IJCMBS

Original Article

doi.org/ 10.5281/zenodo.15337031 JCMBS 2025; 5(3):113-119

Tear Carnitine Analysis After Corneal Crosslinking in Keratoconus

Keratokonus'ta Korneal Crosslinking Sonrasi Gözyaşi Karnitin Analizi

Ayhan Saglık[1*], Mehmed Ugur Isık[2], Ferdag Saglık [3], İsmail Koyuncu[4@]

[1]Department of Ophthalmology, Harran University Faculty of Medicine, Sanlıurfa /Türkiye

[2]Department of Ophthalmology, Lokman Hekim Hospital, Ankara/ Türkiye

[3]Department of Internal Medicine, University of Health Science, Mehmet Akif İnan Training and Research Hospital, Sanlıurfa /Türkiye

[4]Department of Biochemistry, Harran University Faculty of Medicine, Sanlıurfa /Türkiye

Abstract

Background: This study aimed to investigate changes in carnitine levels after CXL treatment, which play an important role in the cell's energy cycle

Materials and Methods: Single eye of 25 patients who underwent crosslinking (CXL) therapy for keratoconus were included in this prospective, nonrandomized study. Patients were divided into 4 different groups based on gender (male/female) and age (<18/≥18) before treatment. Tears were collected with capillary tubes before, and at the 6th postoperative month without anesthetic. From the tear samples, 27 carnitine ester parameters were measured by a mass spectrometer and an ultra-high-performance liquid chromatograph (UHPLC).

Results: The average age of patients comprising **11** (44%) men and 14 (66%) women were 18.28 ± 3.98 years (12-25). After CXL treatment, statistically significant changes were only detected in C12 (Dodecanoyl Carnitine) and C14 (Myristoyl Carnitine) levels. Among all carnitines, only C4 and C6 carnitine levels increased, but this increase was not statistically significant. Among the age groups, the highest difference was in carnitine derivative C6, and the least differing carnitine derivative was C51 and C5DC. The carnitine derivatives that differed most between genders were C6 and C16, while the least differed were C5DC and C8:1. **Conclusions:** In cases where energy needs increase, such as inflammation, there may be a decrease in inflammation severity as carnitine levels decrease. The variation between pre and post CXL carnitine level measurements in keratoconus patients can be used as a useful marker to monitor inflammation and intervene in the event of excessive inflammation.

Keywords: Acylcarnitine; Carnitine; Crosslinking; Keratoconus; Metabolomics.

ÖZ

Amaç: Hücrenin enerji döngüsünde önemli rol oynayan karnitin düzeylerinde CXL tedavisi sonrası oluşabilecek değişiklikleri araştırmak.

Gereç ve Yöntem: Bu prospektif, randomize olmayan çalışmaya keratokonus nedeniyle çapraz bağlama (CXL) tedavisi uygulanan 25 hastanın tek gözü dahil edildi. Hastalar tedavi öncesi erkek, kadın, 18 yaş üstü ve 18 yaş altı olmak üzere 4 farklı gruba ayrıldı. Gözyaşları ameliyat öncesi ve ameliyat sonrası 6. ayda herhangi bir anestezik madde kullanılmadan kılcal tüplerle toplandı. Gözyaşı örneklerinden 27 karnitin ester parametresi bir kütle spektrometresi ve bir ultra yüksek performanslı sıvı kromatografı ile ölçüldü. **Bulgular:** 11'i (%44) erkek, 14'ü (%66) kadından oluşan hastaların yaş ortalaması 18,28 ± 3,98 yıl (12-25) idi. CXL tedavisi sonrasında sadece C12 (Dodecanoyl Carnitine) ve C14 (Myristoyl Carnitine) düzeylerinde istatistiksel olarak anlamlı değişiklikler tespit edildi. Tüm karnitinlerden sadece C4 ve C6 karnitin düzeylerinde artış görüldü ancak bu artış istatistiksel olarak anlamlı değildi. Yaş grupları arasında en yüksek farklılık karnitin türevi C6'da görülürken, en az farklılık gösteren karnitin türevi ise C51 ve C5DC oldu. Cinsiyet gruplarında en fazla farklılık gösteren karnitin türevleri C6 ve C16 olurken, en az farklılık gösterenler ise C5DC ve C8:1 oldu.

