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Research Article

Association Between Serum Vitamin-D Level and Dry Eye Syndrome - A prospective Cross-sectional Study

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Abstract

Aim: This study is to investigate the association between the patients with dry eye syndrome and serum vitamin D level in hospital-based population.

Study Design: Prospective, cross-sectional study.

Methods: 88 participants (including male and female) diagnosed with Dry Eye Syndrome having no ocular diseases or any auto-immune systemic diseases were included. Comprehensive eye examination was done for all subjects. OSDI questionnaire, Schirmer's Test I and II and Tear Break up Time (TBUT) along with the measurement of Serum vitamin D level were performed for all the subjects. Analyses were made using the SPSS.

Results: The mean age of the participants was 37.67 ± 14.17 years (95% CI: 34.92-40.65). Mean ST-I was found to be 8.54 ± 3.43mm (95% CI: 34.92-40.65), mean OSDI score was 45.80 ± 9.74 (95% CI: 43.76-47.80), mean ST-II was 5.86 ± 2.92mm (95% CI: 5.30-6.34), mean TBUT was 5.98 ± 2.23 seconds (95% CI: 5.47-6.47) and Serum Vit-D level was 20.21 ± 9.24 ng/ml (95% CI: 18.06-21.98). Statistically significant difference was observed between ST-I and ST-II ($P < 0.001$). Regression analysis showed a significant association for OSDI ($r = 0.938$, $p < 0.001$), ST-II ($r = 0.28$, $p < 0.04$) and TBUT ($r = 0.64$, $p < 0.001$) with Serum Vit-D level.

Conclusion: In our study, OSDI score, low tear secretion level and tear break up time suggested to show significantly low Serum Vitamin-D concentration in blood which may be the contributory factor for DES. The results of the study suggest that serum Vit-D supplementation can be a useful and effective treatment of DES.

Keywords: Cross Sectional Study; OSDI Questionnaire; Dry Eye Syndrome; Tear Secretion; Serum 25 Hydroxyvitamin-D Level

Introduction

The tear film is a transparent thin fluid layer that is involuntarily controlled by blinking mechanism and covers the outer surface of the cornea. The thickness of tear film is approximately 3µm and 3µl in volume which consists of three layers: lipid is thinnest layer and outer most layer of the tear film secreted by the meibomian gland whereas aqueous layer is the thickest layer and middle layer of tear film secreted by the lacrimal glands and mucin layer is the inner most layer of tear film secreted by the conjunctival goblet cells. The abnormalities of tear film classically comprise dry eye syndrome (DES) of the eyes [1]. The layers of tear film have been illustrated in figure 1.

Dry eye syndrome(DES) has become a growing common ocular disease that is categorized by tear deficiency (instability of tear

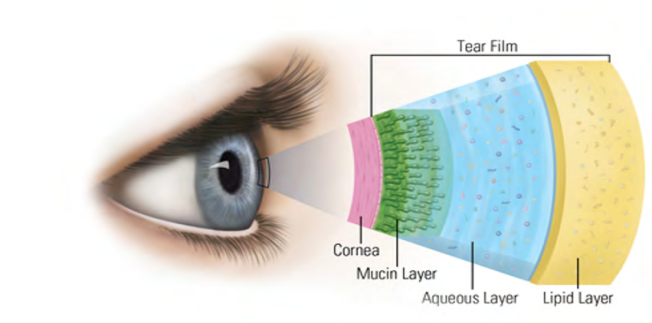

Figure 1: The layers of tear film.

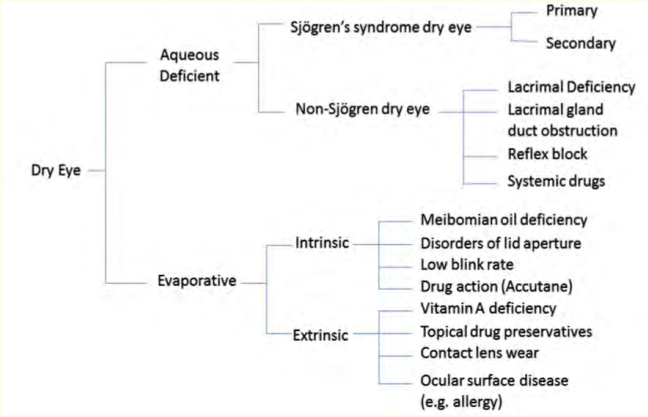

Figure 2: Classification of DES (modified from 2007 DWES report) [4].

film or an escalation in the rate of tear evaporation), ocular surface inflammation, fluctuating visual disturbances and irritational eye symptoms such as burning sensation, sandy or gritty sensation, fb sensation, watering, mild photophobia and itching [1]. DES associated with several common activities of daily life such as reading, computer work, watching television, professional work and mobile use. The projected prevalence of DES for people ≥50 years in United States is 3.2 million or 7.8% among women and 1.6 million or 4.7% among men [2]. The estimated prevalence of dry eye based on OSDI (Ocular Surface disease Index) was 29.25% in India [3].

