Original Article

Serum Vitamin D Levels and Dry Eye Disease in Postmenopausal Women: A Case–Control Study at a Tertiary Care Center in Rural Haryana

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Abstract

Background: Despite the high prevalence of Vitamin-D insufficiency and high susceptibility to dry eye disease (DED) in postmenopausal women (PMW), correlation between DED and Vitamin D has not been explored in PMW in any Indian study. **Aims and Objectives:** To explore the correlation between serum Vitamin D levels in PMW with and without DED, in a hospital-based population in rural Haryana. **Materials and Methods:** Subjective (ocular surface disease index [OSDI] questionnaire) and objective clinical tests were undertaken for DED diagnosis. 25(OH) Vitamin D was measured in serum using enzyme-linked immunosorbent assay; insufficient (10–30 ng/ml) and deficient (<10 ng/ml). Descriptive statistics were analyzed by mean ± standard deviation for continuous and frequencies for the categorical variables; Student's *t*-test used to find out mean difference in Vitamin D levels; $P < 0.05$ was considered statistically significant. **Results:** One hundred and forty PMW (60.1 ± 5.32 years) were included; Group-A (Controls; no DED; *n*: 70); Group-B (Cases; DED diagnosed by OSDI scores; *n*: 70); Subgroup-B1 (clinical tests negative; *n*: 30) and B2 (clinical tests positive; *n*: 40). There was no statistically significant difference in OSDI scores between B1 and B2. Significantly lower mean Vitamin D levels were found in cases (14.36 ± 4.08 ng/ml) as compared to controls (19.19 ± 6.4 ng/ml) ($P = 0.001$) and in B2 (13.15 ± 3.51 ng/ml) as compared to B1 (15.57 ± 4.66 ng/ml) ($P = 0.01$). **Conclusion:** There were significantly low levels of Vitamin-D in clinically established DED. Evaluating Vitamin D levels as a part of the dry eye workup in PMW is recommended. OSDI scores were not aligned with the clinical test scores; questionnaire-based tests alone may not be sufficient for diagnosing DED.

Keywords: *Dry eye disease, postmenopausal women, Schirmer's test, tear break up time, Vitamin D*

Introduction

Dry eye disease (DED) is a multifactorial disease of the lacrimal functional unit, characterized by varying degree of damage to the ocular surface and resulting in symptoms of ocular fatigue, discomfort, pain, and other visual disturbances. The disease, if left untreated, may become chronic and progressive and likely interfere with daily activities and quality of life.[1-4] DED is known to affect 5%–34% of the population worldwide. Its prevalence in India has been reported to be higher than the global prevalence and ranges from 18.4% to 54.3%.[5] Of the multiple systemic and ocular associations of DED, the possible role of Vitamin D insufficiency has been of interest to researchers and various possible explanations have been explored in literature. First and foremost, the tear film is a complex mix of electrolytes, proteins, phospholipids, oligopeptides, glycopeptides, and immunoglobulins along with a variety of surfactants and Vitamin D is reported to have a role in synthesis of these surfactants and also in decreasing tear osmolarity, thus contributing toward stability of the tear film.[3] Another possible explanation explores the very vital anti-inflammatory and immunomodulatory roles of Vitamin-D.[6] The presence of T-cells in the conjunctiva of dry eye and elevated inflammatory cytokines in tears claims the probable inflammatory component of DED. A study estimated inflammatory cytokines in tears of patients with symptoms suggesting severe dry eye and reported interleukins-2, 4, 5, and 6 to be significantly increased in affected patients.[7] By enhancing corneal epithelial barrier function, Vitamin D is also known to act as an immunomodulatory agent. Its supplementation has thus been reported to decrease ocular surface inflammation and improve tear secretion.[5,7]

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Thus, it cannot be a chance association that most of the comorbidities associated with DED including increasing age, female gender, menopause, thyroid disease, diabetes mellitus, arthritis, depression, anxiety; are all associated with decreased Vitamin-D levels.[1,4,8,9] It is also interesting to note that in comparison to men, women report a greater impact of DED on visual quality indicators, and higher prevalence of progression to severe forms of the disease.[4] Despite this, it is ironic that postmenopausal women (PMW), one group which is actually excessively affected by DED as well as Vitamin-D insufficiency, likely due to effect of decreased estrogen, has been the least explored with respect to their mutual association. As we searched literature for this study, we could find only one such study which had targeted only PMW as the target group; and utilized the more reliable objective criteria for diagnosis of DED. Published in 2020, the authors have claimed that it is the first study to compare dry eye parameters with Vitamin D deficiency (VDD) in PMW.[10]

