Association of keratoconus with serum levels of 25-hydroxyvitamin D and antioxidant trace elements: A systematic review and meta-analysis

Parul C Gupta, Mona Pathak1, Bhaskar Thakur1,2, Rajesh Fogla1, Aniruddha Agarwal3, Jagat Ram

The aim of this systematic review and meta-analysis was to summarize and compare the available evidence on the level of vitamin D and antioxidant trace elements between the keratoconus (KC) patients and healthy controls. Seven case–control studies with 830 subjects were found eligible with a systematic search using PubMed, SCOPUS, Web of Science, and EMBASE till November 21, 2021. Data were synthesized with a DerSimonian and Laird random-effects method of meta-analysis. The mean serum vitamin D level was significantly lower in the patients with KC [standardized mean difference (SMD): −0.71; P < 0.001] as compared with the control group. The mean serum vitamin D level decreased more in the progressive patients (SMD: −0.80; P = 0.016) than in the stable patients (SMD: −0.66; P < 0.001) when compared with the control group. The mean serum zinc level was found significantly lower in the patients with KC compared with the control group (SMD: −1.98; P = 0.005). Pooled analysis based on the two studies showed significantly lower mean selenium levels in the KC patients (SMD: −0.34; P = 0.003). Regular evaluation of serum vitamin D, zinc, and selenium levels among the patients with KC at disease onset and future follow-ups could be promising in predicting the progressive disease and disease severity.

Key words: Copper, keratoconus, selenium, vitamin D deficiency, zinc

Keratoconus (KC) is the most commonly occurring primary ectasia. It is a progressive, bilateral (usually asymmetric) disease that produces thinning and conical protrusion of the cornea. It causes blurred vision, myopia, irregular astigmatism, and corneal scarring. Its prevalence in the general population is 54 per 100,000 population, although its prevalence is higher in Asians.1−2 Studies show that genetic, environmental, oxidative stress, and inflammation at the cellular level play a role in the pathogenesis of KC development and progression.3−6 Vitamin D is a fat-soluble prohormone that has immunomodulatory effects on the immune system and is implicated in a variety of diseases. It has been demonstrated to be synthesized de novo in corneal limbal epithelial cell cultures akin to skin after UV-B radiation exposure.7−10 Studies have proposed the antioxidant role of vitamin D in amelioration of oxidative stress in alloxan-induced diabetes and for healing of corneal epithelium and maintenance of corneal tight junctions.5−8 KC has been found to be associated with vitamin D deficiency.3−10 Additionally, the lower level antioxidative trace elements [zinc (Zn), copper (Cu), and selenium (Se)] have also been observed in KC patients.3−12

It is essential to understand how the serum vitamin D levels and antioxidative trace elements are different in the patients of KC as compared with healthy children/adults. Additionally, an understanding of the causal pathways in the relationship between these markers (vitamin D levels and antioxidative trace elements) and progressive KC disease as well as disease severity is also very critical.

In this review, our primary aim was to identify the association between the vitamin D level with KC by performing a systematic review and meta-analysis in children/adults. The secondary aim was to perform a meta-analysis to find out the association of antioxidative trace elements including Zn, Cu, and Se with KC in children/adults. We also aimed to determine how the vitamin D level correlates with the disease progression.

Methods

Data sources and search strategy

Our review is reported according to the reporting guidelines provided in the Meta-analysis of Observational Studies in Epidemiology (MOOSE)13 and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (see PRISMA checklist in the additional file).14 The protocol of systematic review is registered within the PROSPERO database (registration number: CRD42021226360).

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

Cite this article as: Gupta PC, Pathak M, Thakur B, Fogla R, Agarwal A, Ram J. Association of keratoconus with serum levels of 25-hydroxyvitamin D and antioxidant trace elements: A systematic review and meta-analysis. Indian J Ophthalmol 2022;70:2818-24.
We conducted a systematic literature search using electronic databases PubMed, SCOPUS, Web of Science, and EMBASE till November 21, 2021. The search strategy was designed to retrieve the relevant studies to accomplish the study objectives including index terms and free-text terms. Various combinations of Boolean operators were applied by using the following keywords for our search: “Keratoconus,” “vitamin D,” “antioxidant trace elements,” “zinc,” “copper,” “selenium,” “children,” and “adults.” There was no language restriction at the time of the search. The reference lists of the relevant publications for related studies were also manually searched.