Sonuç: İnflamasyon gibi enerji ihtiyacının arttığı durumlarda karnitin düzeyleri azaldıkça inflamasyon şiddetinde de azalma olabilir. Keratokonus hastalarında CXL öncesi ve sonrası karnitin seviyesi ölçümleri arasındaki farklılık, bu inflamasyonun izlenmesi ve aşırı inflamasyon durumunda müdahale edilmesi için yararlı bir belirteç olarak kullanılabilir

Anahtar kelimeler: Açilkarnitin, Karnitin, Crosslinking, Keratokonus, Metabolomics

***Corresponding author:** Ayhan Sağlık, M.D Harran University Faculty of Medicine Department of Ophthalmology Şanlıurfa, Turkiye.
E-mail: saglikayhan@yahoo.com **Received:** 13 February 2025 **Accepted:** 05 May 2025

@One of the authors of this article serves on the journal's editorial board; the peer-review process was conducted by independent editor(s).

Cite as: SAGLIK.A et al. Tear Carnitine Analysis After Corneal Crosslinking in Keratoconus. JCMBS 2025; 5(3):113-119 doi.org/ 10.5281/zenodo.15337031

Highlights

- Carnitine, which plays an important role in the metabolism of fatty acids in the cell.
- A significant decrease in dodecanoyl carnitine and myristoyl carnitine levels was found secondary to the regression of inflammation after CXL treatment.
- Among all carnitines, only C4 and C6 carnitine levels increased.

Introduction

Corneal collagen crosslinking (CXL) treatment is the only treatment method known to stop or slow the progression of keratoconus(1). After this treatment, which creates new covalent bonds between amino acids in collagen fibrils through reactive oxygen radicals, corneal biomechanical resistance and stability increase (2). After CXL treatment, changes occur in fibroblasts of the corneal stroma, and these changes have been identified as markers of treatment efficacy (3). Although biochemical reactions occur mainly in the stroma during this treatment, some changes may occur on the ocular surface and in the tear film (4,5). In addition, the content of the tear film can also be an indicator of the metabolic functions of the cornea (3,6). athological processes occurring in corneal tissue may cause changes in the metabolome, leading to increased or decreased metabolites (7). Metabolomics, including carnitine, can be detected in many body fluids using mass spectrometry (8). Carnitine, a quaternary, non-essential amino acid, is synthesized from essential amino acids lysine and methionine. Carnitine, which plays an important role in the metabolism of fatty acids in the cell, is a quaternary ammonium compound. Carnitine plays a role in the transport of fatty acids to mitochondria and β-oxidation by binding to long-chain fatty acids as acyl residues (9,10). Thus, this study aims to investigate the changes that may occur after CXL treatment in carnitine levels, which has an important role in the cell's energy cycle.

Material and Methods

Study design

The diagnosis of keratoconus was made according to the criteria defined by Rabinowitz et al. (11). One eye of 25 patients who underwent crosslinking therapy for keratoconus was included in this prospective, nonrandomized study. The cases were selected from patients with keratoconus who were admitted to the Cornea Department of Harran University Faculty of Medicine.

atients with an increase in Kmax of more than 1 dioptre in the last two follow-up examinations performed at 6-month intervals were evaluated as progressive keratoconus. The following patients were excluded from the study: those with the thinnest corneal thickness less than 400 μm, corneal scar or infection, uveitis, glaucoma, ocular trauma, history of contact lens use, autoimmune disease, pregnancy, lactation, previous ocular surgery, and those with a history of topical/systemic immunosuppressive or steroid use.

s the surgical technique, conventional CXL (Dresden Protocol) was performed (12). Following topical anesthesia with 0.5% proparacaine hydrochloride, a 9.0 mm central epithelial debridement was performed with 20% alcohol as keratectomy.