In this case–control study, we have statistically explored any possible correlation between serum Vitamin D levels in PMW with dry eye parameters (both subjective and objective) in a hospital-based population in rural Haryana. To the best of our information, this correlation, especially in reference to PMW, has not been explored in any Indian study. We hypothesize that our study will provide a comprehensive baseline data of correlation of Vitamin D levels with DED in this highly vulnerable group, which may further support in charting a treatment plan by the ophthalmologist and exploring newer avenues of treatment for this common, but potentially disabling disease.

Materials and Methods

This case–control study was conducted in the Department of Biochemistry in collaboration with Departments of Ophthalmology and Obstetrics and Gynecology at (BPS GMC for Women) after approval from the Institutional Ethical Committee and in accordance with tenets of the Declaration of Helsinki.

Inclusion and exclusion criteria

PMW, with permanent cessation of menstruation for 12 months at the end of reproductive life, resulting from estrogen deficiency and not associated with any pathology, as established by the gynecologist (author-4) and reporting consecutively in the outpatient services of Obstetrics and Gynecology, from April, 2021 to March, 2022 were enrolled for the study, after obtaining written informed consent. Those on hormone replacement therapy were excluded. All were subjected to detailed work-up by ophthalmologist (author-3) and those with any abnormality of the ocular adnexa, ocular surgery in either eye in last 6 months, active ocular infection or allergy, ocular surface scarring or current contact lens use, current or recent drug use (beta-blockers, antihistaminics, and antipsychotics), and those with history of smoking or any associated systemic disease such as primary Sjogren's syndrome or rheumatic disease, which may affect or alter the lacrimal functional unit, were excluded.

Vitamin D estimation

Venous blood sample was collected from antecubital vein under aseptic conditions after overnight fasting. 25(OH) Vitamin D is the most stable form of Vitamin D and because of its longer plasma half-life and higher circulating concentrations, its measurement in the serum is indicative of the status of Vitamin D in the body. Its quantitative estimation was done using solid phase competitive Enzyme Linked Immunosorbent Assay (ELISA) based on the principle of competitive binding, using Transasia ELISA processor.[11] All samples were analyzed on the same day or stored at −20°C for analysis within 72 h of sampling. Serum levels of >30 ng/mL were considered sufficient; while 10–30 ng/mL and <10 ng/mL were labeled as insufficient and deficient, respectively.

Dry eye work up

The 12 item ocular surface disease index (OSDI) questionnaire was administered in paper form to all participants.[12] Sum of scores for all the questions answered, for each participant, was divided by the number of questions answered and then multiplied by 25 to give an OSDI score that could range from 0 to 100. Based on this score, participants were assigned into test and control groups. Score of 0–12 was labeled as controls and those with higher scores were subjected to tear film breakup time (TBUT) and Schirmer's test, interval between each test being at least 5 min. TBUT is a quantitative test for the measurement of tear film stability and is the time required for the first random spot to appear on cornea following a complete blink due to breaking up of tear film.[13] Fluorescein strip (2% Fluorescein Sodium ophthalmic strip; Pricon) was applied on the inferior palpebral conjunctiva of both eyes and participants asked to blink several times to uniformly distribute fluorescein over the cornea. Interval between a complete blink and the first appearance of a dry spot on the cornea was measured on a slit lamp biomicroscope. Mean TBUT of both eyes was calculated; score of ≤5 s was taken as diagnostic criteria for DED. Schirmer's test quantitatively measures both the reflex and basal tear secretion, a value of ≤5 mm of strip wetting in 5 min is considered abnormal and suggests dysfunctional basal aqueous component of tear secretion by lacrimal gland.[14] The test (nonanesthetic) was performed utilizing strips (Schirmer tear test strips; Pricon) hooked over the lateral two-thirds of the lower eyelids of both eyes under the standard conditions.[13] Strips were removed after 5 min; ≤5 mm of wetting was taken as the diagnostic criteria for DED. Based on OSDI scores and scores of objective tests, participants were classified into groups and subgroups [Figure 1].