Classification

Dry eye is classified into two types: Evaporative dry eye (EDE) and Aqueous deficient dry eye (ADDE)by the International Dry Eye Workshop [4]. Aqueous dry eye usually occurs with the abnormalities of aqueous layer of tear film and evaporative dry eye because of abnormalities of lipid layer of the tear film. Depending on the severity, dryness is classified into three types: Mild, moderate and severe dry eye. The classification is demonstrated in figure 2.

Dry eye patient questionnaire like the ocular surface disease index (OSDI®) [5], have played an important role on quality of life and helped to investigate the symptoms in response to DES treatment strategy. The artificial tears, anti-inflammatory drugs, punctal occlusionetc. are commonly used as modes of treatment of DES. Artificial tears are a lubricating agent that helps to relief the irritation of dry eye symptoms, which could consist of either carboxymethylcellulose polyethylene glycol 400 (PEG 400), polysorbate, polyvinyl alcohol, povidone, or propylene glycol or hyaluronate sodium [6].

Low levels of Serum 25-hydroxyvitamin D (Serum VIt-D) have been documented in bone diseases which symptomatically presents with body pain, muscle pain and fatigue with muscle weakness. Sunlight has been accepted as a key provider of Vitamin D and the role of vitamin D in calcium absorption and its mechanism in the body has been reported [7]. Vitamin D transpires in two types: Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is produced from plant sources and vitamin D3 is synthesized when ultraviolet B strikes the skin with the exposure of sunlight. Good food sources of vitamin D3are egg yolk, fish (not only fatty fish), and offal such as liver.

Recent studies have found that deficiency in serum vitamin D is a risk factor for dry eye syndrome [8]. However, the effect of vitamin D supplementation on DES has not been reported [9].

The target cells of the vitamin D were demonstrated by calbindine (a calcium binding protein) in the retinal tissues and also present in nerve cell in central nervous system. Later, vitamin D receptors (VDR) were demonstrated in corneal epithelium, lens, ciliary body, retinal pigment epithelium and retinal photoreceptors by immunohistochemical staining [10]. Recently the presence of Vit-D hydroxylase activity has been shown in corneal epithelial and endothelial cells. The corneal limbal epithelial cell cultures has been shown to produce de novo Vit-D, similar to skin cells following UV-B exposure [11]. Other vitamin D sources in the eye are the tear film layers, aqueous and vitreous [12].

In this study, we investigated the correlation between the patients with dry eye syndrome and lower vitamin D level in hospital-based population. This study supports the hypothesis that Serum 25 hydroxyvitamin D plays an important role in patients with dry eye syndrome. Another study has hypothesised that vitamin D may help to prevent dry eye by inducing cathelicidin (LL-37), an antimicrobial peptide that can be produced by cell in the eyes and also heal the eye wound [13].

Aim

Primary objective

This study is to determine the association between the patients with dry eye syndrome and lower vitamin D level in hospital-based population.

Secondary objective

- To assess the prevalence of dry eye syndrome among gender.
- To determine the prevalence of dry eye syndrome in different age groups.
- To investigate the severity of dry eye syndrome (DES) based on its standard classification.

Methodology

Study design

A hospital based prospective cross-sectional study conducted at the premises of Bansara Eye Care Center, Laitumkhrah, Shillong, Meghalaya.

Participants and setting

All study participants were literates including the patients and attendants, who came for routine eye examination in the hospital. All of the participants was informed regarding the safety and health aspects of the study. Any subject not willing to participate was excluded. A subject's participation in the study was decided after informing about the procedure.

The experimental various tests described here are well-established methods and therefore the minimal risks are posed to the study participants. Should any problematic medical condition be revealed during the study, subjects has been informed and referred to an appropriate health care professional for diagnosis and relevant treatment. The study was approved by the ETHICS committee of Bansara Institute of Ophthalmic Sciences, Shillong and Martin Luther Christian University, Shillong. No reward was offered to the participants in the study.

This study included subject's demographic details, a complete ophthalmological examination including detailed ocular and medical history; best corrective visual acuity, slit lamp examination to rule out any obstruction in the optical media obstructing the visual axis of the participant's eye, applanation tonometry, fundus examination.