Riboflavin instillation was initiated with a solution containing 0.1% riboflavin + 20% dextran T500 (Collagex, Taipei, Taiwan), applied at 2-minute intervals for 30 minutes. Corneal thickness was measured with an ultrasonic pachymeter before ultraviolet A (UVA) application and was confirmed to be over 400 μm. For the next 30 minutes, UVA was applied with LightLink-CXL (LIGHTMED, Taiwan) at 365 nm and 3.0 mW/cm², and riboflavin instillation was continued at 2-minute intervals during the procedure.

ontact lenses were placed after the procedure. Topical antibiotics were used 4 times a day for 1 week (0.05% moxifloxacin, Moxai® Abdi Ibrahim). After the epithelium had healed, topical steroid treatment was started 4 times a day, then tapered after 2 weeks and continued for 3 months (0.5% loteprednol etabonate, Lotemax®, Bausch + Lomb).

Tears were collected with capillary tubes as defined by Posa et al. (13) before and at the 6th postoperative month without any anesthetic agent. Collected tears were kept at -80°C until the date of analysis. Patients were divided into four groups based on gender (male/female) and age (<18/≥18) before treatment.

Laboratory analysis

Chemicals and Reagents

The reagents used were the internal standard set of Labeled Carnitine Standards-Set B from Cambridge Isotope Laboratories (UK). Mobile phase modifiers such as formic acid and acetonitrile were used in combination with high-performance liquid chromatography (HPLC) gradient-grade methanol (J.T. Baker, Center Valley, PA, USA) and deionized water (Millipore Simplicity UV water purification system, Waters Corporation, Milford, MA, USA).

LC-MS / MS carnitine determination

From the tear samples, 27 carnitine ester parameters were measured by a Shimadzu-8040 triple-quad mass spectrometer (MS/MS) (Shimadzu-8040) and a Shimadzu Nexera X2 ultra-high-performance liquid chromatograph (UHPLC). The carnitine profile was studied by modifying the neonatal screening method developed by LaMarca and Azzari (19,20). Filter paper (Whatman filter paper 10538018), cut into 3.2 mm discs, was placed in 96 well plates. 5 μL of tear samples was then placed in each well and allowed to dry at room temperature overnight. Sample extraction was performed using a methanol mixture of approximately 66.6%/33.3% (v/v) and a 300 μL extraction solution of 3 mmol/L hydrazine hydrate aqueous solution, respectively. The extraction solution included internal standards and several stable heavy isotope analogs of carnitine and acylcarnitines. The extracted sample was injected into the Shimadzu LCMS-8040. Mass spectral data for amino acids were obtained using a neutral loss scan of 46 Da in positive mode (CE = 15 V). Mass spectral data for acylcarnitines were obtained using an 85 m/z precursor ion scan in positive mode (CE = 25 V). The percent recovery for each analyte was determined by comparison to an internal standard~~ for each analyte~~. Standard concentrations were in the range of 7.6–152 μmol/L for acylcarnitines. Spiked samples containing different concentrations of analytes were used as daily quality control tests.

Analysis Condition

A 2.2-minute run in FIA flow at 0.070 μL/min (A: water + 0.05% formic acid, B: acetonitrile, A/B: 30%/70%). Column oven 30 °C, desolvation line 300 °C, sample injection volume 40 μL, interface temperature 500 °C, nebulizing gas 3 L/min, and drying gas 20 L/min. All data were reprocessed using Shimadzu Neonatal Software, which automatically calculates the concentration of each compound.

Statistical analysis

The data were analyzed using SPSS for Windows version 22.0 software (IBM SPSS Inc, Chicago, IL, USA). Shapiro-Wilk test was used to analyze the normal distribution. Paired sample t-test was applied using mean ± standard deviation values for normally distributed data. Wilcoxon test was performed using median and interquartile (IQR) value for non-normally data. A value of $p < 0.05$ was accepted as statistically significant.

Ethical Approval

This study was approved by the Harran University Faculty of Medicine Ethics Committee (number: HRU/20.08.07, date: 27.04.2020). Informed consent was obtained from all patients. All procedures were carried out in accordance with the Declaration of Helsinki.

Results

The average age of patients comprising **of** 11 (44%) men and 14 (66%) women was 18.28 ± 3.98 years (12-25). Twelve (48%) of the patients were under the age of 18 and 13 (52%) were over 18 years. Of those under the age of 18, 7 were female and 5 were male. In the investigation for twenty-seven carnitine derivatives, tear levels of 15 carnitine derivatives (free carnitine and 14 acyl carnitine) could be measured. The distribution of all carnitine derivative levels before and after CXL treatment is shown in **Table 1.** Accordingly, after CXL treatment, statistically significant changes were only detected in C12 (Dodecanoil Canitine) and C14 (Myristoil Canitine) levels. A decrease was detected in both carnitine derivatives (p: 0.045, 0.038, respectively).

