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Research Articles

Invited Review

Case Reports

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On Behalf of the Turkish Ophthalmological Association Owner

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Contributions submitted to the Journal must be original and not published elsewhere or under consideration for publication by another journal.

Reviewed and accepted manuscripts are translated either from Turkish to English or from English to Turkish by the Journal through a professional translation service. Prior to publication, the translations are sent to the authors for approval or correction requests, to be returned within 3 days. If no response is received from the corresponding author within this period, the translation is checked and approved by the editorial board.

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All individuals and organizations from which the authors received any form of assistance and other support should be declared, and the Conflicts of Interest Form should be used to explain any conflicts of interest.

All contributions are evaluated by the editor-in-chief, associate editors, and independent referees.

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The editorial policies and general guidelines for manuscript preparation specified below are based on the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" from the International Committee of Medical Journal Editors (ICMJE) (2013, archived at http://www.icmje.org/).

Research articles, systematic reviews, and meta-analyses should be prepared according to the relevant guidelines:

CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/);

PRISMA statement of preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/);

STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al., for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/);

STROBE statement, a checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/);

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, 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).

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All submissions to TJO are made electronically through the Journal Agent website (http://journalagent.com/tjo/). After creating an account, authors can use this system for the online submission and review process. Manuscripts collected in the system are archived according to the rules of the ICMJE, Index Medicus (Medline/PubMed) and Ulakbim-Turkish Medicine Index.

Format: Manuscripts should be prepared using Microsoft Word, size A4 with 2.5 cm margins on all sides, 12 pt Arial font, and 1.5 line spacing.

Abbreviations: Abbreviations should be defined at first mention and used consistently throughout the text thereafter. Internationally accepted abbreviations should be used; refer to scientific writing guides as necessary.

Cover letter: The cover letter should include the manuscript type, a statement confirming that the article is not under consideration for publication by another journal, declaration of all sources of funding and equipment (if applicable) and a conflict of interest statement. In addition, the authors should confirm that articles submitted in English have undergone language editing and that original research articles have been reviewed by a biostatistician.

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Citing presentations given at scientific meetings, unpublished manuscripts, theses, Internet addresses, and personal interviews or experiences should be avoided. If such references are used, they should be indicated in parentheses at the end of the relevant sentence in the text, without a reference number and written in full, in order to clarify their nature.

References section: References should be numbered consecutively in the order in which they are first mentioned in the text. All authors should be listed regardless of number. The titles of journals should be abbreviated according to the style used in the Index Medicus.

Reference Format

Journal: Last name(s) of the author(s) and initials, article title, publication title and its original abbreviation, publication date, volume, the inclusive page numbers.

Example:

Collin JR, Rathbun JE. Involutional entropion: a review with evaluation of a procedure. Arch Ophthalmol. 1978;96:1058-1064.

Book: Last name(s) of the author(s) and initials, chapter title, book editors, book title, edition, place of publication, date of publication and inclusive page numbers of the extract cited.

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Example: Herbert L. The Infectious Diseases (1st ed). Philadelphia; Mosby Harcourt; 1999:11;1-8.

Book Chapter: Last name(s) of the author(s) and initials, chapter title, book editors, book title, edition, place of publication, date of publication and inclusive page numbers of the cited piece. Example:

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Books in which the editor and author are the same person: Last name(s) of the author(s) and initials, chapter title, book editors, book title, edition, place of publication, date of publication and inclusive page numbers of the cited piece.

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All visual materials together with their legends should be located on separate pages following the main text.

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To ensure controllability of research findings, the study design, study sample, and methodological approaches and practices should be explained with appropriate sources referenced.

The "p" value defined as the limit of significance along with appropriate indicators of measurement error and uncertainty (confidence interval, etc.) should be specified. Statistical terms, abbreviations, and symbols used in the article should be described and the software used should be stated. Statistical terminology (random, significant, correlation, etc.) should not be used in non-statistical contexts.

All data and analysis results should be presented as tables and figures and summarized in the text of the Results section. Details of the biostatistical methods and procedures used should be presented in the Materials and Methods section or under a separate Statistics heading before the Results section.

MANUSCRIPT TYPES

Original Research Articles

Includes clinical studies, clinical observations, new techniques, and experimental and in vitro studies. Original research articles should include a title, structured abstract, keywords relevant to the content of the article, and introduction, materials and methods, results, discussion, study limitations, conclusion, references, tables/figures/images, and acknowledgements sections. The main text should not exceed 3000 words, excluding references.The title, abstract, and keywords should be written in both Turkish and English.

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4. Each author's full name (without abbreviations and academic titles) and affiliation
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Abstract: The article should be summarized in a Turkish abstract not exceeding 250 words and a corresponding English abstract up to 285 words in length. References should not be cited in the abstract. The use of abbreviations should be avoided as much as possible; any abbreviations in the abstract should be defined and used independently of those used in the main text. For original research articles, the structured abstract should include the following 5 subheadings:

Objectives: The aim of the study should be clearly stated.

Materials and Methods: The study should be described, including selection criteria, design (randomized, retrospective/prospective, etc.), and statistical methods applied, if applicable.

Results: The main results of the study should be stated and the statistical significance level should be indicated.

Conclusion: The results of the study should be summarized and the clinical applicability of the results should be defined.

Keywords: The abstract should be followed by 3 to 5 keywords. Keywords in English should be consistent with the Medical Subject Headings (MESH) terms (www.nlm.nih.gov/mesh/MBrowser.html). Turkish keywords should be direct translations of MESH terms.

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Materials and Methods: The study plan should be clearly described, including whether the study was randomized and retrospective or prospective, the inclusion and exclusion criteria applied, the patient/sample number and characteristics, and statistical methods used.

Results: The results of the study should be stated, with tables/figures given in numerical order; the results should be evaluated according to the statistical analysis methods applied. See the Tables, Graphics, Figures, And Images section of the General Guidelines for details about the preparation of visual material.

Discussion: The study results should be discussed in terms of their favorable and unfavorable aspects and they should be compared with the literature. The conclusion of the study should be highlighted.

Study Limitations: This section should state which data and analyses could not be included in the study, discuss limitations of the study, and give recommendations for future studies.

Conclusion: Highlights the results obtained and conclusions that can be drawn from the study.

Acknowledgements: Any technical or financial support or editorial contributions (statistical analysis, English/Turkish evaluation) towards the study should appear at the end of the article.

References: Authors are responsible for the accuracy of the references. See the General Guidelines for details about the usage and formatting required.

Case Reports

Case reports should present cases which are rarely seen, feature novelty in diagnosis and treatment, and contribute to our current knowledge. The first page should include the title in Turkish and English, an unstructured Turkish summary up to 150 words in length and a corresponding English abstract not exceeding 175 words, and keywords in both languages. The main text should include the introduction, case presentation, discussion, and references. The main text should not exceed 1500 words, excluding references. For case series of 3 or more, the main text should not exceed 2000 words, excluding references.