By the end of the evaluation patients were given a dry eye questionnaire to rule out Dry Eye Syndrome (DES).

Patients suspected to have Dry Eye underwent additional dry eye test of Schirmer's Test I (without local anesthesia), Schirmer's Test II (with local anesthesia) and Tear Break up Time (TBUT) along with the measurement of Serum (OH) vitamin D level.

After these above-mentioned assessments, all the patients with dry eye syndrome were asked to get their Serum (OH) vitamin D level evaluated. All patients diagnosed to have DES with Vitamin-D deficiency were managed with artificial tear substitutes and Vitamin-D supplement.

Patient selection

Inclusion criteria	Exclusion criteria
Patients diagnosed with DES Age limit 15 years -65 years Spectacle wearer Computer Users Patients with CVS	Patients with Infected/contagious eye diseases. Regular Contact lens wearer. Auto-immune diseases: Rheumatoid arthritis, Thyroid disease, Sjogren's syndrome, Rosacea, lupus and Type-1 Diabetes Mellitus. Patients who has undergone Ocular surgery within 3 months of examination Patients with Vitamin -A deficiency Pregnancy and Lactation Post-Menopausal Women.

Table a

Exclusion questionnaire

To rule out any auto-immune disease, questionnaire relating to different types of auto-immune diseases has been used. Based on

their response to the questionnaire, the participants were classified as having auto immune diseases or not. Participants with auto immune diseases were excluded from this study.

Dry Eye parameters

- **OSDI Questionnaire**: Each participant was given an Ocular Surface Disease Index (OSDI) questionnaire sheet which is a standardised questionnaire to rule out the severity of dryness and was asked to fill the questionnaire to their best knowledge. The OSDI comprises of a set of 12 questions that is related to ocular dryness where for each set of questions the participants is scored based on their severity of the symptoms. The total score were obtained from the OSDI formula([sum of scores for all questions answered]×100)/([total number of questions answered]×4) which gives the overall score on the severity of dryness. The OSDI questionnaire has 3 categories, that is., mild, moderate and severe. Based on the overall score, the participants were categorised as having mild, moderate or severe dryness.
- **Schirmer Test:** The test was performed by using schirmer paper strips, inserted in the lateral side of the lower eyelid (inferior fornix) for five minutes to measure the tear secretion of the eye. Schirmer Test can be classified into two types: Schirmer test- I (without Anaesthesia) and Schirmer test –II (with Anaesthesia). Schirmer test- I evaluates reflex tearing along with basal secretion whereas ST-II evaluates true basal secretion of the eye.

For Schirmer Test- I, before inserting the paper strips into the eye, the participants were instructed to look up and with an index finger, gently pulled down the lower lid and strip was placed. Same procedure repeated for other eye. The participants were asked not to squeeze the eyes and to keep the eye gently closed. After 5 minutes, the participants were asked to open both eyes and look upwards and the length of the moistened area on the strip was measured. Less than 15mm in 5 minutes has been considered as dryness whereas 15-10mm, 10mm-5mm and <5mm has considered as mild, moderate and severe dryness respectively [14].

5 minutes later, one drop of topical anaesthesia (0.5% proparacaine) in both eyes were instilled followed by the placement of schimer paper strips at the lateral side of inferior for nices. Again, the length of the moistened area on the strip was measured. Less than 5 mm as abnormal wetting of basal tearing and less than 10mm in 5 minutes have been considered as borderline of dryness [15] (Figure 2).

TBUT

Tear Break-Up Time (TBUT) was performed to determine the stability of tear film of the eye. To measure first tear break up time, fluorescein dye was installed into the tear film for all the participants and asked not to blink while the tear film was observed under a broad beam of cobalt blue illumination of slit lamp. The appearance of the first dry spot in the tear film was recorded in seconds. Normal value of5 to 10 seconds was considered as marginal and <5 has considered as low [16] (Figure 3).

Figure 3: Schirmer Test.

Detection of serum Vit-D

As Serum Vit-D were chosen a main parameter of this study so at the end of DES assessment, peripheral blood samples were collected from each participant and Serum Vit-D level were measured by using a reverse phase high performance liquid chromatography method. Serum Vit-D level was classified into four types whereas <20 ng/ml was considered as Serum Vit-D deficiency, 20-29 ng/ml as Serum Vit-D insufficiency, 30-100 ng/ml as sufficient Serum Vit-D level and potential toxicity considered as >100 ng/ml [17] (Figure 4).

