabilization.

Our Clinical Experience with the Treatment of DED with Vitamin D Supplementation

At our dry eye clinic (at a tertiary referral center for DED), 423 patients with "pain without stain," those with symptoms of DED but no corroborative clinical signs, were evaluated. Among these, 345 patients had high OSDI scores at presentation, and 296 of them had serum vitamin D levels lower than 20ng/ml. They were treated with vitamin D supplementation as per the algorithm mentioned in Fig. 3 along with topical lubricants (sodium hyaluronate 0.1%). The OSDI scores significantly improved in 275 patients, three months following treatment with vitamin D supplements. In addition to these cohort-based analyses, anecdotally we observed improvement in Schirmer and TBUT scores in patients with both aqueous deficiency and evaporative DED. This improvement could be due the augmentation of endogenous immuno-inflammatory dampening process by vitamin D, as these immune-inflammatory factors are critical contributors

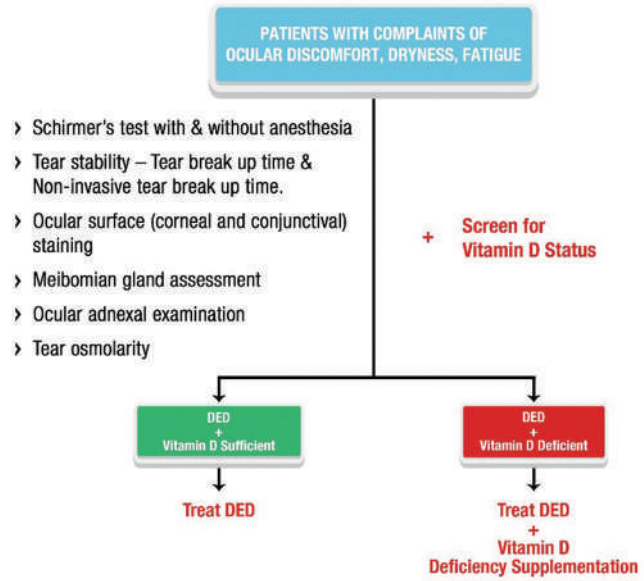

Figure 1: Algorithmic approach to patients with DED. The figure depicts the approach to manage patients with DED and vitamin D deficiency

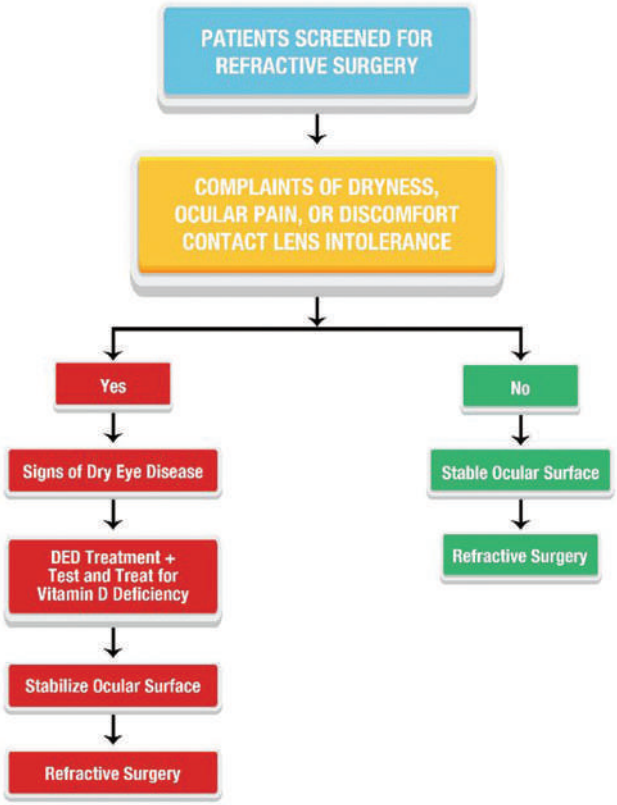

Figure 2 : Preoperative management of patients undergoing refractive surgery. Guideline to preoperative management of patients undergoing refractive surgery to ensure ideal post-surgical outcomes