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Reviews articles analyze topics in depth, independently, and without bias. The first section should include Turkish and English titles, unstructured summaries, and keywords. All cited literature should be referenced. The main text should not exceed 5000 words, excluding references.

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AT A GLANCE

2023 Issue 6 at a Glance:

Esteemed colleagues,

In our last issue of 2023, the Turkish Journal of Ophthalmology features six original studies, one invited review, and three case reports.

In their study titled "Revisiting Pentacam Parameters in the Diagnosis of Subclinical and Mild Keratoconus Based on Different Grading System Definitions," Toprak et al. aimed to reassess the performance of Pentacam parameters in the diagnosis of subclinical keratoconus (KC) and mild KC according to the different definitions in the Amsler-Krumeich (AK), Collaborative Longitudinal Evaluation of Keratoconus (CLEK), and ABCD systems. The cross-sectional study included 24 eyes with subclinical KC, 144 eyes with mild KC (according to AK in 101 eyes, CLEK in 28 eyes, and ABCD in 15 eyes), and 70 normal eyes and evaluated minimum pachymetry, KISA% index, inferior-superior keratometric asymmetry, corneal aberrations, Pentacam indices, front/back elevations, pachymetric progression index, Ambrosio-Relational Thickness (ARTmax), and Belin/Ambrosio Enhanced Ectasia Display scores (Df, Db, Dp, Dt, Da and D-final). Of these, ARTmax, minimum pachymetry, Dt, and Da were found to have the highest ability to distinguish eyes with subclinical KC from normal eyes. The authors emphasized that uniform and definitive criteria for the classification of subclinical and clinical KC are needed to reach a diagnostic and therapeutic consensus in KC (See pages 324-335).

A study by Kazancı et al. titled "The Effect of Autografts from the Inferior and Superior Bulbar Conjunctiva on the Ocular Surface in Primary Pterygium Surgery: A Cytology Study" investigated the impact of using a superior or inferior conjunctival autograft in primary pterygium surgery on the ocular surface. The study included 40 eyes of 40 patients who underwent pterygium surgery with autografting. Before and 1 year after the surgery, cell counts were performed on impression cytology samples obtained from the bulbar conjunctiva, and Schirmer 1 test, tear breakup time (TBUT), conjunctival staining with lissamine green, and corneal staining with fluorescein were evaluated. Corneal and conjunctival staining scores, TBUT, and Schirmer test data showed significant improvement in both patient groups after surgery ($p<0.05$), with no differences between the groups ($p>0.05$). In both preoperative and postoperative impression cytology, the number of goblet cells was higher in the lower bulbar conjunctiva than in the superior bulbar conjunctiva ($p<0.001$). However, no difference was observed in terms of epithelial cells or mucin spots. The authors reported that although there was no significant difference in cytologic parameters between the groups postoperatively ($p>0.05$), obtaining autografts from the inferior bulbar conjunctiva may be a good option in cases where the superior bulbar conjunctiva cannot be used or glaucoma surgery may be performed later (See pages 336-342).

Saracaloğlu et al. present another article on pterygium in this issue, titled "Expression Analysis of the Small GTP-Binding Protein Rac in Pterygium." This study aimed to determine *Rac1, Rac2,* and *Rac3* protein expression in pterygium tissue and compare these expression levels with those in normal conjunctival tissue. Tissue samples from 78 patients with primary pterygium and healthy conjunctival graft samples taken during pterygium surgery were used in the study. RAC1, RAC2, and RAC3 gene expressions in the pterygium tissues did not differ from those in control samples ($p>0.05$). In addition, there was no significant difference in Rac2 or Rac3 protein expressions in pterygium tissues compared to normal tissues in western blot and immunohistochemical analyses ($p>0.05$) (See pages 343-348).

In a retrospective study titled "Evaluation of Central and Peripheral Retinal Vascular Changes in the Fellow Eyes of Patients with Unilateral Retinal Vein Occlusions," Ertop et al. aimed to detect vascular changes in the peripheral retina and macula in the fellow eyes of patients with unilateral retinal vein occlusion (RVO) by examining 53 patients with unilateral RVO and 44 age-matched control subjects. They examined the presence of peripheral retinal vascular pathology in both eyes using high quality ultra-widefield fundus fluorescein angiography, as well as laser flare photometry values and macular vascular density, flow area, and foveal avascular zone measurements on optical coherence tomography angiography. Peripheral retinal vascular pathologies were detected in the fellow eye in 36 patients (67.9%) (See pages 349-355).

The study titled "Real-World Outcomes of Intravitreal Anti-Vascular Endothelial Growth Factor Treatment for Diabetic Macular Edema in Türkiye: MARMASIA Study Group Report No. 1" is a real-life study by 21 ophthalmologists working in 8 tertiary hospitals on the Asian side of the Marmara Region of Türkiye (MARMASIA study group). This comprehensive study included 1,372 eyes (854 patients) treated using a pro re nata protocol in routine practice. The authors aimed to determine the demographic and clinical characteristics and treatment outcomes of diabetic macular edema (DME) patients who underwent anti-vascular endothelial growth factor (anti-VEGF) intravitreal injection (IVI). Five groups were

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formed, with each cohort including the previous one, by collecting patients' baseline and follow-up data at 3, 6, 12, 24, and 36 months. The number of eyes (patients) in the 3, 6, 12, 24, and 36-month cohorts were 1372 (854), 1352 (838), 1185 (722), 972 (581), and 623 (361), respectively. The mean change in best corrected visual acuity (BCVA) and central macular thickness (CMT) from baseline at 3, 6, 12, 24, and 36 months were +7.6, +9.1, +8.0, +8.6, and +8.4 letters and -115.4, -140.0, -147.9, -167.3, and -215.4 μm, respectively ($p<0.001$). The median number of IVIs in the cohorts were 3.0, 3.0, 5.0, 7.0, and 9.0, while the overall rates of anti-VEGF switch and intravitreal dexamethasone implant (IDI) combination were calculated as 18.5% and 35.0%, respectively. The largest real-life DME study reported from Turkey to date, this study showed that anti-VEGF IVI numbers and letter gains were lower than in randomized controlled studies. The authors emphasized that because of the lower baseline BCVA and higher IDI combination rate, these gains differed from those in other real-life studies (See pages 356-368).