It was observed that there was some decrease in free carnitine levels after CXL treatment, but this decrease was not significant (p = 0.346). Among all carnitines, only C4 and C6 carnitine levels increased, but this increase was not statistically significant (p: 0.0707, 0.086, respectively), **(Figure-1), (Table-1).**

Table 1. The acylcarnitines in the tear samples of the two studied groups quantified using the LC-MS/MS method

Acylcarnitines	m/z	Formula	Status	Pre-CXL (n:) Mean ± SD	Post-CXL (n:) Mean ± SD	Fold change	p
C0 (free carnitine)	218.20>103.00	C7H15NO3	↓	14.81 ± 8.90	12.65 ± 6.21	-1.17	0.346

C2 (acetyl carnitine)	260.20>85.00	C9H17NO4	↓	1.72 ± 1.09	1.59 ± 1.20	-1.08	0.656
C3 (propionyl carnitine)	274.20>85.00	C10H19NO4	↓	1.12 ± 0.69	0.91 ± 0.60	-1.23	0.287
C4 (butyryl carnitine)	288.20>85.00	C11H21NO4	↑	0.38 ± 0.20	0.40 ± 0.19	0.95	0.707
C4DC (methylmalonyl carnitine)	374.30>85.00	C11H19NO6		Ø	Ø		
C5 (isovaleryl carnitine)	302.20>85.00	C12H23NO4	↓	1.68 ± 1.15	1.41 ± 1.35	-1.19	0.360
C5:1 (tiglyl carnitine)	300.20>85.00	C12H21NO4	↔	0.03 ± 0.02	0.03 ± 0.02	0	0.841
C5OH (isovaleryl carnitine)	318.20>85.00	C12H23NO4	↔	0.03 ± 0.01	0.03±0.02	0	0.452
C5DC (glutaryl carnitine)	388.30>85.00	C12H21NO6	↔	0.04 ± 0.02	0.04 ± 0.02	0	0.959
C6 (hexanoyl carnitine)	316.20>85.00	C13H25NO4	↑	0.06 ± 0.04	0.09 ± 0.05	0.66	0.086
C6DC (adipoyl carnitine)	344.20>85.00	C13H23NO6	↔	Ø	Ø		
C8 (octanoyl carnitine)	342.20>85.00	C15H29NO4	↓	0.02 ±0.01	0.01 ± 0.01	-2	0.151
C8:1 (octenoil carnitine)	302.20>85.00	C15H27NO4	↓	0.07 ± 0.06	0.05 ± 0.06	-1.4	0.367
C8DC (suberyl carnitine)	430.40>85.00	C15H27NO6		Ø	Ø		
C10 (decanoyl carnitine)	372.30>85.00	C17H33NO4	↔	0.01±0.01	0.01±0.008	0	0.092
C10:1 (decenoil carnitine)	370.30>85.00	C17H31NO4		Ø	Ø		
C10DC (sebacoyl carnitine)	458.40>85.00	C17H31NO6		Ø	Ø		
C12 (dodecanoyl carnitine)	400.30>85.00	C19H37NO4	↓	0.02 ± 0.01	0.01 ± 0.009	-2	0.045
C14 (myristoyl carnitine)	428.40>85.00	C21H41NO4	↓	0.02 ± 0.01	0.01 ± 0.008	-2	0.038
C14:1(tetradecenoyl carnitine)	426.40>85.00	C21H39NO4		Ø	Ø		
C14:2(tetradecadienoyl carnitine)	424.40>85.00	C21H37NO4		Ø	Ø		
C16 (palmitoyl carnitine)	456.40>85.00	C23H45NO4	↓	0.06 ± 0.04	0.04 ± 0.02	-1.5	0.457
C16:1 (hexadecenoyl carnitine)	454.40>85.00	C23H45NO4		Ø	Ø		
C18 (stearoyl carnitine)	484.40>85.00	C25H49NO4		Ø	Ø		
C18:1 (oleoyl carnitine)	482.40>85.00	C23H45NO4		Ø	Ø		
C18:2 (linoleyl carnitine)	480.40>85.00	C23H45NO4		Ø	Ø		
C18:1OH (hydroxoleoyl carnitine)	498.40>85.00	C25H49NO4		Ø	Ø		