Study selection
All studies were eligible for inclusion in our systematic review if they meet the following criteria: (1) observational study (including case report and case series with smaller numbers); (2) contains information on the association/comparison of either serum vitamin D level or any of the antioxidant trace elements (zinc, copper, and selenium) between with and without KC; (3) confirms the diagnosis of KC; (4) pediatric/adult human subjects; (5) published as either an original investigation or short communication/commentary/letter or availability of pre-print or conference presentation. However, review/personal view or guideline/recommendation were excluded. Also, the studies that were not primarily centered on the association between serum levels of vitamin D/antioxidant trace elements and KC conducted in other than pediatric/adult population and animal studies were excluded. The criterion of the definition of disease severity and progression varied between studies.[10-12,15,16]

Data extraction
Two reviewers independently searched and screened the studies based on the titles and abstracts of the studies obtained by the searches. Those studies that were found relevant were subjected to a detailed appraisal for full text. Three senior authors verified the searches and eligible studies. Data were extracted by two reviewers from the eligible studies. An Excel data collection sheet was developed to extract all relevant information (study characteristics) from the included eligible studies. Study characteristics including study identity number, study title, the name of the first author, the country where the study was conducted, setting, study design, the total number of patients/subjects, the number of patients/subjects in the study population and healthy control, average age [with standard deviation (SD)/inter-quartile range (IQR)], gender (frequency of male and female), average body mass index (BMI) (with SD/IQR), status of comorbidity, disease duration (mean/median with SD/IQR), status of the disease progression, average serum levels of vitamin D/antioxidant trace elements (with SD/IQR) separately in the study population and healthy control, percentage of vitamin D deficiency in the study population and healthy control, type of vitamin D estimation test used, amount of sun exposure, season/sampling month, and treatment status for the vitamin D deficient subjects were extracted. Vitamin D was measured in ng/mL, whereas the measurement unit for antioxidant trace elements was changed into µg/dL when they were reported in other units. Both the reviewers independently extracted the above-mentioned study characteristics on the Excel data collection sheet. Observed discrepancies were resolved through the consensus among all the authors.

Quality assessment
Methodological Index for Non-Randomized Studies (MINORS) instrument was used to assess the study level methodological quality on 12 different items of the included studies.[17] The items were scored from 0 to 2 as follows: 0 (not reported), 1 (reported but inadequate), or 2 (reported and adequate). The ideal global score for comparative observational studies is 24. Two reviewers performed the quality assessment which was further verified by another reviewer. Discussions on the qualitative assessment were carried out in consensus of all the authors to resolve any disagreements.

Statistical analysis
The analysis was carried out to find out the pooled standardized mean difference (SMD) in the exposures (serum levels of vitamin D, zinc, copper, and selenium) between KC patients and the control group in the children/adult population using Hedges’ g method. All the reported IQRs were converted into SD by dividing IQR by 1.35.[23]

The I² statistic was used to assess heterogeneity among effect sizes (ES). An I² > 70% considered significant for high heterogeneity.[19] The fixed effect method was used in case of no/low heterogeneity; however, a random-effect meta-analysis with DerSimonian and Laird (D&L) method was preferred in case of substantial heterogeneity. All the ES were summarized with their 95% confidence interval (CI) and P value. A P value of <0.05 was considered statistically significant. A subgroup analysis was carried out to find out the association between the serum vitamin D levels in stable and progressive KC patients. An Egger’s test was used to assess the publication bias. A funnel plot was not conducted as the number of studies was <10. All statistical analyses were conducted using standard statistical software Stata 17.0 MP—Parallel Edition (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

Results
A total of 121 unique studies were identified after merging and removing duplicates identified through searching PubMed (n = 48), SCOPUS (n = 88), EMBASE (n = 95), and Web of Science (n = 82) [Fig. 1]. Out of these 127 studies, altogether 15 studies were found eligible for full-text review based on screening of the title and abstract. After a full-text review, eight studies were excluded. Among the excluded studies, two studies were unrelated to the topic,[20,21] one study focused on Vit D treatment and receptors,[22] and one study centered on the treatment of KC with topical copper sulfate eye drops (IVMED-80).[23] A study by McMillan was the report of 10 Vit-D-dependent cases which benefitted from vitamin D supplementation (including only one KC patient).[24] One was an animal study[25] and another study assessed the effect of Vit D supplementation.[26] In another study, we were not able to extract data because of the unavailability of the full text.[27] We have contacted the authors of this study but could not get a response. Finally, seven case–control studies were found eligible for quantitative synthesis.