Tekcan et al. conducted a study titled "Anterior Segment Changes and Refractive Outcomes after Cataract Surgery Combined with Gonioscopy-Assisted Transluminal Trabeculotomy in Open-Angle Glaucoma" aiming to compare the accuracy of intraocular lens (IOL) calculation formulas and identify factors affecting refractive error in patients undergoing combined phacoemulsification and gonioscopy-assisted transluminal trabeculotomy (phaco-GATT). They retrospectively reviewed 53 eyes of 53 patients who underwent phaco-GATT surgery, comparing anterior segment (AS) parameters measured by Scheimpflug camera preoperatively and at postoperative 3 months and the mean prediction error (PE) and absolute PE using the Sanders Retzlaff-Kraft/theoretical (SRK/T), Barrett-Universal II, Hill-radial-based function (Hill-RBF), and Kane formulas. There was significant shortening of axial length (AL) and enlargement of anterior chamber depth (ACD), anterior chamber angle (ACA), and anterior chamber volume postoperatively ($p<0.001$), while the closest deviation to zero was obtained with the Kane formula (0.001 diopters). Preoperative AL was significantly correlated with mean PE in all formulas except Kane, and Barrett was the only formula in which PE was not significantly correlated with postoperative ACD and ACA (See pages 369-376).

Özkan penned a review titled "Golden Indications and an Overview on the Use of Botulinum Toxin in Strabismus" discussing the current indications of botulinum A toxin (BAT) in strabismus in light of the author's more than 30 years of clinical experience with BAT, focusing especially on ideal first-choice practices, referred to as "golden indications" (See pages 377-385).

In the case reports section, the first case is presented by Bayramoğlu et al. under the title "Extraretinal Fibrovascular Proliferation in a Neonate Possibly Associated with an *ESAM* Gene Variant." They comprehensively examined the diagnostic process of a female infant born at postmenstrual 35 weeks whose fundus examination revealed venous dilatation and arterial tortuosity in both eyes and advanced extraretinal fibrovascular proliferation (See pages 386-389).

Another case report titled "Neurofibromatosis Type 1 Vasculopathy Presenting as Branch Retinal Vein Occlusion: Case Report and Review of the Literature" by Özdemir Zeydanlı and Özdek presents the findings of a 2-year-old girl with neurofibromatosis type 1 (NF1) who had retinal vein branch occlusion secondary to NF1 and peripheral retinal ischemia in the light of the relevant literature. The authors emphasized that NF1-induced retinal occlusions may occur even at very young ages and that detailed fundus examination with fluorescein angiography was necessary in all patients with NF1 (See pages 390-394).

The final article in this issue is a case report by Özdemir et al. titled "Surgical Treatment of Bullous Exudative Retinal Detachment Secondary to Atypical Bilateral Central Serous Chorioretinopathy." This report examined the diagnosis, treatment, and follow-up of a 28-year-old woman with bullous exudative retinal detachment (RD) associated with an atypical variant of bilateral central serous chorioretinopathy (CSCR). The authors emphasized that bullous exudative RD may occur secondary to CSCR, albeit rarely, and that a favorable outcome can be obtained with pars plana vitrectomy, subretinal fluid drainage, and laser photocoagulation (See pages 395-398).

As we bid farewell to 2023 with articles featuring examples of the comprehensive diagnosis and successful treatment of even rare and challenging diseases, we hope the new year brings peace and tranquility to the world.

Respectfully on behalf of the Editorial Board,
Hakan Özdemir, MD

Original Article

Revisiting Pentacam Parameters in the Diagnosis of Subclinical and Mild Keratoconus Based on Different Grading System Definitions

İbrahim Toprak*, Çiğdem Martin*, Celal Emre Güneş*, Jorge Alio**

*Pamukkale University Faculty of Medicine, Department of Ophthalmology, Denizli, Türkiye
**Department of Research and Development, VISSUM, Alicante, Spain; Cornea, Cataract and Refractive Surgery Department, VISSUM, Alicante, Spain; Division of Ophthalmology, Department of Pathology and Surgery, Faculty of Medicine, Miguel Hernández University, Alicante, Spain

Abstract

Objectives: To retest the performance of Pentacam parameters in the detection of eyes with subclinical keratoconus (KC) and mild KC based on different definitions from the Amsler-Krumeich (AK), Collaborative Longitudinal Evaluation of Keratoconus (CLEK), and ABCD systems.

Materials and Methods: This cross-sectional university-based study comprised 24 eyes with subclinical KC, 144 eyes with mild KC (based on AK in 101 eyes, CLEK in 28 eyes, and ABCD in 15 eyes), and 70 controls. Diagnostic ability of the thinnest point (TP) pachymetry, KISA% index, inferior-superior asymmetry, corneal aberrations, Pentacam indices, front/back elevations, pachymetric progression index, Ambrósio-Relational Thickness (ARTmax), and Belin/Ambrósio Enhanced Ectasia Display scores (Df, Db, Dp, Dt, Da, and D-final) were evaluated.

Results: ARTmax (83.3% sensitivity/74.3% specificity) had the highest ability in distinguishing subclinical KC from normal, followed by TP pachymetry, Dt, and Da. D-final showed excellent sensitivity/specificity in mild KC diagnosis based on AK (98%/100%) and CLEK (97.4%/100%) descriptions. In the mild KC-ABCD group, index of vertical asymmetry accurately detected all eyes with mild KC and 97.1% of the controls.

Conclusion: This study points out the gray zone in the detection of eyes with subclinical and mild KC due to overlapping terminology and grading criteria. Pentacam parameters seem to have modest capability in subclinical KC detection, indicating the necessity for additional diagnostic modalities. However, eyes with mild KC can be diagnosed with high accuracy using Pentacam parameters, although the strongest parameters may vary according to the definition of "mild KC." Nevertheless, uniform and definitive criteria for subclinical and clinical KC classification are required for a diagnostic and therapeutic consensus in KC.

Keywords: Diagnosis, Pentacam, Scheimpflug, subclinical keratoconus

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Introduction

Keratoconus (KC) is an asymmetrically bilateral progressive corneal ectasia characterized by visual deterioration and stromal thinning. In moderate and advanced stages, the diagnosis of KC can easily be made based on apparent clinical and topographical findings, whereas detecting eyes with KC in its earliest form remains a challenge.[1]

There is growing interest in developing a powerful parameter or formula for distinguishing subclinical cases of KC to avoid iatrogenic post-laser ectasia.[2,3,4,5,6,7] However, there is no consensus in the literature on the nomenclature for early stages of KC.[2,3,4,5,6,7]

A recent systematic review reported that the most commonly used definition of subclinical KC is an eye with topographic signs of KC and/or suspicious topographic findings with normal slit-lamp examination and KC in the fellow eye.[8,9,10,11,12,13] Regarding clinical KC, various classification systems such as the Amsler-Krumeich (AK), KC severity score, Collaborative Longitudinal Evaluation of Keratoconus (CLEK), ABCD, and RETICS (*Red Temática de Investigación Cooperativa en Salud)* were introduced to grade disease severity.[14,15,16,17,18] The RETICS was developed by Alió et al.[16] as a visual limitation-based KC classification.