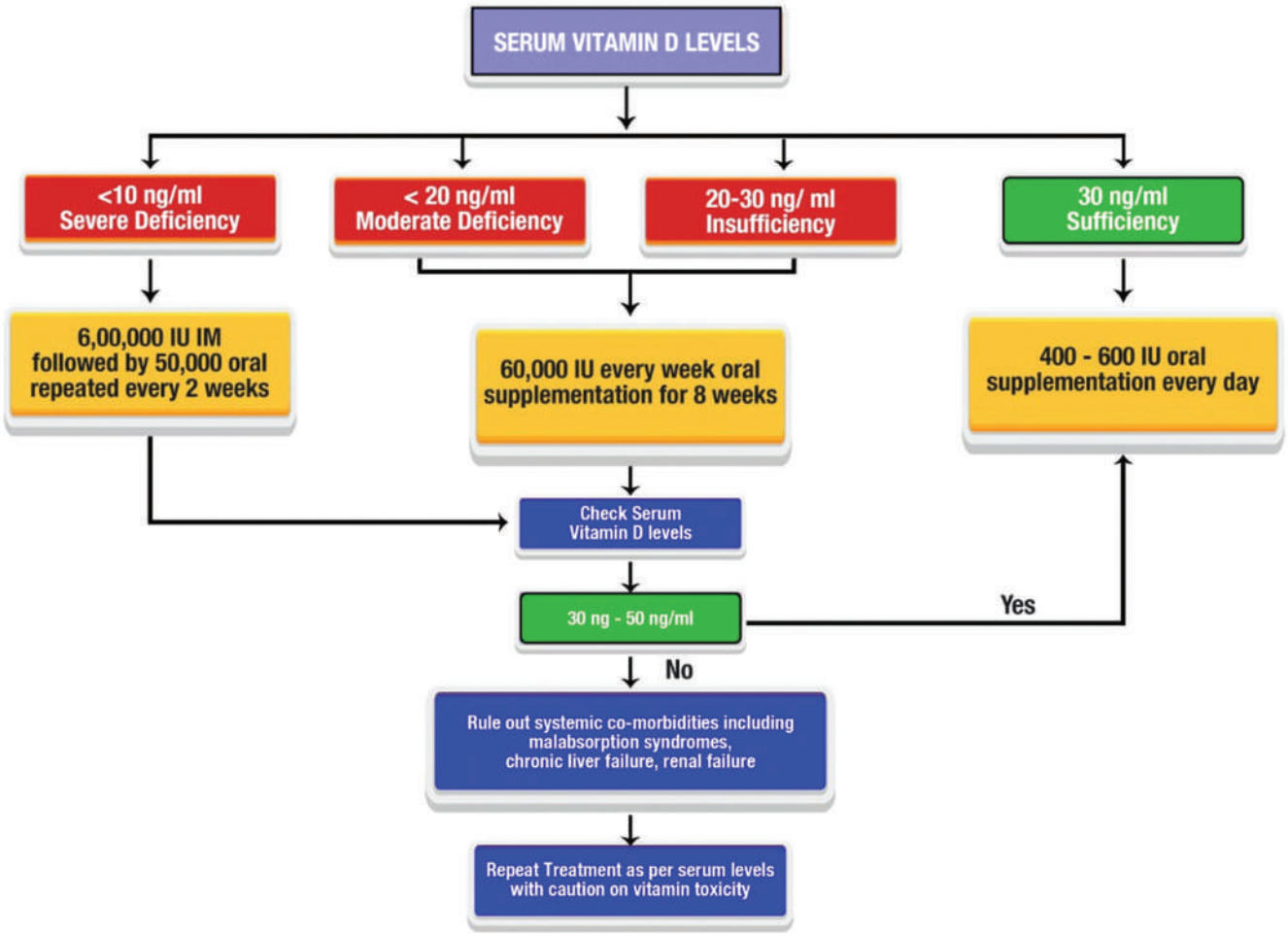

Figure 3: Approach to management of vitamin D deficiency. Guidelines to treat vitamin D deficiency in adults with DED

to the pathogenic mechanisms that result in reduction in tear secretion, tear film stability, and threshold for pain.

Conclusion

To conclude, VDD is one of the significant contributors to the pathogenesis of DED. Following these guidelines, and effectively managing VDD in individuals with DED, will prevent worsening of DED and post-operative complications. Ensuring vitamin D sufficiency in patients with ocular ailments is a clinically wise prophylactic strategy as we are augmenting human body's own defense and disease resolution mechanism.

Acknowledgement

This work was supported by Narayana Nethralaya Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of this abstract.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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