Characteristics of included studies
These seven case–control studies were published between 2012 and 2020 and conducted in three countries, including 830 participants (421 KC patients and 409 healthy controls). Out of the total, 266 subjects were male and 306 were female. The weighted mean age of included participants (combined with cases and controls) was 29 years (SD: 5.63 years). Among the eligible seven studies, four studies were conducted in Turkey [Akkaya S (2020), Aslan MG (2020), Kılıç B (2016), Ortlak H (2012)],[10,15,16,28] two in Iran [Bamdad S (2018), Zarei-Ghanavati S (2019)] and one in Brazil [Leca R (2020)].[29] Five out of

August 2022
Gupta, et al.: Association between keratoconus and 25-Hydroxyvitamin D 2819
seven studies [Akkaya S (2020), Aslan MG (2020), Kılıç R (2016), Ortak H (2012), Zarei-Ghanavati S (2019)] had matched case-control design.\[10,11,15,16,28\]

Four studies compared vitamin D levels among 277 KC patients and 266 healthy controls. Four studies involving 244 KC patients and 243 healthy controls reported zinc levels for both groups. Further, two studies compared copper and selenium levels among KC patients and healthy controls. Details of the study characteristics are made available in Table 1.

Risk-of-bias assessment
Study quality was assessed by MINORS criteria and was good for all the seven studies, with a quality score ranging from 18 to 21 [Table 2]. The ES for selenium had no between-study heterogeneity while it was moderate for vitamin D. For zinc and copper, the heterogeneity was very high. There was no publication bias for all four parameter comparisons.

Association of vitamin D and trace elements with KC patients
KC patients on an average had 4.5 ng/mL lower level of vitamin D (mean: 14.0 ng/mL; 95% confidence interval (CI): 8.4, 19.6) compared to the control (mean: 19.4 ng/mL; 95% CI: 13.2, 25.7). Meta-analysis of four studies showed this difference statistically significant with a moderate level of heterogeneity (SMD: −0.71; 95% CI: −1.04, −0.38; P value: <0.001 and I²: 67.0%) [Fig. 2, Table 3]. Subgroup analysis showed that the mean serum vitamin D level was decreased more in the progressive group patients than in the non-progressive group patients when compared with the healthy controls [Fig. 3]. When we performed a meta-analysis on two studies that informed the severity of KC, we found that the deficient vitamin D level had an increased odds of severe KC [odd ratio (OR): 2.07; 95% CI: 0.85, 5.05] depicted in Fig. 4; however, the association was not significant (P = 0.111).

The average zinc level was significantly lower (mean: 81.7 ng/mL; 95% CI: 27.4, 136.0) in KC patients as compared to healthy controls. The SMD showed that the KC patient had a significantly lower zinc level as compared to the control group (SMD: −1.98; 95% CI: −3.35, −0.60; P value: 0.005; I²: 97.4%). Pooled effect estimates of three studies revealed that the serum level of copper was lower among KC patients than in healthy controls (SMD: −0.79; 95% CI: −1.96, 0.38; P value: 0.19; I²: 96.6%) but given a small number of studies and large variability, this difference was not statistically significant. Only two studies were reporting a comparison of the selenium level. Both studies showed a significantly lower level of selenium among KC patients. Based on these two studies, our pooled analysis showed that the serum selenium level is significantly lower in the KC patients (SMD: −0.34; 95% CI: −0.57, −0.12; P value: 0.003) as compared to the control group [Table 3].\[18\]