Recently, Belin and Duncan[18] proposed the ABCD system, which incorporates anterior/posterior corneal curvature data, thinnest point (TP) on pachymetry, and corrected distance visual acuity (CDVA) for progression follow-up in eyes with KC. The

ABCD system has been also integrated into the Pentacam HR software (Oculus Optikgerate GmBH, Wetzlar, Germany).[18]

Pentacam technology has an important place in the diagnosis of KC because it provides a variety of quantitative parameters, and the utility of these parameters in early KC detection is still being tested. However, previous studies reported diverse sensitivity and specificity values for Pentacam parameters in the diagnosis of subclinical and mild KC due to overlaps among these definitions and a lack of globally accepted uniform criteria.[1,7,10,11,12,13,14,17,18]

This study aimed to re-evaluate the performance of Pentacam parameters in the discrimination of eyes with subclinical KC and mild KC using different definitions of "mild KC" from the AK, CLEK, and Belin ABCD systems against the backdrop of previously published similar studies.

Materials and Methods

The Pamukkale University Non-Interventional Clinical Research Ethics Committee approved the study protocol (decision no: 23, date: 08.12.2020) and the tenets of Declaration of Helsinki on the use of human subjects in research were followed. This retrospective university-based, single-center, cross-sectional study included 24 eyes with subclinical KC (24 patients), 144 eyes with mild KC (144 patients), and 70 control eyes with normal tomography (70 subjects).

All included subjects had reliable records for ophthalmological examinations including CDVA (Snellen) measurement, slit-lamp biomicroscopic examination, dilated fundus examination, and Pentacam imaging. Contact lens wearers were requested to remove their contact lenses prior to the measurements (at least 2 weeks for soft contact lenses and 3 weeks for hard contact lenses).

Each patient was only included in one group to ensure independence of the groups. One eye per patient was used for the statistical analysis. The randomization function of SPSS Statistics for Windows version 24.0 (IBM Corp., Armonk, NY, USA) was used for eye selection in bilateral cases.

Study Groups and Selection Criteria

Subclinical KC

An eye with suspicious topographical alterations but normal biomicroscopy and manifest KC in the contralateral eye was classified as subclinical KC.[7,8] Eyes included in this group also met all of the following criteria (Figure 1):

- CDVA (spectacle correction) ≤0 logarithm of the minimal angle of resolution (logMAR),
- Presence of any suspicious patterns on axial curvature map such as superior steep, inferior steep, irregular, inferior-steep asymmetric bowtie, superior-steep asymmetric bowtie, symmetric or asymmetric bowtie with SRAX >21 degrees and/or localized front (5-7 µm) and/or back (10-17 µm) elevation at the TP,
- Corneal thickness at the TP >470 µm,
- 3-mm inferior-superior keratometric asymmetry (I-S) <1.4 diopters (D),
- Central keratometry (K) <47.2 D.

Manifest KC in the contralateral eye was defined using the following criteria in combination: presence (if any) of biomicroscopic signs of KC (Vogt's striae, Fleischer's ring, Munson's sign or Rizzuti's phenomenon) and/or topographical map patterns typical for KC (round, oval, superior steep, inferior steep, irregular, inferior-steep asymmetric bowtie, superior-steep asymmetric bowtie, and symmetric or asymmetric bowtie with SRAX >21 degrees) accompanied by focal steepening (front elevation >7 µm and/or back elevation >17 µm at the TP) and corresponding corneal thinning, 3-mm I-S keratometric difference >1.4 D, central K >47.2 D, and TP pachymetry <470 µm.

Mild KC Group

In eyes with confirmed diagnosis of KC (based on the above-mentioned topographic criteria for manifest KC), three independent mild KC groups were extracted using definitions of "mild KC" from the AK (mild KC-AK; corresponds to stage 1, induced myopia and/or astigmatism <5 D, corneal radii ≤48 D, and no corneal scarring), CLEK (mild KC-CLEK; steep K <45 D and TP pachymetry >450 µm), and ABCD (mild KC-ABCD; corresponds to stage 0, anterior average radii of curvature in the 3-mm zone >7.25 mm, posterior average radii of curvature in the 3-mm zone >5.90 mm, TP pachymetry >490 µm, CDVA ≤0 logMAR, and no corneal scarring) classification systems.[14,17,18]

Control Group

Eyes included in the control group met the following criteria:

- Bilateral normal corneal tomography and ophthalmological examination,
- None of the above-mentioned pathological findings,
- Normal front and back corneal surface (front elevation <5 µm and back elevation <10 µm at the TP),
- CDVA (spectacle) ≤0 logMAR,
- No history of persistent eye rubbing, atopy or vernal keratoconjunctivitis and no family history of KC.

Exclusion Criteria

Poor Pentacam scan quality (defined as the presence of any quality specification score other than "OK" displayed on the screen; e.g., "data gaps," "model," "fix," "align") and history of corneal pathology (e.g., infection, trauma, scarring, surgery, and other corneal thinning disorders) were defined as the exclusion criteria.

Pentacam Imaging and Main Outcome Measures

Pentacam (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany) measurements were performed by the same single experienced technician (F.K.) under scotopic conditions without pharmacological pupil dilation. Scans were obtained in the same way for all individuals in automatic release mode, and the best-quality scan with an "OK" score was utilized for statistical analysis. Throughout the study, the same Pentacam software version (1.25r15) was used, and all patient data was stored in an SPSS (IBM Corp., Armonk, NY, USA) database.