Statistical analysis

The sample size was calculated using proportion formula, taking standard deviation (SD) in group A (without DED) 6 and 6.07 in group B (with DED) and pooled SD 36.42 at 2% precision and 95% confidence interval (CI).[10] The required sample size was 140 subjects, 70 subjects in each group (Group A and B). All the data were collected and entered into Microsoft Excel Spread sheet. Statistical analysis was done by using the Statistical Package for the Social Sciences software (SPSS) version 26 (SPSS Inc, Chicago, IL, USA) by SPSS by trial version and same value calculated by R software which is available for free. Descriptive statistics were analyzed in the form of mean ± SD for the continuous and frequencies for the categorical variables. Student ' t ' test (parametric data) was used to find out the mean difference of Vitamin D level in two groups (with and without dry eye in PMW). $P < 0.05$ was considered as statistically significant.

Results

A total of 140 females (mean age 60.1 ± 5.32 years; 50–74 years) fulfilling the inclusion and exclusion criteria were included in the study and assigned to the study groups and sub-groups [Figure 1]. PMW in both groups was comparable with respect to age and the difference in the mean age was statistically nonsignificant ($P = 0.765$).

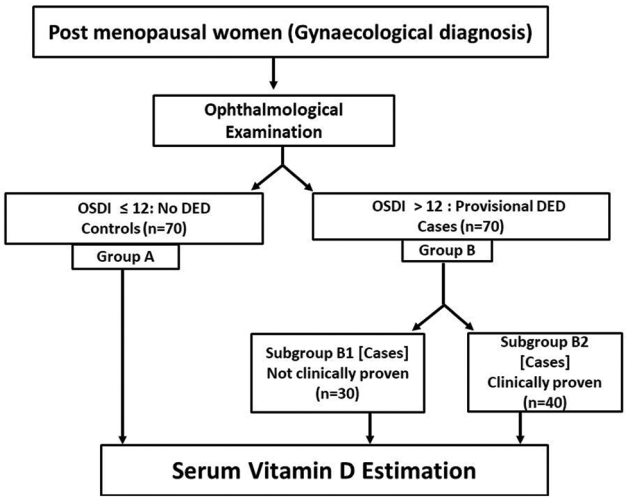

Figure 1: Flowchart showing the division of participants in groups and subgroups based on the objective and subjective tests

A total of 128 (91.4%) participants were insufficient and 10 (7.1%) deficient with reference to their Vitamin-D levels and with respect to cutoff values discussed in the section of Methodology. Table 1 summarizes the mean values of age, OSDI, Schirmer's test score, TBUT, and serum Vitamin D levels in Groups A and B as well as in subgroups B1 and B2. Shapiro–Wilk test was used to check the normality of the data. Skewness and kurtosis was also calculated to measure the symmetry and peakedness of the normal distribution of the data. Difference in OSDI scores between subgroups B1 and B2 was not statistically significant ($P = 0.523$). However, comparing the objective tests, the mean Schirmer's scores and TBUT scores were found to be significantly lower in B2 than in B1 ($P = 0.001$ for both). PMW with clinically confirmed DED (B2) had mean serum Vitamin D levels (13.15 ± 3.51 ng/ml) significantly lower than PMW with no DED (A) (19.19 ± 6.41 ng/ml) (95% CI; $P = 0.01$) and those with only subjective diagnosis of DED (B1) (15.57 ± 4.66) (95% CI; $P = 0.01$); the values were also significantly lower in cases (B) (14.36 ± 4.08 ng/ml) when compared to controls (A) (19.19 ± 6.41); (95% CI; $P = 0.001$).