Data preparation and statistical analysis

All data were entered in Microsoft Excel version 2010 and all statistical analyses were performed with the help of IBM SPSS

Figure 4: TBUT.

version 23.0. To compare between the dry eye parameters across groups ANOVA test was used. Linear regression analysis was used to explain the relation between Dry eye Parameters (OSDI, Schirmer tests, TBUT) and Serum Vit-D level. Pearson correlation was performed to estimate the strength of association between the Dry Eye Parameters and Serum Vit-d level. The mean value of the individual groups was reported. $P < 0.05$ was considered as statistically significant.

Literature Review

Most theories regarding dry eye parameters were described and measured by using Schirmer I (without anesthesia) and Schirmer II (with the anesthesia) and TBUT (Tear break-up time). With time there have been developments of new generation dry eye syndrome evaluation tests or diagnostic tests with much improvisation from their prototype or their early generation. Thereby the procedure by measuring tear osmolarity from tear film, the instrument is called TearLab® Osmolarity System. Because of high cost of tear osmolarity test of tear-lab, still majority of literature for dry eye evaluation using schirmer test and TBUT (Tear break-up time) has been used for wide range of tear-lab emerged.

Vitamin D deficiency or insufficiency has been suggested as an important-factor of dry eye syndrome [18]. Although we know that serum 25 hydroxyvitamin D supplementation helps to improve vitamin D deficiency or insufficiency, recently it has been suggested that vitamin D supplementation is useful and an effective treatment in patients with dry eye syndrome [9].

Serum 25 hydroxyvitamin D plays a important role in patients with dry eye syndrome and it has also been explained that vitamin D may help to prevent dry eye by inducing cathelicidin, an antimicrobial peptide that can be produced by cell in the eyes and also heal the eye wound [19].

A study was done by Seok Hyun Bae, Young Joo Shin, Ha Kyoung Kim., *et al*. [9]. Where TBUT, and tear secretion test showed an improvement at 2 and 6 weeks after vitamin D supplementation compared to pre-treatment values ($p < 0.05$ for all, paired t-test). They also found that eyelid margin hyperemia and the severity of symptoms showed improvement at 2, 6, and 10 weeks after vitamin D supplementation ($p < 0.05$ for all). Compared to pre-treatment values, Fluorescent staining score (FSS), ocular surface disease index (OSDI) score and visual analogue pain (VAS) score were decreased at 2 weeks ($p < 0.05$ for all).

Another study done by Yi-Fang, Meng Jiong, Lu Qian Xing., *et al*. [20] showed that when the Schirmer I test was considered, they found the DES group demonstrated a significantly lower value compared with the control group ($p < 0.001$) and a significantly reduced TBUT was detected in the DES cases (DES group, control group, ($p < 0.001$). Compared with the control group, the mean OSDI value was significantly higher in the DES group ($p < 0.001$). A significant association between serum 25(OH)D level and DES incidence was detected in this study.

The prospective clinical study conducted by BE Kurtul, PA Özer and MS Aydinli., *et al*. [21]. Showed TBUT scores and Schirmer-1 results of the study group were significantly lower than the control group (P= 0.01 and 0.007, respectively). The mean vitamin D levels were 11.50 ± 1.8 ng/ml in the study group and 32.8 ± 8.72 ng/ml in control group (P =0.001).

The cross-sectional study conducted by Anshu Sahai, Pankaj Malik [21] showed Dry eye prevalence was maximum in those above 70 years of age (36.1%) followed by the age group 31-40 years (20%) (P = 0.007). It was significantly higher (P = 0.024) in females (22.8%) than in males (14.9%), more common in rural residents (19.6%) than in urban (17.5%) (P = 0.553) and Furthermore, subjects exposed to excessive wind (P = 0.004), sunlight/high temperature (P = 0.014) and drug exposure (P = 0.002) were at higher risk of developing dry eye.

In the study conducted by Hwang JS, Lee YP, Shin YJ [22]. The OSDI and visual analog pain scale scores of both vitamin D deficiency (VDD) and non- vitamin D deficiency (VDD) groups decreased after application of topical carbomer-based lipid-containing (CLAT) and hyaluronate (HU) compared with baseline values ($P < 0.05$ for all, paired t test). TBUT, corneal fluorescein staining score, and lid hyperemia in the VDD group remained unaffected by topical CLAT and HU, whereas those in the non-VDD group were improved ($P = 0.001$, 0.030, and 0.012, respectively). OSDI score, TBUT, and lid margin hyperemia were improved in the intramuscular group after cholecalciferol supplementation compared with pretreatment ($P < 0.05$).