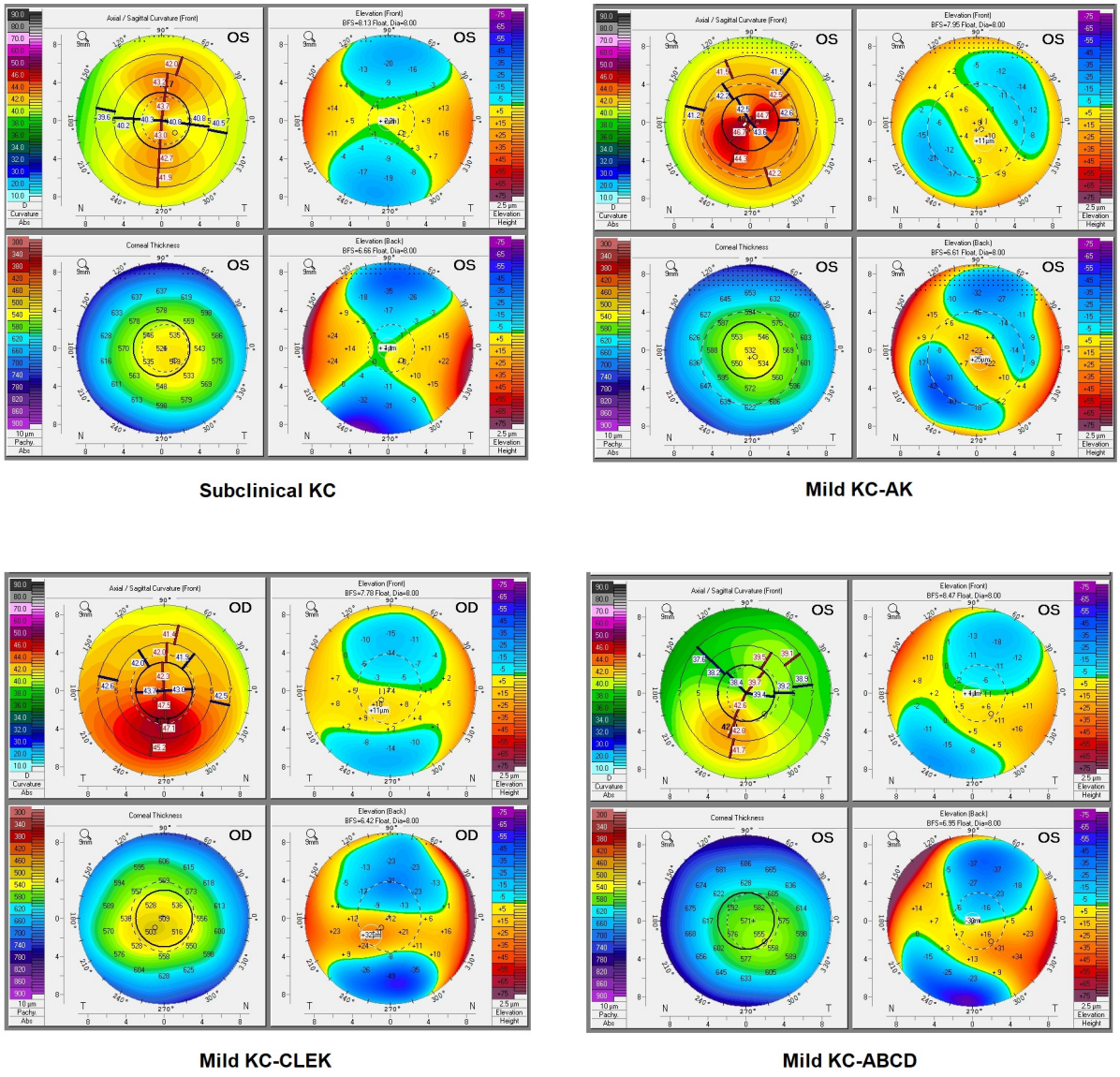

Figure 1. Representative Pentacam images (axial/sagittal curvature, corneal thickness and anterior/posterior elevation maps) of eyes with subclinical KC and mild KC based on the AK, CLEK, and ABCD classification systems

The Pentacam HR utilizes a 360° rotating Scheimpflug camera that captures high-resolution cross-sectional images of the anterior segment. These images are transformed into a three-dimensional (3-D) form to obtain qualitative data for anterior/posterior corneal topography, elevation, pachymetry, corneal power distribution, Zernike corneal wavefront analysis, anterior chamber anatomy, and KC detection/staging.

Mean and maximum keratometry (Kmean and Kmax), the TP pachymetry, KC percentage index (KISA, automatically calculated by the Pentacam system), I-S (automatically calculated by the Pentacam system), root-mean-square (RMS) values for higher order (HOA), spherical, vertical coma, and total aberrations, index of surface variance (ISV), index of vertical asymmetry (IVA), keratoconus index (KI), center keratoconus index (CKI), index of height asymmetry (IHA), index of height decentration (IHD), minimum radius of curvature (Rmin), front elevation at the TP (F.Ele.Th), back elevation at the TP (B.Ele.Th), pachymetric progression index (PPI-min, max and avg), maximum Ambrósio Relational Thickness (ARTmax), and Belin/Ambrósio Enhanced Ectasia Display (BAD-D) scores were noted. ARTmax was calculated by the Pentacam system according to the following formula: ARTmax = TP pachymetry/PPI-max.

Regarding BAD-D scores, Pentacam software generates an "enhanced reference image (best fit sphere)" for the anterior and posterior corneal surfaces by excluding a 3.0-mm area centered on

the TP. The difference between standard and enhanced surfaces are mapped on the screen and highlighted by color code to facilitate visualization of the suspected areas. BAD-D values representing the standard deviation (SD) of front elevation difference (Df), back elevation difference (Db), average pachymetric progression (Dp), TP thickness (Dt), and ARTmax (Da), as well as a final D score (D final) are provided by the system. A D value <1.6 SD is accepted as "normal" (white), ≥1.6 SD (up to 2.6 SD) is indicated as "suspicious" (yellow) and a D value ≥2.6 SD (for D final, ≥3.0 SD) indicates "abnormality" (red).

The ABCD KC grading system, which uses the anterior (A) and posterior (back) (B) radius of curvature taken from the 3.0-mm exclusion zone (centered on the TP), corneal thickness at the TP (C), CDVA (D), and presence or degree of corneal scarring is available in the Pentacam software.[18]

All of the above-mentioned Pentacam parameters were compared among the control, subclinical KC, mild KC-AK, mild KC-CLEK, and mild KC-ABCD groups. Furthermore, we tested the ability of the Pentacam parameters to discriminate subclinical KC and mild KC from normal. For "mild KC" classification, the AK, CLEK, and ABCD systems were used separately to assess the effect of different definitions of "mild KC" on the diagnostic performance of Pentacam parameters.

Sample Size Calculation

Assuming an effect size (d) of 0.4, at least 162 total cases were required to achieve 95% power at 95% confidence level (G*Power version 3.1.9.4 computer software, Universität Düsseldorf, Germany).