Abbreviations: * p<0.05 and **p<0.001 values for the comparison of the variables between two groups were calculated according to the Mann-Whitney U test. (m/z: mass of main ion and product ion)

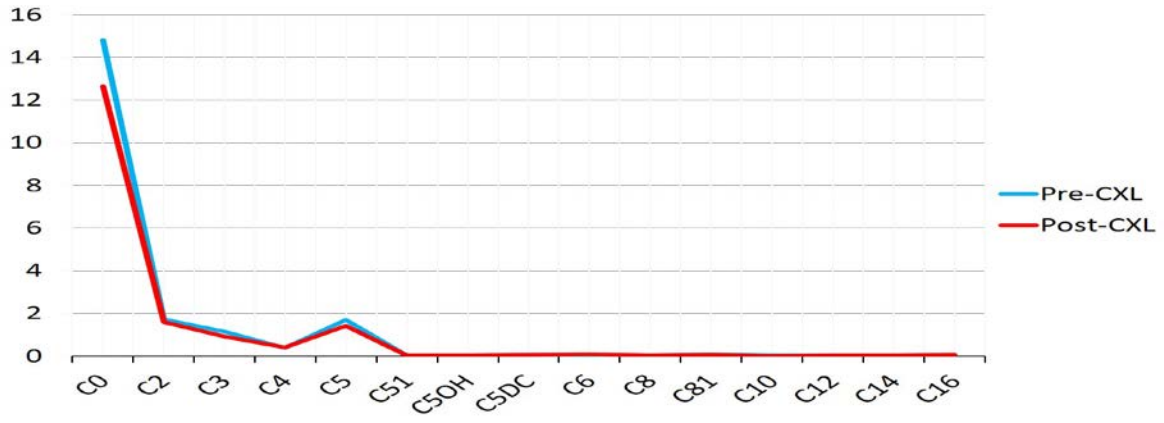

Figure 1. Tear acylcarnitine levels pre- and post- crosslinking

While the C6 level increased statistically significantly only in the group under the age of 18 (0.048 μg / ml increase, p: 0.032), it was not significant even though there was an increase in the group over the age of 18 (0.007 μg / ml increase, p: 0.691). Among the age groups, the highest difference was in carnitine derivative C6, and the least differing carnitine derivative was C51 and C5DC **(Figüre-2)**.

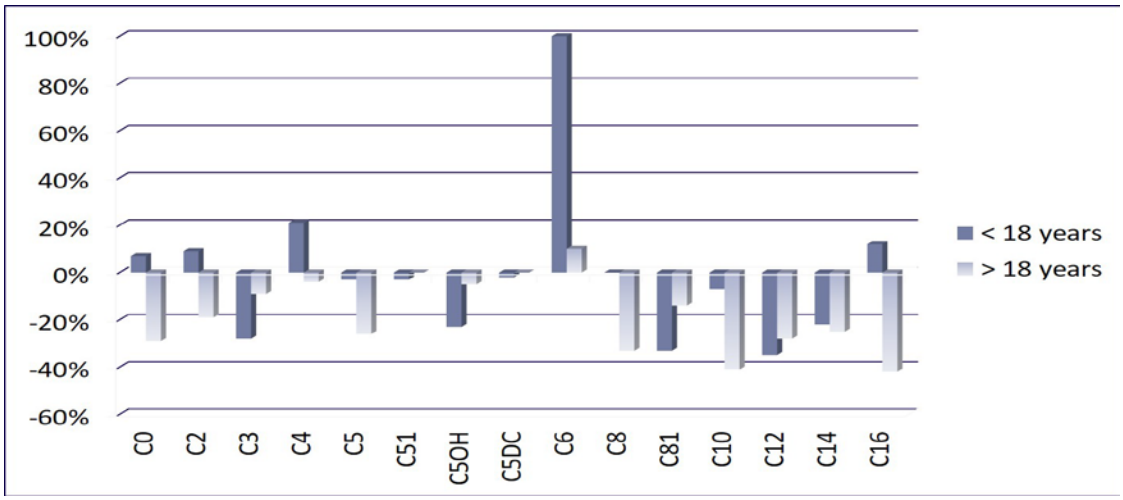

Figure 2. Distribution of the difference in tear acylcarnitine levels before and after crosslinking by age groups