The study conducted by Kizilgul M, Kan S, Ozcelik O., *et al*. [23]. Vitamin D Replacement Improves Tear Osmolarity in Patients with Vitamin D Deficiency. All of the patients underwent tear function osmolarity (TFO) measurement initially and eight weeks after vitamin D replacement. The mean TFO was significantly decreased ($p < 0.001$). The mean Ca level was 2.37 ± 0.07 mmol/L initially and 2.35 ± 0.07 mmol/L after vitamin D replacement ($p < 0.05$). The change of TFO was negatively correlated with the variation of $25(OH)D_3$ before and after replacement in patients with dry eye disease ($r=-0.390$, $p=0.049$). As a consequence of the presence of vitamin D receptor (VDR) and 1α-hydroxylase in different parts of the eye, vitamin D replacement improves tear hyperosmolarity that is considered to be induced by ocular surface inflammation.

In the cross sectional study done by Kim MJ, Hwang HR, Kim YJ., *et al*. [24] they included adults aged >19 years who underwent ophthalmologic interviews and examinations, it found that the serum 25(OH)D levels were lower in the dry eye group than in the normal group ($P = 0.01$). A significant association was found between severe vitamin D deficiency and dry eye syndrome ($P = 0.04$).

The study conducted by Jin KW, Ro JW, Shin YJ., *et al*. [25]. As result they found that Tear break-up time (TBUT) and tear secretion were positively correlated with serum 25(OH)D levels ($r = 0.389$, $p = 0.001$; and $r = 0.428$, $p < 0.001$, Pearson correlation test). Tear break-up time (TBUT) and tear secretion were shorter in the vitamin D-deficient group compared to the sufficient group ($p = 0.022$ and $p = 0.004$).

Another study done by Da-Hye Jeon, Hyungseon Yeom, and Hyeon Chang Kim Are [26]. They found that higher serum vitamin D levels were associated with a non-significantly reduced risk of DED in the crude analysis (odds ratio [OR], 0.991; 95% confidence interval [CI], 0.971 to 1.011) and in the adjusted analysis (OR, 0.988; 95% CI, 0.966 to 1.010). In the crude analysis of no/mild DED vs. moderate/severe DED, men exhibited a decreased risk with increasing serum vitamin D levels (OR, 0.999; 95% CI, 0.950 to 1.051), while women exhibited an increased risk (OR, 1.003; 95% CI, 0.979 to 1.027).

The study conducted by Yang CH, Albietz J, Harkin DG., *et al*. [27], they found that Among older adult participants, vitamin D levels were negatively correlated with dry eye symptoms, the severity of dry eye associated with tired eye symptoms. Vitamin D levels of people with dry eye diagnosis were not correlated with OSDI scores and IL-6 levels; while IL-6 levels showed correlation with tear production. In supplement study, vitamin D levels increased by 29mol/l, while dry eye symptoms and grading of corneal staining showed significant reductions but no significant changes in IL-6 levels.

Results

One hundred-seventy six (176) eyes of eighty eight (88) subjects were included in this study irrespective of their gender and race. There were 46.6% (N = 41) and 53.4% (N = 47) males to female population in the study (Figure 5). Age of subjects ranged from 15 to 65 years with a mean age of 37.67 ± 14.17 years (95% CI: 34.92-40.65).

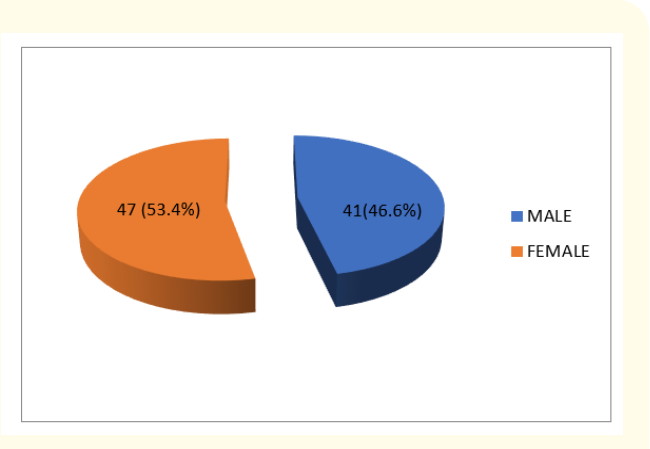

Figure 5: Pie chart representing the gender population.

In order to find out the DES frequency, the age groups were classified into five groups. 36-45 years (29.5%) were more likely to have DES shown in figure 6. Depending upon OSDI questionnaire, the most common level of severity was moderate (63.6%) followed by mild (20.5%) and severe (15.9%).