Statistical Analysis

Statistical analysis was performed using SPSS statistics version 24.0 (IBM Corp., Armonk, NY, USA). Age and quantitative Pentacam parameters were given as mean ± SD. The Kolmogorov-Smirnov test was used to assess normal distribution of the variables. Bonferroni-corrected Kruskal-Wallis test was used to compare age and Pentacam parameters among the control, subclinical KC, mild KC-AK, mild KC-CLEK, and mild KC-ABCD groups, as none of the variables were normally distributed and met the parametric test conditions. Receiver operating characteristic (ROC) curve analysis was performed and area under the ROC curve (AUC) was calculated to test the ability of the Pentacam parameters to discriminate eyes with subclinical KC, mild KC-AK, mild KC-CLEK, and mild KC-ABCD from normal controls. AUC values were interpreted as excellent (0.90-1.00), good (0.80-0.89), fair (0.70-0.79), poor (0.60-0.69), and worthless (0.50-0.59). The ROC curve plots the true positives (sensitivity) against false positives (1-specificity) for different threshold values. The value with the best sensitivity/specificity pair on the ROC curve was accepted as the cut-off value based on the Youden index. Sensitivity/specificity values for a variable with an AUC value <0.80 were not presented in the article due to its low clinical importance. The DeLong test was conducted (MedCalc® Statistical Software version 20.009, MedCalc Software Ltd, Ostend, Belgium) to assess the statistical significance between the ROC curves for the relevant Pentacam parameters in distinguishing subclinical KC from normal. A p value <0.05 indicated statistically significance at 95% confidence interval for the Kruskal-Wallis test, whereas a p value <0.005 was accepted as statistically significant for pairwise comparisons among the five groups (Bonferroni correction), as the Mann-Whitney U test was performed 10 times.

Results

There was no statistically significant difference in age among the control (n=70 eyes; 27.1±9.9 years), subclinical KC (n=24 eyes; 26.2±6.1 years), mild KC-AK (n=101 eyes; 30.2±9.5 years), mild KC-CLEK (n=28 eyes; 29.4±14.4 years), and mild KC-ABCD (n=15 eyes; 29.3±7.5 years) groups (p=0.060, Kruskal-Wallis test). However, Kmean, TP pachymetry, Kmax, ISV, IVA, KI, CKI, IHA, IHD, Rmin, I-S, KISA, RMS values (total, HOA, spherical, and vertical coma aberrations), F.Ele.Th, B.Ele.Th, PPI (min, avg, and max), ARTmax, and BAD-D scores (all) showed significant differences among the five groups (p<0.05, Kruskal-Wallis test, Tables 1, 2).

Pairwise Comparisons of Pentacam Parameters Between the Groups

Control vs. Subclinical KC Group

Eyes with subclinical KC had lower TP pachymetry, spherical aberration (more negative), and ARTmax but higher IVA, KISA, PPI (min, avg, and max), Df, Dp, Dt, Da, and D final values when compared to the control group (Bonferroni correction, p<0.005 for all).

Control vs. Mild KC-AK Group

Compared to the control group, the mild KC-AK group had significantly higher Kmean, Kmax, ISV, IVA, KI, CKI, IHA, IHD, I-S, KISA, total RMS, RMS-HOA, F.Ele.Th, B.Ele.Th, PPI (min, avg, and max), and BAD-D scores (all) and lower TP pachymetry, Rmin, ARTmax, spherical and vertical coma aberration RMS values (Bonferroni correction, p<0.005 for all).

Control vs. Mild KC-CLEK Group

Eyes with mild KC-CLEK had higher Kmax, ISV, IVA, KI, IHA, IHD, I-S, KISA, total RMS, RMS-HOA, F.Ele.Th, B.Ele.Th, PPI (min, avg, and max), and BAD-D scores (all) when compared to those of the control group (Bonferroni correction, p<0.005 for all). In contrast, TP pachymetry, Rmin, ARTmax, and vertical coma RMS values were lower in the mild KC-CLEK group (Bonferroni correction, p<0.005 for all).

Control vs. Mild KC-ABCD Group

ISV, IVA, IHA, IHD, KISA, RMS-HOA, F.Ele.Th, B.Ele.Th, PPI (min, avg, and max) and BAD-D scores (all) were higher, whereas TP pachymetry and ARTmax were lower in the mild KC-ABCD group than in the control group (Bonferroni correction, p<0.005 for all).

Subclinical KC vs. Mild KC-AK Group

Eyes with subclinical KC had lower Kmean, Kmax, ISV, IVA, KI, CKI, IHA, IHD, I-S, KISA, total RMS, RMS-HOA, F.Ele.Th, B.Ele.Th, PPI (min, avg, and max) and BAD-D scores

Table 1. Comparison of keratometry, pachymetry, topographic indices, inferior-superior asymmetry, KISA, and front/back elevation among the study groups

	Control (A)	Subclinical KC (B)	Mild KC-AK (C)	Mild KC-CLEK (D)	Mild KC-ABCD (E)	p*(KW)	Statistically significant pairwise comparisons (p<0.005) (KW with Bonferroni correction)**
Kmean (D)	43.0±1.3	42.7±1.4	45.8±1.2	42.9±.9	43.4±1.4	<0.0001	C vs. all groups (p<0.0001)
TP (µm)	553.7±30.1	515.8±27.5	464.9±30.9	483.5±33.3	517.7±25.2	<0.0001	A vs. all groups (p<0.0001); B vs. C (p<0.0001) and D (0.002); C vs. E (p<0.0001)
Kmax (D)	44.6±1.9	44.2±1.7	50.9±2.6	46.6±1.7	45.7±1.8	<0.0001	A vs. D (p=0.001); C vs. all groups (p<0.0001); B vs. D (0.001)
ISV	22.2±9.5	20.4±6.9	56.0±22.8	43.0±16.8	33.2±10.2	<0.0001	A vs. C, D (p<0.0001 for both) and E (p=0.001); B vs. C, D and E (p<0.0001 or all); C vs. E (p<0.0001)
IVA	0.10±0.04	0.15±0.07	0.59±0.34	0.51±0.26	0.32±0.09	<0.0001	A vs. B (p=0.004), C, D and E (p<0.0001 for all); B vs. C, D and E (p<0.0001 for all); C vs. E (p=0.001)
KI	1.02±0.02	1.03±0.02	1.14±0.08	1.09±0.06	1.04±0.04	<0.0001	A vs. C and D (p<0.0001 for both); B vs. C (p<0.0001) and D (p=0.003); C vs. D (p=0.002) and E (p<0.0001)
Center KI	1.01±0.01	1.01±0.01	1.03±0.02	1.01±0.01	1.01±0.01	<0.0001	A vs. C (p<0.0001); C vs. all groups (p<0.0001)
IHA	4.71±3.78	5.49±4.33	25.37±18.69	22.14±20.39	14.65±9.50	<0.0001	A vs. C, D (p<0.0001 for both) and E (p=0.001); B vs. C, D and E (p<0.0001 for all)
IHD	0.01±0.01	0.01±0.01	0.07±0.05	0.05±0.03	0.03±0.01	<0.0001	A vs. C, D (p<0.0001 for both) and E (p=0.001); B vs. C, D and E (p<0.0001 for all); C vs. E (p<0.0001)
Rmin (mm)	7.49±0.85	7.65±0.29	6.64±0.34	7.24±0.27	7.39±0.29	<0.0001	A vs. D (p<0.0001); B vs. D (p<0.0001); C vs. all groups (p<0.0001)
I-S (D)	0.06±0.51	0.26±0.54	3.78±2.76	2.79±1.83	0.84±1.60	<0.0001	A vs. C and D (p<0.0001 for both); B vs. C and D (p<0.0001 for both); C vs. E (p<0.0001)
KISA (%)	3.24±2.0	10.70±11.4	228.25±307.19	136.27±164.93	35.77±22.13	<0.0001	A vs. all groups (p<0.0001); B vs. C, D and E (p<0.0001 for all); C vs. E (p=0.004)
F.Ele.Th (µm)	2.84±1.66	3.29±1.46	13.93±6.57	10.61±6.21	5.87±2.95	<0.0001	A vs. C, D and E (p<0.0001 for all); B vs. C, D (p<0.0001 for both) and E (p=0.001); C vs. E (p<0.0001)
B.Ele.Th (µm)	6.31±2.78	8.04±3.00	34.69±12.93	32.61±17.60	13.87±7.10	<0.0001	A vs. C, D and E (p<0.0001); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)