Discussion

Vitamin D has two forms D2 (Ergocalciferol) and D3 (Cholecalciferol), both of which are biologically inactive and further activated in liver and kidney by enzymatic hydroxylation. Estrogen is said to increase the activity of the enzyme that activates Vitamin D to its active form 1,25 dihydroxycholecalciferol and therefore, less estrogen production in menopause can alter its expression. Vitamin D which has been identified to play a role not only in calcium metabolism and bone health but also has anti-inflammatory and immunoregulator roles.[2,10] Studies have reported that a large proportion of PMW have inadequate Vitamin D levels.[15,16] In our study, more than 90% of participants had insufficient Vitamin-D levels, despite the fact that our institute caters to mostly rural agricultural population having plenty of outdoor exposure. However, the outdoor exposure could be the possible explanation for a very less proportion of PMW with deficient Vitamin D levels (about 7%) in our study. Similar observation was reported by Aparna *et al*. who summarized various Indian studies to assess Vitamin D status in India and observed that even though most of the

Table 1: Mean values (estimated marginal means reported) of all the parameters in Groups (A and B) and subgroups (B1 and B2)

	Group A (70)	Group B (70)	Subgroup B1 (30)	Subgroup B2 (40)
Age (years), mean±SD	60.25±5.47	60.85±6.69	59.65±7.34	62.06±6.04
OSDI score	9.84±2.02	20.89±5.37	20.48±4.24	21.30±6.50
Schirmer's test score (mm/5 min)	-	7.71±2.31	10.89±3.61	4.53±1.01
TBUT (s)	-	6.21±1.46	7.80±1.71	4.63±1.21
25(OH) Vitamin D (ng/mL)	19.19±6.41	14.36±4.08	15.57±4.66	13.15±3.51

OSDI: Ocular surface disease index; TBUT: Tear break up time; SD: Standard deviation

Indian population lives in areas with adequate sunlight throughout the year, there is a high prevalence of VDD.[8]

Another important association of Vitamin D is that with DED which, as discussed in section of Introduction, is more than an incidental association.[1] For the present study, we did an extensive literature search and settled on some systematic reviews and meta-analyses to explicate the overall relationship between Vitamin D and DED. Most of these have reported a significantly lower levels of Vitamin D in DED patients.[17,18] On the other hand, there is a literature that reports no significant association between Vitamin-D and DED.[19]

So now there are two factors; firstly, Vitamin D levels are decreased in menopause, and second, Vitamin D has a high association with DED. These two, combined together, raise a new research question which we have tried to address in this study; what is the association of Vitamin D levels and DED in PMW? Estrogen, besides having role in Vitamin D metabolism, is known to have a role in production of surfactants, a probable reason for a higher susceptibility to DED in women after menopause.[2] Postmenopause, gynecological manifestations are an important concern and ironically, ophthalmological manifestations, although having a vital role in quality of life, have been highly ignored. We had similar observation in the process of recruiting PMW for the study. Owing to the lack of awareness of dry eye work-up, there was resistance and many a times, even denial of ophthalmic examination by many PMW, thus leading to loss of many potential participants. In our study, significantly lower mean Vitamin D levels were found in the test group as compared to controls. Furthermore, much lower mean Vitamin D levels were found in clinically proven dry eye patients as compared to patients with negative clinical tests. By generating a correlation between decreased Vitamin D levels and DED parameters in PMW, our study opens newer avenues of research that includes a protective role of Vitamin D in the development of DED, through prospective interventional studies.