Discussion
Despite detailed research globally, the true etiology of KC remains elusive. In our meta-analysis, we observed a decreased vitamin D level in the patients with KC compared to the
Vitamin D is a multifunctional fat-soluble steroid derivative that can be synthesized endogenously and plays a pivotal role in calcium homeostasis and bone metabolism. Its role in skeleton outcomes is robust, and now recently, its beneficial effect on non-skeletal outcomes, including the immune system, cardiovascular health, tumorigenesis, and ocular health, is gaining importance. The human eye is excessively dependent on adequate vitamin D levels for many physiological, optical, and structural activities. Vitamin D has been studied in different ophthalmic conditions, including dry eye, glaucoma, diabetic retinopathy, ocular inflammation, myopia, and optic neuritis. There has been a worldwide increase in myopia, and KC represents the extreme end of this continuum of myopia. It is seen that KC is significantly benefited by the availability of Vitamin D.

Insufficiency or deficiency of Vitamin D worldwide has become a global public health issue. The majority of the clinical studies in the literature opine that values of serum 25(OH)D levels <20 ng/mL (50 nmol/L) indicate vitamin D deficiency. Levels below 30 ng/mL indicate insufficiency, while levels between 30 and 60 ng/mL (75 and 150 nmol/L) signify normal values. There is decreased expression of vitamin D receptors (VDR) in KC corneas. In vitro studies suggest that vitamin D upregulates VDR in corneas and helps in autophagic lysosomal-mediated scavenging of oxidatively damaged diseased human corneal epithelial cells. The ability of vitamin D supplementation to arrest and even reverse findings of KC in patients was demonstrated by Knapp in 1939. He observed a decrease in the height of the cone in six patients supplemented with vitamin D.

Table 1: Study characteristics

| Author (Year)       | Country | Study design (Matching) | n   | KC cases | Control | Age (mean±SD) | Gender (Male/Female) |
|---------------------|---------|-------------------------|-----|----------|---------|---------------|----------------------|
| Akkaya S (2020)     | Turkey  | Case–control (matched)  | 200 | 100      | 100     | 25.19±6.15    | 96/104               |
| Aslan MG (2020)     | Turkey  | Case–control (matched)  | 85  | 55       | 30      | 22.52±3.57    | 36/49                |
| Bamdad S (2018)     | Iran    | Case–control (unmatched)| 100 | 50       | 50      | 29.1±4.7      | 55/45                |
| Kılıç R (2016)      | Turkey  | Case–control (matched)  | 111 | 58       | 53      | 35.35±7.2     | 42/69                |
| Leca R (2020)       | Brazil  | Case–control (matched)  | 58  | 22       | 36      | 36.47±5.46    | 37/39                |
| Ortak H (2012)      | Turkey  | Case–control (matched)  | 76  | 36       | 40      | 36.47±5.46    | 37/39                |
| Zarei-Ghanavati S (2019) | Iran | Case–control (matched)  | 200 | 100      | 100     | 25.19±6.15    | 96/104               |
| Total/average       |         |                         | 830 | 421      | 409     | 28.95±5.63    | 266/306              |

Abbreviations: n=total number of subjects, KC=keratoconus, SD=standard deviation, wtAge=weighted mean age

Figure 2: Association of serum 25-hydroxyvitamin D deficiency and keratoconus. SMD, standardized mean difference, CI, confidence interval

Figure 3: Association of serum 25-hydroxyvitamin D deficiency in the patients with stable and progressive keratoconus. SMD, standardized mean difference, CI, confidence interval

Figure 4: Association of serum 25-hydroxyvitamin D deficiency in the patients with severe keratoconus. OR, odds ratio, CI, confidence interval
Initially, he had found findings consistent with KC in enucleated eyes of dogs fed on a vitamin D deficient diet.\(^{[20]}\) Other anecdotal reports suggest arrest and reversal of KC and subsequent myopic progression with vitamin D supplementation.\(^{[24]}\) The study by Choi et al.\(^{[27]}\) has shown that a 1 ng/mL increase in serum vitamin D levels caused a 0.03 hyperopic change.

In the study by Akkaya et al.,\(^{[10]}\) patients with a deficient vitamin D level had an increased probability of having KC by 2.9-folds. However, the deficient vitamin D level was not associated with the KC severity group.\(^{[10]}\) Another study by Zarei-Ghanavati et al.\(^{[11]}\) could not find a significant difference in the vitamin D level when compared across the various severity stages. Our pooled analysis based on these two studies showed that the vitamin D level had a promisingly increased risk of severe KC. This can be an invaluable target for future research investigating the potential association between vitamin D and KC and between vitamin D levels and disease severity.