Considering the overall change in carnitine derivatives, the change in male patients was greater than that of female patients **(Figure 3)**. The carnitine derivatives that differed most between the gender groups were C6 and C16, while those that differed least were C5DC and C8. C6 and C16 levels were higher in males than in females.
It was observed that a postoperative corneal scar developed in one case, which did not cause vision loss. In this case, a significant increase was observed in all carnitine derivatives except for C14 (0.01 μg/mL) and C16 (0.04 μg/mL).

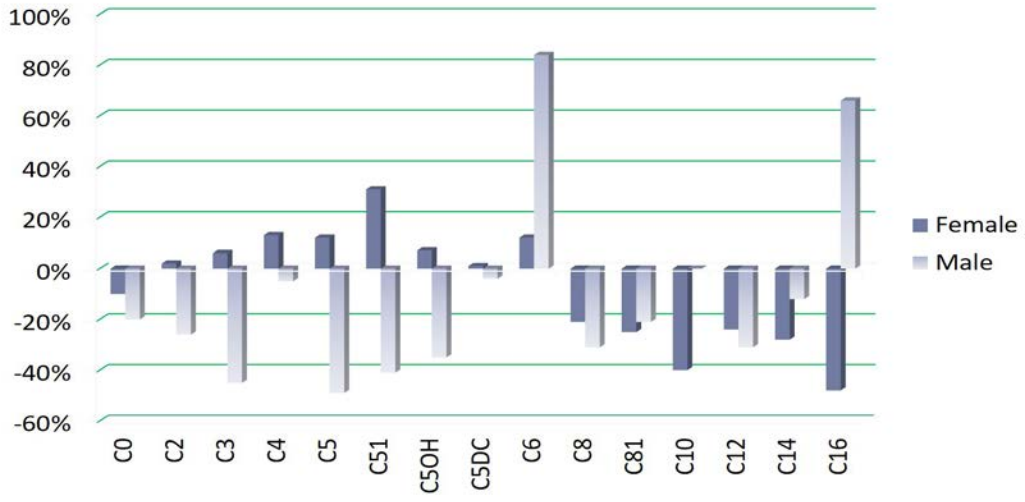

Figure 3. Distribution of difference in tear acylcarnitine levels before and after crosslinking by gender

Discussion

Fatty acids are used as an energy substrate in all tissues except the brain (14). Carnitine is an amino acid derivative that facilitates the transport of long-chain fatty acids into mitochondria and provides a substrate for oxidation and energy production. A decrease in carnitine levels in the cell is expected with inflammation and increased energy