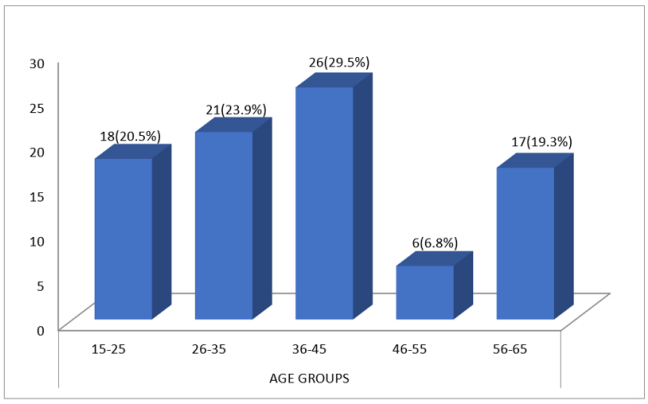

Figure 6: Bar Graph showing the frequency of different age-groups.

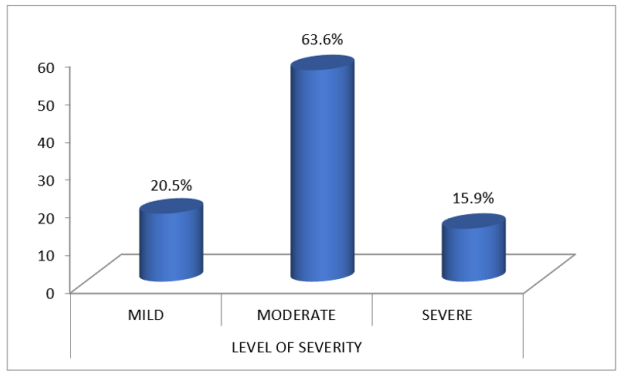

Figure 7: Bar Graph representing the level of severity of DES by OSDI.

Based on the level of severity of Serum Vit-D, it was observed that the subjects with Serum Vit-D deficiency was the highest accounting 45.5%, followed by Serum Vit-D insufficiency (43.1%) and the least common prevailed to be Serum Vit-D sufficient (11.4%) shown in figure 8.

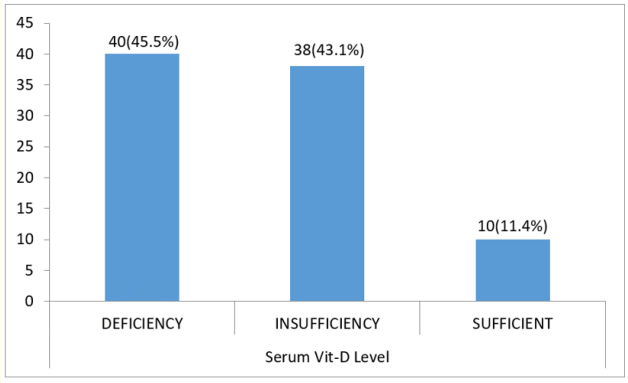

Figure 8: Bar Graph representing the level of severity of Serum Vit-D.

Mean ST-I was found to be 8.54 ± 3.43mm (95% CI: 34.92-40.65), mean ST-II was 5.86 ± 2.92 (95% CI: 5.30-6.34) and OSDI mean was 45.80 ± 9.74(95% CI: 43.76-47.80). The mean for TBUT was 5.98 ± 2.23 seconds (95% CI: 5.47-6.47) and Serum Vit-D level was 20.21 ± 9.24 ng/ml (95% CI: 18.06-21.98), depicted in table 1.

Parameters	Mean ± SD	95% CI
Age	37.67 ± 14.17	34.92-40.65
OSDI	45.80 ± 9.74	43.76-47.80
ST-I	8.54 ± 3.43	7.85-9.15
ST-II	5.86 ± 2.92	5.30-6.34
TBUT	5.98 ± 2.23	5.47-6.47
Serum-Vit D	20.21 ± 9.24	18.06-21.98

Table 1: Description Analysis.

ANOVA showed no significant difference for ST-I, ST-II and TBUT for both eyes respectively and but a statistically significant difference found between ST-I and ST-II {$t(87)=5.6$, $P < 0.001$}, as shown in table 2.

Table 3 and 4 revealed a significant positive association for both ST-II and TBUT with Serum vit-D level ($p \leq 0.004$ for both) show-

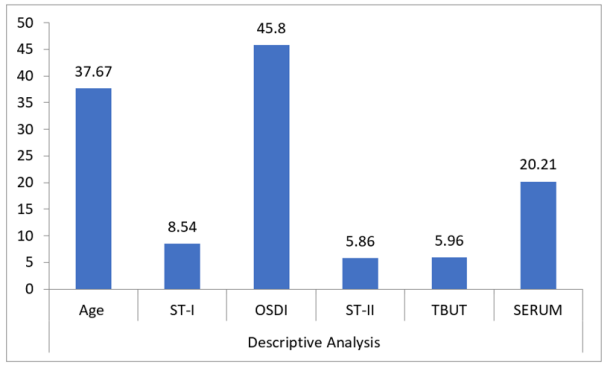

Figure 9: Bar Graph showing the mean of Descriptive Analysis.