In the study by Aslan et al.,\(^{[15]}\) serum vitamin D levels were decreased significantly in both progressive and non-progressive KC groups as compared to healthy non-KC controls. The probability of progressive and non-progressive KC increased by 1.29 and 1.23 times in patients having deficient serum vitamin D levels in comparison with the control group. There was also a significant correlation between corneal topographic parameters and serum vitamin D levels. However, the serum vitamin B12, folic acid, and calcium levels were similar in all the groups.\(^{[15]}\)

Another study carried out by Akkaya et al.,\(^{[10]}\) demonstrated that the lower vitamin D level is associated with progressive KC patients. Our pooled analysis based only on these two studies revealed a significant association between deficient vitamin D and progressive KC disease. The predominant goal of KC treatment consists of halting its progression at an earlier stage. The antiproliferative effect of vitamin D on the progression of scleral elongation in myopia may be considered akin to the progression of corneal protrusion in KC.\(^{[18]}\)

In addition to decreased vitamin D levels in keratoconic patients, numerous copper-dependent enzymes like superoxide dismutase, cytochrome c oxidase, and lysyl oxidase, and copper levels are altered in keratonic tissues.\(^{[19]}\) It has been postulated that there is inhibition of migration of copper ions to the center of the cornea in KC patients due to an increase in tear alkalinity. Decreased concentration of cuprate ion in the middle of the cornea leads to inactivation of a copper-dependent enzyme such as lysyl oxidase, which promotes collagen cross-linking and subsequently promotes KC.\(^{[21]}\) In the study by Avetisov et al.,\(^{[20]}\) an excessive accumulation of iron, copper, and zinc was demonstrated in the periphery of the corneal buttons but a complete deficiency in the center. This leads to the hypothesis that the etiopathogenesis of KC is associated with the formation of an invisible physiochemical barrier in corneal tissues, which subsequently leads to ectasia. Micronutrients such as zinc and selenium can protect the cornea by amelioration of oxidative stress in the epithelium, and downregulation of proinflammatory cytokines,\(^{[30,31]}\) Bamdad and Zarei-Ghanavati reported lower levels of zinc, copper, and selenium in KC patients as compared to controls.\(^{[11,12]}\) Kilic and Ortak demonstrated a lower level of zinc in KC patients. An elevated copper/zinc ratio is a marker for increased oxidative stress, as Zarei-Ghanavati et al.\(^{[11]}\) have described.

Our study contributes additional information on the strategy management of antioxidant trace elements. The KC...

---

**Table 2: Quality appraisal of included studies according to MINORS assessment**

| Author (Year) | Total | Clearly stated aim | Prospective collection of data | Inclusion of consecutive patients | Adequate statistical analyses | Bias | Loss to follow-up <5% | Follow-up period appropriate to the aim of the study | Follow-up assessment of the group | Study size |
|---------------|-------|--------------------|-----------------------------|-------------------|-----------------------------|------|---------------------|--------------------------------|-----------------------------|--------|
| Akkaya S (2020) | 21    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Aslan MG (2020) | 20    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Bamdad S (2018) | 19    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Kılıç R (2016)  | 18    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Leca R (2020)   | 19    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Ortak H (2012)  | 19    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
| Zarei‑Ghanavati S (2019) | 21    | 2                  | 2                           | 2                 | 2                           | 2    | 2                   | 2                              | 2                           | 2      |
A patient had a significantly lower level of zinc and selenium compared to the control group. Serum copper was also lower in KC patients but not significant.

There can be many clinical implications of decreased serum vitamin D and other trace elements. The adequate replacement of vitamin D3 in a study has resulted in the arrest and reversal of KC and myopic progression.[44] Considering KC to be more severe in pediatric patients as compared to adults,[42] they should be monitored closely for serum vitamin D and other antioxidant trace elements levels. Since pregnancy has been considered to be a risk factor for KC progression,[43] women with preexisting KC before planning pregnancy may be considered for supplementation with vitamin D and other trace elements in a possible attempt to decrease KC progression. KC patients with a family history of KC have more severe diseases.[44] Supplementation with these elements and vitamin D can probably be carried out earlier in childhood of patients with other family members having KC. KC patients have a thicker choroidal thickness as compared to healthy controls, possibly due to inflammatory choroidal mechanisms.[45] Vitamin D has anti-inflammatory properties as it decreases the production of inflammatory cytokines, prostaglandins, and other immune cells.[46] Vitamin D-rich diet (egg yolk, animal liver, and milk products) or additional supplementation can help control these inflammatory mechanisms.