consumption of the cell (15). Although the pathogenesis of keratoconus has not been clarified, metabolomic changes in tears have been demonstrated in pre- and post-CXL comparisons (16). In another study investigating the activity of metabolomics related to energy production in keratoconus and healthy corneas, increased carnitine synthesis activity was reported in corneas with keratoconus (17). In fact, significantly reduced carnitine levels in keratoconus corneas were detected in a study comparing keratoconus corneas to post-mortem corneas (18). The most important function of biological fluids circulating in the body is the transport of nutrients to tissues and the removal of metabolic waste. On the other hand, apart from the function of carnitine in energy metabolism, it has been reported that carnitine enhances the activity of the enzymes that scavenge free radicals in the tear, such as catalase and glutathione peroxidase (19). Also, lower GSH levels have been reported in tear samples from patients with KC (20). In the study by Snytnikova et al comparing aqueous humor in eyes with keratoconus and post-mortem normal corneas, carnitine concentrations in aqueous humor were shown to be decreased in the group with keratoconus (18). n a study investigating the relationship between tear film carnitine and dry eye, patients with dry eye disease were shown to have significantly lower carnitine levels in tear fluid compared to controls; the authors suggested using solutions containing carnitine to reduce ocular surface damage in these patients (21). As a result of active transport of ocular tissues through the cell membrane, the reduction of carnitine levels in the tear film has been shown (22,23). In addition, inflammation negatively affects carnitine metabolism in the cells. Previous studies have reported that plasma carnitine levels are reduced in inflammatory processes in particular (24). Therefore, we think that increased carnitine levels in the tear film after CXL in the current study are also compatible with these results and that CXL may be effective in suppressing possible inflammation. In asthmatic patients, whose pathophysiology is similar to keratoconus, where inflammatory mechanisms play a role, serum myristoyl carnitine and dodecanoyl carnitine were found to be significantly higher in asthmatic patients than in healthy controls (25). Similarly, in our study, a significant decrease in dodecanoyl carnitine and myristoyl carnitine levels was found secondary to the regression of inflammation after CXL treatment. In the cornea, keratocytes or fibroblasts play a central role in mediating the corneal response after injury. After injury, the keratocytes next to the wound undergo apoptosis, while those farther away from the wound transform into fibroblasts and/or myofibroblasts. At the end of these processes, scar tissue develops (26). Therefore, high carnitine concentrations in the tear film in the case with corneal scar development in this study can be explained by the increase in energy demand due to the increase in the activity of keratocytes, and an increase in carnitine transport through the tear film to compensate for this need. With a similar approach, in the study in which we previously investigated carnitine levels in pterygium tissue, where energy metabolism was accelerated due to premalignant cells and chronic inflammation, we demonstrated increased carnitine levels compared to normal conjunctival tissue (27). albis-Estrada et al, by prescribing oral nutraceutical supplements in dry-eyed patients, concluded that the tear metabolomic profile in patients with dry eye disease can be modified by appropriate oral supplements containing antioxidants and essential fatty acids (28). With this approach, we believe that it would be useful to investigate whether it would be beneficial to enhance carnitine production in order to minimize or prevent scar tissue that may develop after CXL.

Study limitations

One of them is that no tear samples were collected from the healthy population, and no comparison was made with healthy controls. Another limitation is the relatively small sample size, especially the small number of patients who developed corneal scars. A longer postoperative follow-up period could provide more robust insights into variations in carnitine levels over time.

Conclusion

The results of this study showed that the change in carnitine levels before and after CXL in KC patients may be a useful marker for monitoring inflammation and intervening in case of excessive inflammation.

Acknowledgements: *None.*

Ethical Approval: *This Study approval was obtained from the Harran University Faculty of Medicine, Ethics Committee (number: HRU/20.08.07 date: 27.04.2020). Informed consent was obtained from all patients.*

Author Contributions: *Concept: F.D, O.D. Literature Review: F.D, O.D. Design: F.D, O.D. Data acquisition: F.D, O.D. Analysis and interpretation: F.D, O.D. Writing manuscript: F.D, O.D. Critical revision of manuscript: F.D, O.D.*

Conflict of Interest: *The author(s) do not have any potential conflict of interest regarding the research. authorship and/or publication of this article.*

Data Availability: *The data used to support the findings of this study are available from the corresponding author upon reques*