Herein we would like to highlight the diagnostic criteria for DED in our study, both objective and subjective. The OSDI questionnaire, first introduced in 1997, at Allergan Inc.(Irvine, CA) is one of the most widely used symptom based questionnaire for diagnosis of DED.[12,20-22] However, there is a literature which proves that use of clinical signs is more clinically relevant for the diagnosis of DED than OSDI alone.[23] In our study, all 70 cases diagnosed with DED by OSDI underwent clinical tests and interestingly, about half of them (30 participants) did not test positive for any of the two clinical tests for DED, thus highlighting the fact that if we had used only OSDI for DED diagnosis, we would have probably over diagnosed DED. Moreover, the difference in the OSDI score between B1 and B2 was not statistically significant; again emphasizing that subjective OSDI scores alone might not be sufficient for DED diagnosis. This lack of correlation between subjective and objective tests has also been observed in another study by Jin *et al*.[24] Yazdani *et al*. utilizing the ROC curve analysis in a large sample size of 1090 DED patients, concluded that both symptom-based questionnaires and clinical signs should be considered while diagnosing DED.[21] After subjecting participants to OSDI questionnaire, we utilized both Schirmer's test and TBUT in our study for clinical diagnosis. Both of these are fast and easy standard diagnostic tests, being used in dry eye clinics.[5,6,13,18,24,25] Theoretically, those with low Schirmer's and TBUT scores should have decreased tear production accounting for worse symptoms, replicated by higher OSDI scores. However we could not find any such association. In a meta-analysis, the authors observed that effect of Vitamin D is more on results of Schirmer's test (associated with less tear production) than TBUT (associated with tear film stability).[18] However, in our case, both Schirmer's and TBUT scores had a significant correlation with Vitamin D levels individually. Confirmation of DED by objective tests and its further classification into subgroups, based on clinical tests, is the strength of our study. There are other studies in literature which have used serum Vitamin D as the exposure and Schirmer's test and TBUT as outcome measures but results have been controversial.[1,5,6,24,25] A systematic review and meta-analysis on ten previous studies reported that patients with DED had significantly lower levels of serum Vitamin D, mean TBUT and Schirmer's test scores as compared to healthy controls.[18] On the other hand, evaluation of data from participants in a Study Group for Environmental Eye Disease, did not find any significant association between serum Vitamin D levels and DED.[20]

Another strength of our study is a well-defined target group including only PMW, a group which ironically has been much less explored with respect to dry eye parameters. While there is abundant literature correlating Vitamin D levels with DED.[1,5,6,18,25] there is hardly any on this correlation in PMW, especially with the more objective tests for diagnosis of DED. As mentioned earlier, we could only find one such study which had inclusion criteria same as ours and utilized objective criteria for diagnosis of DED. This is a cross-sectional study conducted in Turkey; however their study did not find any statistically significant difference between the Vitamin-D levels and dry eye parameters.[10] Interestingly, in a similar cohort of PMW, we found a significant correlation between DED and serum Vitamin D levels. A possible explanation to this fact can be the difference in cut offs used for diagnostic criteria for DED and for VDD. A meta-analysis has concluded that the heterogeneity of the DED diagnostic criteria and their measurement as well as the applied pathological threshold definition may explain the large variability in the prevalence reported.[26]

Since the last decade, there has been an effort to investigate the non-calcemic roles of Vitamin D such as

its anti-inflammatory and immuno-regulatory roles which may help in addressing ocular discomfort and vision related complaints in relation to DED.[10] Vitamin D enters the eye via circulation or through UVB induced synthesis and its action is mediated by Vitamin D receptor, which has been found in most tissues and cells in human body, including corneal epithelial cells.[27] Tear hyperosmolarity is considered to be induced by ocular surface inflammation and is one of the main causes of discomfort in dry eye patients.[6] Improvement in tear hyperosmolarity has been reported in Vitamin D deficient study subjects 8 weeks after administration of Vitamin D.[28] In another convincing study, the authors concluded that Vitamin D supplementation resulted in earlier and significant improvement in DED parameters in patients with VDD.[29]

While our study clearly establishes correlation between decreased Vitamin D levels and DED parameters, there are certain limitations. Firstly it is a hospital based study where most of the menopausal women visited for some gynaecological problems. This, combined with the limited sample size in our study, may not allow our results to be generalized to the population. As mentioned earlier, there was resistance and in some cases even denial of ophthalmic work-up from menopausal women, thus leading to loss of some potential participants. Moreover, correlation between Vitamin-D levels and severity of DED should also have been explored. Still our results have been promising and awareness about correlation of menopause and DED amongst the medical fraternity, especially from the ophthalmic side, may pave the way for further research in this field. All PMW with insufficient Vitamin D levels need to be explained the risk of DED and screened for the same; an early identification and timely initiation of treatment may prevent dry eye sequelae in this vulnerable group of PMW. Similarly, in PMW presenting with dry eye symptoms, evaluation of Vitamin D levels should be undertaken. In future, an additional therapeutic modality of Vitamin D supplementation may open a new avenue in the treatment of DED patients, refractory to conventional treatment.

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Conflicts of interest

There are no conflicts of interest.

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