This meta-analysis of seven observational studies documented a significant association between vitamin D and antioxidant trace elements, namely zinc and selenium, in patients with KC. These findings suggest the requirement of regular monitoring of vitamin D and trace elements and related interventions among the KC patients. Evaluation of vitamin D levels in KC patients at the onset and follow-up examination may assist in predicting the course of the disease.

There are several strengths of this study, including (a) the first reporting of meta-analysis on the association between vitamin D and the patients with KC; (b) the association of vitamin D with the disease progression; (c) this study also revealed the association between antioxidant trace elements and the patients with KC; (d) we used a systematic and comprehensive search strategy utilizing various electronic databases to identify a wide coverage of available eligible studies; and (e) all the included studies are of fair quality with no publication bias. Our findings should be interpreted in light of several study limitations. First, we performed a limited number of available eligible studies with limited sample sizes. For example, we performed a pooled effect analysis on the association between serum selenium and the patients with KC based only on the two studies. Second, a subgroup analysis was carried out only based on two-two studies for the severe and non-severe patient populations. Although we observed substantial heterogeneity in the fewer associations, dealing with the small number of studies, we could not perform the sensitivity analysis here. Owing to the high heterogeneity in the meta-analysis, it is tough to understand if these findings suggest the “true point” estimate. Third, the criterion of the definition of disease severity and progression varied between studies. Fourth, the mean vitamin D level of controls was also in the deficient range (19.4 ng/mL); however, it was significantly higher than the cases. Lastly, since progression in KC is likely multifactorial, the implication that vitamin D, zinc, and selenium are the source of progression may need further justification. However, such findings may also be useful to frame the multiple hypotheses to conduct future research in a more pragmatic setting.

Conclusion

To conclude, our study indicates that the patients with KC could have lower serum vitamin D levels than those in the control group. Other antioxidant trace elements, namely serum zinc level and serum selenium level, are also lower among the patients with KC. Regular monitoring of vitamin D levels and trace elements for KC patients at disease onset and follow-ups could be used to predict the disease severity. Future trials demonstrating the effect of vitamin D, zinc, and selenium supplemenations could be worth attempting among the patients with KC as well as in the progressive group as a possible curative option. Imbalance in serum vitamin D, zinc, and selenium levels may prove the role of environmental factors influencing KC development.

Financial support and sponsorship Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS. Keratoconus: A review. Contact Lens Anterior Eye 2010;33:157–66.
2. Georgiou T, Funnell CL, Cassels-Brown A, O’Conor R. Influence of ethnic origin on the incidence of keratoconus and associated atopic disease in Asians and white patients. Eye Lond Engl 2004;18:379–83.
3. Gordon-Shaag A, Millodot M, Shneor E, Liu Y. The genetic and environmental factors for keratoconus. BioMed Res Int 2015;2015:795738. doi: 10.1155/2015/795738.
4. Wojakowska A, Pietrowska M, Widlak P, Dobrowolski D, Wylegala E, Tarnawska D. Metabolic signature discriminates normal human cornea from keratoconus-A pilot GC/MS study. Mol Basel Switz 2020;25:2933. doi: 10.3390/molecules25122933.
5. Chwa M, Atlano SR, Hertzog D, Zheng H, Langberg J, Kim DW, et al. Hypersensitive response to oxidative stress in keratoconus corneal fibroblasts. Invest Ophthalmol Vis Sci 2008;49:4361–9.
6. Balasubramanian SA, Mohan S, Pye DC, Willcox MDP. Proteases, proteolysis and inflammatory molecules in the tears of people with keratoconus. Acta Ophthalmol (Copenh) 2012;90:e303–9. doi: 10.1111/j.1755-3768.2011.02369.x.
7. Alsalem JA, Patel D, Susarla R, Coca-Prados M, Bland R, Walker EA, et al. Characterization of Vitamin D production by human ocular barrier cells. Invest Ophthalmol Vis Sci 2015;55:2140. doi: 10.1167/iovs.14-19740.
8. Iqbal S, Khan S, Nasime I. Antioxidant role of Vitamin D in mice with alloxa-induced diabetes. Can J Diabetes 2018;42:412–8.
9. Elizondo RA, Yin Z, Lu X, Watsky MA. Effect of vitamin D receptor knockout on cornea epithelium wound healing and tight junctions. Invest Ophthalmol Vis Sci 2014;55:5245–51.
10. Akkaya S, Ulusoy DM. Serum vitamin D levels in patients with keratoconus. Ocul Immunol Inflamm 2020;28:348-53.