Parameters	t	df	P-Value
STIOD-STIOS	.903	87	.369
STIIOD-STIIOS	1.385	87	.170
TBUTOD-TBUTOS	-.296	87	.768
STI-STII	5.689	87	0.001

Table 2: ANOVA test among ST-I, ST-II, TBUT (OD and OS), ST-I and ST-II.

Parameters	Correlation	P-Value
OSDI-SERUM VIT-D	-0.938	0.001
STI-SERUM VIT-D	0.087	0.210
STII-SERUM VIT-D	0.284	0.004
TBUT- SERUM VIT-D	0.587	0.001

Table 3: Pearson's correlation: OSDI, ST-I, ST-II, TBUT with Serum Vit-D.

ing moderate significant association for TBUT (r = 0.64) and weak but significant association for ST-II(r = 0.28). Meanwhile, negative strong significant association was noted for OSDI score with Serum Vit-D level (r=-0.938, p < 0.001). Also no association was noted between ST-I and Serum Vit-D level. Also, we can observe these associations of ST-I, OSDI, ST-II and TBUT with Serum Vit-D level in the scatter plot shown in figure 10.

Parameters	Regression(R)	P-Value
OSDI-SERUM VIT-D	0.938	0.001
STI-SERUM VIT-D	0.087	.421
STII-SERUM VIT-D	0.284	0.004
TBUT- SERUM VIT-D	0.641	0.001

Table 4: Linear Regression: OSDI, ST-I, ST-II, TBUT with Serum Vit-D level.

Discussion

DES is common a ocular multifactorial disorder in the general population that affects our vision related quality of life like visual disturbance, visual discomfort, burning sensation, and itching, watering and strain etc [1]. Recently more studies are going on and there is more interest in the association of Serum Vit-D and DES Parameters. Most of the available studies in the literature had shown the association of Serum Vit-D and DES [8,24,29,30]. This study is intended mainly to investigate the association between the patients with DES and lower Serum Vit-D level in hospital-based population.

This study suggested female were more likely to have DES with male: female (0.8:1), also the mean age of study group was 37.67 ± 14.17 years (95% CI: 34.92-40.65).

It's also been observed that 36-45 years(29.5%) were more likely to have DES which differs from study done by by Anshu Sahai, Pankaj Malik [26] where it demonstrated Dry eye prevalence was maximum in those above 70 years of age (36.1%) which may be due to their rural resident participants, whereby this study's population were urban residents and more exposed to electronic gadgets, limiting them indoors, with less exposure to direct sunlight, thereby increasing the chance of Vitamin D deficiency.

This study revealed a significant association for both ST-II and TBUT with Serum Vit-D level (p≤0.004 for both) which has also been seen in two other studies; one conducted byJin KW, Ro JW, Shin YJ., *et al*. [30] where it demonstrated TBUT and tear secretion were positively correlated with serum 25(OH)D levels (r = 0.389, p = 0.001; and r = 0.428, p < 0.001)and second done by Yi-Fang., *et al*. [24] showed a significantly reduced TBUT and ST-II in the DES cases (p < 0.001).This similar association was due to the inclusion of only DES participants in all of the above studies.