Financial Disclosure: *No financial support was received for this study.*

References

1. Hersh PS, Stulting RD, Muller D, et al. United states multicenter clinical trial of corneal collagen crosslinking for keratoconus treatment. Ophthalmol. 2017:124(9): 1259-70.
2. Raiskup F, Spoerl E. Corneal crosslinking with riboflavin and ultraviolet ai principles. The ocul surf. 2013:11(2): 65-74.
3. Sharif R, Sejersen H, Frank G, et al. Effects of collagen cross-linking on the keratoconus metabolic network. Eye. 2018:32(7): 1271-81.
4. Uysal BS, Akcay E, Kilicarslan A, et al. Tear function and ocular surface changes following corneal collagen cross-linking treatment in keratoconus patients: 18-month results. Int Ophthalmol. 2020:40(1): 169-77.
5. Recalde JI, Acera A, Rodríguez-Agirretxe I, et al. Ocular surface disease parameters after collagen cross-linking for keratoconus. Cornea. 2016:36(2): 148-52.
6. Lema I, Durán JA. Inflammatory molecules in the tears of patients with keratoconus. Ophthalmol. 2005:112(4): 654-9.
7. Kryczka T, Ehlers N, Nielsen K, et al. Metabolic profile of keratoconic cornea. Curr Eye Res. 2013:38(2): 305-9.
8. Yuan M, Breitkopf SB, Yang X, et al. A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. Nat Protoc. 2012:7(5): 872-81.
9. Keshani M, Alikiaii B, Babaei Z, et al. The effects of L-carnitine supplementation on inflammation, oxidative stress, and clinical outcomes in critically Ill patients with sepsis: a randomized, double-blind, controlled trial. Nutr J. 2024;23(1):31.
10. Guerreiro G, Deon M, Becker GS, et al. Neuroprotective effects of L-carnitine towards oxidative stress and inflammatory processes: a review of its importance as a therapeutic drug in some disorders. Metab Brain Dis. 40.2 (2025): 127.
11. Rabinowitz YS, McDonnell PJ. Computer-assisted corneal topography in keratoconus. J Refract Surg. 1989:5(6): 400-408.
12. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a–induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol. 2003:135(5): 620-7.
13. Posa A, Bräuer L, Schicht M, et al. Schirmer strip vs. Capillary tube method: Non-invasive methods of obtaining proteins from tear fluid. Ann Anat. 2013;195(2): 137-42.
14. Berg JM, Tymoczko JL, Stryer L. Each organ has a unique metabolic profile. Biochemistry. 2002:5
15. Adeva-Andany, Maria M, Calvo-Castro I, et al. AM. Significance of l-carnitine for human health. IUBMB Life. 2017;69(8): 578-94.
16. Sağlik A, Koyuncu İ, Soydan A, et al. Tear organic acid analysis after corneal collagen crosslinking in keratoconus. Eye Contact Lens. 2020;46 Suppl 2: S122-8.
17. Wojakowska A, Pietrowska M, Widlak P. et al. Metabolomic signature discriminates normal human cornea from keratoconus—a pilot gc/ms study. Molecules. 2020;25(12): 2933.
18. Snytnikova OA, Yanshole LV, Iskakov IA, et al. Quantitative metabolomic analysis of the human cornea and aqueous humor. Metabolomics. 2017;13(12):152.
19. Augustin AJ, Spitznas M, Kaviani N, et al. Oxidative reactions in the tear fluid of patients suffering from dry eyes. Graefes Arch Clin Exp Ophthalmol. 1995;233(11): 694-8.
20. Saijyothi AV, Fowjana J, Madhumathi S, et al. Tear fluid small molecular antioxidants profiling shows lowered glutathione in keratoconus. Exp Eye Res. 2012; 103:41-46.
21. Pescosolido N, Imperatrice B, Koverech A, et al. L-carnitine and short chain ester in tears from patients with dry eye. Optom Vis Sci. 2009:86(2): E132-8.
22. Xu S, Flanagan JL, Simmons PA., et al. Transport of l-carnitine in human corneal and conjunctival epithelial cells. Mol Vis. 2010; 16:1823-31.
23. Cammarata PR, Xu GT, Huang L, et al. Inducible expression of na+/myo-inositol cotransporter mrna in anterior epithelium of bovine lens: Affiliation with hypertonicity and cell proliferation. Exp Eye Res. 1997:64(5): 745-57.
24. Flanagan JL, Simmons PA, Vehige J, et al. Role of carnitine in disease. Nutr Metab (Lond). 2010; 7:30.
25. Zhu T, Ma Y, Wang J, et al. "Serum metabolomics reveals metabolomic profile and potential biomarkers in asthma." Allergy Asthma Immunol Res. 16.3 (2024): 235.
26. Karamichos D, Hutcheon AE, Zieske JD. Transforming growth factor-beta3 regulates assembly of a non-fibrotic matrix in a 3d corneal model. J Tissue Eng Regen Med. 2011:5(8): e228-38.
27. Saglik A, Koyuncu I, Yalcin H, et al. Carnitine analysis in pterygium. Arq Bras Oftalmol. 2020:83(1): 5-10.
28. Galbis-Estrada C, Pinazo-Durán MD, Martínez-Castillo S, et al. A metabolomic approach to dry eye disorders. The role of oral supplements with antioxidants and omega 3 fatty acids. Mol Vis. 2015:21:555-67.