11. Zarei-Chanavati S, Yahaghi B, Hassanzadeh S, Ghayour-Mobarhan M, Hakimi HR, Eghbali P. Serum 25-hydroxyvitamin D, selenium, zinc and copper in patients with keratoconus. J Curr Ophthalmol 2020;32:26-31.

12. Bamdad S, Owji N, Bolkheir A. Association between advanced keratoconus and serum levels of zinc, calcium, magnesium, iron, copper, and selenium. Cornea 2018;37:1306-10.

13. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: A proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. JAMA 2000;283:2008-12.

14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ 2009;339:b2700-b2700.

15. Aslan MG, Findik H, Okutucu M, Aydin E, Oruç Y, Arpa M, et al. Serum 25-hydroxy vitamin d, vitamin b12, and folic acid levels in progressive and nonprogressive keratoconus. Cornea 2021;40:334-41.

16. Ortak H, Soğüt E, Taş U, Mesci C, Mendil D. The relation between keratoconus and plasma levels of MMP-2, zinc, and SOD. Cornea 2012;31:1048-51.

17. Slim K, Nini E, Forestier D, Kviatkovski F, Panipon J. Methodological index for non-randomized studies (minors): Development and validation of a new instrument. ANZ J Surg 2003;73:712-6.

18. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 2014;14:135. doi: 10.1186/1471-2288-14-135.

19. Pathak M, Dwivedi S, Thakur B, Vishnubhatla S. Methods of estimating the pooled effect size under meta-analysis: A comparative appraisal. Clin Epidemiol Glob Health 2020;8:105-12.

20. Avetisov SE, Mamikonyan VR, Novikov IA, Pateyl YS, Osipyan GA, Korushchenkova NP. [Abnormal distribution of trace elements in keratoconic corneas]. Vestn Oftalmol 2015;131:34-42.

21. Avetisov SE, Mamikonyan VR, Novikov IA. [The role of tear acidity and Cu-cofactor of lysyl oxidase activity in the pathogenesis of keratoconus]. Vestn Oftalmol 2011;127:3-8.

22. Shivakumar S, S R, Ghosh A, Jeyabalan N. Vitamin D enhances the autophagic lysosomal clearance in oxidatively stressed human corneal epithelial cells: A therapeutic intervention for keratoconus. Invest Ophthalmol Vis Sci 2019;60:2819-9.

23. Molokhia S, Muddana SK, Hauritz H, Qiu Y, Burr M, Chayet A, et al. IVMED 80 eye drops for treatment of keratoconus in patients -Phase 1/2a. Invest Ophthalmol Vis Sci 2020;61:2587-7.

24. McMillan J. Spectrum of darkness, agent of light: Myopia, keratoconus, ocular surface disease, and evidence for a profoundly vitamin D-dependent eye. Cureus 2018;10:e2744. doi: 10.7759/ cureus.2744.

25. Knapp AA. Vitamin D complex in keratoconus: Etiology, pathology and treatment of conical cornea: Preliminary report. J Am Med Assoc 1938;10:1993. doi: 10.1001/jama.1938.0790240017006.

26. Knapp AA. Results of vitamin-D-complex treatment of keratoconus*. Am J Ophthalmol 1939;22:289-92.

27. Yolton DP. Calcium: II. Role in keratoconus. J Am Optom Assoc 1983;54:135-8.

28. Kılıç R, Bayraktar AC, Bayraktar S, Kurt A, Kavuçu M. Evaluation of serum superoxide dismutase activity, malondialdehyde, and zinc and copper levels in patients with keratoconus. Cornea 2016;35:1512-5.