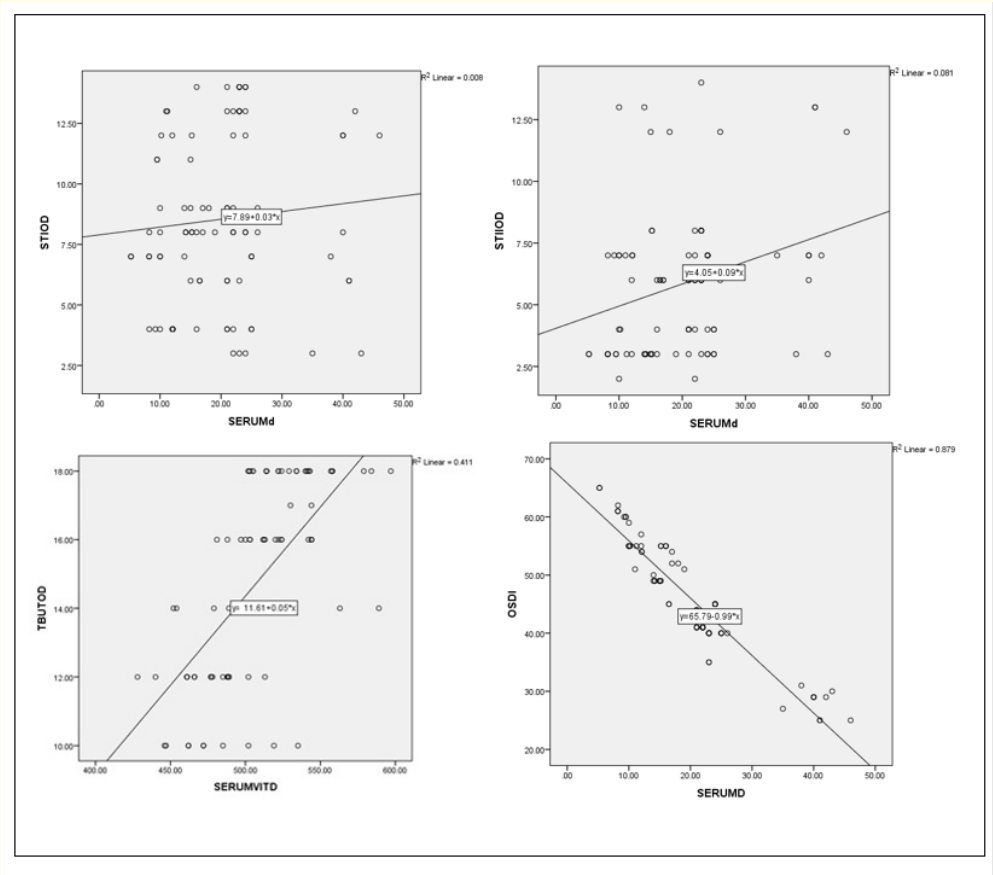

Figure 10: Scatter plot showing the association of OSDI, ST-I, ST-II and TBUT with Serum Vit-D level.

In this study no association was observed between ST-I with Serum Vit-D level because theoretically, ST-I evaluates reflex tearing along with basal secretion whereas ST-II evaluates true and exact basal secretion of the eye [29] similar study done by Na Li., *et al*. [17] represents that ST-II is more reliable and objective comparing to ST-I ($p < 0.001$)which differed from the results by BE Kurtul., *et al*. [25] where they found positive association between Serum Vit-D level and ST-I ($p < 0.007$) may be because their study participants were Serum Vit-D deficiency patients and also differed in terms of their study design being case-control study.

Donghyun Jee., *et al*. [29] illustrated no statistically significant association between Serum Vit-D level and DES (95%CI: 0.55–1.30, P = 0.076) as they hypothesized that serum vitamin D has a limited effect on DES. Serum vitamin D may have difficulty in reaching the cornea, due to its lack of vasculature. However other studies have been shown that Vit-D receptors are demonstrated in the cornea and various other structure of the eye. Recently the presence of Vit-D hydroxylase activity has been shown in corneal epithelial and endothelial cells. The corneal limbal epithelial cell cultures has been shown to produce de novo Vit-D, similar to skin cells following UV-B exposure [30,31].

A study done Tovey, A [13] proposes that Serum Vit-D plays an important role in patients with dry eye syndrome and it has also been explained that vitamin D may help to prevent dry eye by inducing cathelicidin (LL-37), an anti-microbial peptide that can be produced by cell in the eyes and also heal the eye wound. Depend-

ing upon that study, the association between Serum Vit-D and DES parameters observed in this study.

Another study by Rohit Shetty., *et al*. [11] mentioned that apart from finding the association between Vitamin D and DES, it is important to investigate into the etiopathological effects of Vitamin D deficiency and DES for better targeted management of DES.

This study holds a few limitations being the following, small study sample size, no standard investigation was performed to rule out auto-immune diseases and the cross-sectional study design. Another limitation to our study is that we did not do a post vitamin-D treatment evaluation of DES parameters. This would have helped us to strengthen the correlation between DES and Serum Vit-D deficiency.

The strength of this study was that it was the first of its kind in BECC showing the importance of performing ST-II rather than ST-l being the gold-standard test to evaluate DES.

Further studies can be done by including more sample size and healthy control group along with the DES group in order to have a better understanding of the effect of the association between DES and Serum Vit-D level.

Conclusion

In our study, OSDI score, low tear secretion level and tear break up time suggested to show significantly low Serum Vitamin-D concentration in blood which may be the contributory factor for DES. The results of the study suggest that Serum Vit-D supplementation can be a useful and effective treatment of DES.

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