All values given as mean ± standard deviation. *KW: Kruskal-Wallis test (used to compare Pentacam parameters among the 5 groups; p<0.05 was accepted as statistically significant); **p<0.005 was accepted as statistically significant for pairwise comparisons after Bonferroni correction (Mann-Whitney U test)

(all) but higher TP pachymetry, Rmin, ARTmax, and vertical coma aberration RMS values compared to eyes with mild KC-AK (Bonferroni correction, p<0.005 for all).

Subclinical KC vs. Mild KC-CLEK Group

In the subclinical KC group, Kmax, ISV, IVA, KI, IHA, IHD, I-S, KISA, total RMS, RMS-HOA, F.Ele.Th, B.Ele.Th, PPI (min, avg, and max), Db, Dp, Dt, Da and D final scores were lower while TP pachymetry, Rmin, ARTmax, and vertical coma aberration RMS values were higher than in the mild KC-CLEK group (Bonferroni correction, p<0.005 for all).

Subclinical KC vs. Mild KC-ABCD Group

The subclinical KC group had lower ISV, IVA, IHA, IHD, KISA, total RMS, RMS-HOA, F.Ele.Th, Db, and D final values when compared to the mild KC-ABCD group (Bonferroni correction, p<0.005 for all).

Mild KC-AK vs. Mild KC-CLEK Group

Kmean, Kmax, KI, CKI, total RMS, Df, Db, and D final scores were significantly higher in the mild KC-AK group than in the mild KC-CLEK group (p<0.005), whereas Rmin and spherical aberration RMS values were lower in the mild KC-AK group (Bonferroni correction, p<0.005 for all).

Mild KC-AK vs. Mild KC-ABCD Group

Eyes with mild KC-AK had higher Kmean, Kmax, ISV, IVA, KI, CKI, IHD, I-S, KISA, total RMS, RMS-HOA, F.Ele. Th, B.Ele.Th, PPI (min, avg, and max) and BAD-D scores (all) but lower TP pachymetry, Rmin, ARTmax, and vertical coma aberration RMS values when compared to eyes with mild KC-ABCD (Bonferroni correction, p<0.005 for all).

Table 2. Comparison of corneal aberrometry, progression index, Ambrósio Relational Thickness and Belin/Ambrósio Enhanced Ectasia Display scores among the study groups

	Control (A)	Subclinical KC (B)	Mild KC-AK (C)	Mild KC-CLEK (D)	Mild KC-ABCD (E)	p* (KW)	Statistically significant pairwise comparisons (p<0.005) (KW with Bonferroni correction)**
RMS-total (µm)	1.97±1.11	1.26±0.60	4.94±2.44	3.37±1.90	2.41±1.34	<0.0001	A vs. C (p<0.0001) and D (p=0.001); B vs. C (p<0.0001), D (p=0.001) and E (p<0.0001); C vs. D (p=0.001) and E (p<0.0001)
RMS-HOA (µm)	0.26±0.13	0.28±0.11	1.29±0.77	0.90±0.52	0.63±0.22	<0.0001	A vs. C, D and E (p<0.0001 for all); B vs. C, D and E (p<0.0001 for all); C vs. E (p<0.0001)
Spherical aberration (µm)	0.14±0.07	0.09±0.07	-0.03±0.23	0.13±0.15	0.12±0.11	<0.0001	A vs. B and C (p<0.0001 for both); C vs. D (p=0.001)
Vertical coma (µm)	-0.01±0.12	-0.06±0.13	-1.01±0.76	-0.69±0.52	-0.17±0.43	<0.0001	A vs. C and D (p<0.0001 for both); B vs. C and D (p<0.0001); C vs. E (p<0.0001)
PPI-min	0.68±0.10	0.77±0.13	1.28±0.36	1.25±0.36	0.78±0.11	<0.0001	A vs. B (p=0.002), C, D (p<0.0001 for both) and E (p=0.004); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)
PPI-avg	0.96±0.10	1.09±0.19	1.74±0.35	1.66±0.34	1.13±0.13	<0.0001	A vs. B (p=0.002), C, D and E (p<0.0001 for all); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)
PPI-max	1.20±0.15	1.49±0.32	2.56±0.62	2.43±0.51	1.57±0.25	<0.0001	A vs. B, C, D and E (p<0.0001 for all); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)
ARTmax	465.2±70.7	362.8±88.0	190.7±53.7	209.3±52.0	338.0±56.8	<0.0001	A vs. all (p<0.0001); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)
BAD-Df	0.09±0.79	0.77±0.68	5.24±3.00	2.85±1.76	2.04±1.48	<0.0001	A vs. B, C, D and E (p<0.0001 for all); C vs. all groups (p<0.0001)
BAD-Db	-0.26±0.58	0.06±0.81	4.53±2.54	2.88±1.45	1.10±0.87	<0.0001	A vs. C, D and E (p<0.0001 for all); C vs. all groups (p<0.0001); B vs. D (p=0.001) and E (p=0.001)
BAD-Dp	0.35±0.65	1.28±1.25	5.68±2.39	5.08±2.32	1.51±0.90	<0.0001	A vs. B (p=0.001), C, D and E (p<0.0001 for all); B vs. C and D (p<0.0001); C vs. E (p<0.0001); D vs. E (p<0.0001)
BAD-Dt	-0.36±0.86	0.71±0.89	2.51±1.18	1.81±1.20	0.63±0.75	<0.0001	A vs. B, C, D and E (p<0.0001 for all); B vs. C (p<0.0001) and D (p=0.002); C vs. E (p<0.0001)
BAD-Da	0.21±0.65	1.15±0.80	2.70±0.46	2.55±0.48	1.37±0.52	<0.0001	A vs. B, C, D and E (p<0.0001 for all); B vs. C and D (p<0.0001 for both); C vs. E (p<0.0001); D vs. E (p<0.0001)
BAD-D final	0.81±0.52	1.60±0.78	5.83±1.81	4.54±1.25	2.36±0.51	<0.0001	A vs. B, C, D and E (p<0.0001 for all); C vs. all groups (p<0.0001); B vs. D (p=0.001) and E (p=0.002); D vs. E (p<0.0001)