29. Leca R, Lima ALH, Scorza F, Scorza C, Almeida F, Fonsceca F. Evaluation of tear and blood vitamin D3 levels in patients with keratoconus. Invest Ophthalmol Vis Sci 2020;61:PB0044.

30. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21:319-29.

31. Cankaya C, Cumurcu T, Gunduz A. Corneal endothelial changes in patients with vitamin D deficiency. Indian J Ophthalmol 2018;66:1256. doi: 10.4103/ijo.IJO_238_18.

32. Lr W, L T, Kh H, Sa LN. Vitamin D deficiency as a public health issue: Using vitamin D2 or vitamin D3 in future fortification strategies. Proc Nutr Soc 2017;76. doi: 10.1017/S0033583317000349.

33. Jin KW, Ro JW, Shin YJ, Hyon JY, Wei WR, Park SG. Correlation of vitamin D levels with tear film stability and secretion in patients with dry eye syndrome. Acta Ophthalmol (Copenh) 2017;95:e230-5. doi: 10.1111/aos.13241.

34. Shetty R, Sethu S, Cheuvor P, Deshpande K, Pahuja N, Nagaraja H, et al. Lower vitamin D level and distinct tear cytokine profile were observed in patients with mild dry eye signs but exaggerated symptoms. Transl Vis Sci Technol 2016;5. doi: 10.1167/tvst.5.6.16.

35. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.

36. Rosen CJ. Clinical practice. Vitamin D insufficiency. N Engl J Med 2011;364:248-54.

37. Choi JA, Han K, Park YM, La TY. Low serum 25-hydroxyvitamin D is associated with myopia in Korean adolescents. Invest Ophthalmol Vis Sci 2014;55:2041-7.

38. Mutti DO, Marks AR. Blood levels of vitamin D in teens and young adults with myopia. Optom Vis Sci Off Publ Am Acad Optom 2011;88:377-82.

39. Dudakov L, Liskova P, Jirsova K. Is copper imbalance an environmental factor influencing keratoconus development? Med Hypotheses 2015;84:518-24.

40. Prasad AS, Bao B, Beck FWJ, Kucuk O, Sarkar FH. Antioxidant effect of zinc in humans. Free Radic Biol Med 2004;37:1182-90.

41. Higuchi A, Inoue H, Kawakita T, Ogishima T, Tsubota K. Selenium compound protects corneal epithelium against oxidative stress. PLoS One 2012;7:e45612. doi: 10.1371/journal.pone.0045612.

42. Naderan M, Rajabi MT, Zarrinbakhsh P, Farjadnia M. Is keratoconus more severe in pediatric population? Int Ophthalmol 2017;37:1169-73.

43. Naderan M, Jahanrad A. Topographic, tomographic and biomechanical corneal changes during pregnancy in patients with keratoconus: A cohort study. Acta Ophthalmol (Copenh) 2017;95:e291-6.

44. Naderan M, Rajabi MT, Zarrinbakhsh P, Naderan M, Bakhshi A. Association between family history and keratoconus severity. Curr Eye Res 2016;41:1414-8.

45. Pinheiro-Costa J, Viana Pinto J, Pertesrolo S, Beato JN, Torrão L, Brandão E, et al. Increased choroidal thickness in keratoconus patients: Perspectives in the disease pathophysiology. J Ophthalmol 2019;2019:2453931. doi: 10.1155/2019/2453931.

46. Liu W, Zhang L, Xu HJ, Li Y, Hu CM, Yang JY, et al. The anti-inflammatory effects of vitamin D in tumorigenesis. Int J Mol Sci 2018;19. doi: 10.3390/ijms19092736.

47. Yin K, Agrawal DK. Vitamin D and inflammatory diseases. J Inflamm 2014;7:69-87.

48. Logan VF, Gray AR, Peddie MC, Harper MJ, Houghton LA. Long-term vitamin D3 supplementation is more effective than vitamin D2 in maintaining serum 25-hydroxyvitamin D status over the winter months. Br J Nutr 2013;109:1082-8.