All values given as mean ± standard deviation. *Kruskal-Wallis test (used to compare Pentacam parameters among the 5 groups; p<0.05 was accepted as statistically significant); **p<0.005 was accepted as statistically significant for pairwise comparisons after Bonferroni correction (Mann-Whitney U test)

Mild KC-CLEK vs. Mild KC-ABCD Group

B.Ele.Th, PPI (min, avg, and max), Dp, Da, and D final values were higher and ARTmax was lower in the mild KC-CLEK group than in the mild KC-ABCD group (Bonferroni correction, p<0.005 for all).

The full range of data for pairwise comparisons is provided in Tables 1 and 2.

Diagnostic Ability of Pentacam Parameters

Discrimination of Subclinical KC from Normal

ARTmax, TP pachymetry, Dt, Da, D final, PPI-max, spherical aberration, KISA, Df, KI, IVA, and Dp had good to fair diagnostic ability in distinguishing subclinical KC from normal (listed from highest to lowest AUC, ranging from 0.831 to 0.702, p<0.05) (Table 3 and Figure 2). The DeLong test revealed no statistically significant differences in diagnostic power of AUC values for subclinical KC among the best-performing (AUC >0.800) Pentacam parameters (ARTmax, TP pachymetry, Dt, and Da) (p=0.970).

Discrimination of Mild KC-AK from Normal

D final, ARTmax, Da, Db, PPI-max, IVA, Dp, PPI-avg, B.Ele.Th, HOA, PPI-min, KISA, Df, F.Ele.Th, TP pachymetry, Dt, IHD, KI, vertical coma, I-S, ISV, Kmax, Rmin, IHA, Rmin, total RMS, Km, CKI, and spherical aberration RMS value had AUC values ranging from 0.999 to 0.724 (listed from highest to lowest, p<0.05) in the diagnosis of mild KC-AK.

Table 3. Diagnostic ability of Pentacam parameters in distinguishing subclinical KC and mild KC (based on AK classification) from normal based on the receiver operating characteristic analysis

	Subclinical KC vs. control				Mild KC-AK vs. control			
	AUC	Sensitivity and specificity (%)	Cut-off value	p*	AUC	Sensitivity/ specificity (%)	Cut-off value	p*
Kmean (D)	<0.700	NA	NA	p>0.05	0.819	74.8/70	≥43.8	<0.0001
TP pachymetry (µm)	0.828	87.0/71.4	≤544	<0.0001	0.956	87.8/90	≤506.5	<0.0001
Kmax (D)	<0.700	NA	NA	p>0.05	0.909	84.4/88.6	≥46.2	<0.0001
ISV	<0.700	NA	NA	p>0.05	0.915	80.3/81.4	≥31.5	<0.0001
IVA	0.714	NA	NA	0.003	0.988	98/95.7	≥0.165	<0.0001
KI	0.727	NA	NA	0.001	0.943	87.8/92.9	≥1.045	<0.0001
Center KI	<0.700	NA	NA	p>0.05	0.781	NA	NA	<0.0001
IHA	<0.700	NA	NA	p>0.05	0.884	81/84.3	≥8.15	<0.0001
IHD	<0.700	NA	NA	p>0.05	0.949	91.8/94.3	≥0.019	<0.0001
Rmin (mm)	<0.700	NA	NA	p>0.05	0.906	83.7/90	≤7.245	<0.0001
I-S asymmetry (D)	<0.700	NA	NA	p>0.05	0.935	90.5/82.9	≥0.605	<0.0001
KISA (%)	0.757	NA	NA	<0.0001	0.966	89.8/100	≥8.83	<0.0001
RMS-total (µm)	<0.700	NA	NA	p>0.05	0.839	74.8/70	≥2.585	<0.0001
RMS-HOA (µm)	<0.700	NA	NA	p>0.05	0.969	93.2/94.3	≥0.365	<0.0001
Spherical aberration (µm)	0.762	NA	NA	<0.0001	0.724	NA	NA	<0.0001
Vertical coma (µm)	<0.700	NA	NA	p>0.05	0.934	85.7/95.7	≤ -0.226	<0.0001
F.Ele.Th (µm)	<0.700	NA	NA	p>0.05	0.962	87.8/95.7	≥5.50	<0.0001
B.Ele.Th (µm)	<0.700	NA	NA	p>0.05	0.983	93.2/100	≥13.50	<0.0001
PPI-min	<0.700	NA	NA	p>0.05	0.966	91.2/97.1	≥0.835	<0.0001
PPI-avg	<0.700	NA	NA	p>0.05	0.986	93.2/100	≥1.155	<0.0001
PPI-max	0.777	NA	NA	<0.0001	0.988	93.2/100	≥1.525	<0.0001
ARTmax	0.831	83.3/74.3	≤424	<0.0001	0.990	93.2/100	≤329.50	<0.0001
BAD-Df	0.756	NA	NA	<0.0001	0.963	91.2/88.6	≥0.960	<0.0001
BAD-Db	<0.700	NA	NA	p>0.05	0.990	91.8/100	≥0.985	<0.0001
BAD-Dp	0.702	NA	NA	0.004	0.987	93.2/100	≥1.680	<0.0001
BAD-Dt	0.820	87/70	≥-0.165	<0.0001	0.952	86.4/90	≥1.00	<0.0001
BAD-Da	0.817	82.6/74.3	≥0.585	<0.0001	0.990	92.5/100	≥1.475	<0.0001
BAD-D final	0.788	NA	NA	<0.0001	0.999	98/100	≥1.985	<0.0001

*p<0.05 indicates statistical significance. NA: Not analyzed (sensitivity and specificity values not presented for variables with a p value >0.05 and AUC <0.800)

Discrimination of Mild KC-CLEK from Normal

D final, KISA, Db, ARTmax, Da, PPI-max, IVA, Dp, PPI-avg, B.Ele.Th, Df, HOA, IHD, F.Ele.Th, PPI-min, TP pachymetry, Dt, I-S, KI, vertical coma, ISV, IHA, Kmax and Rmin had excellent to fair ability to discriminate mild KC-CLEK from normal (listed from highest to lowest AUC, ranging from 0.997 to 0.715, p<0.05) (Table 4 and Figure 3).

Discrimination of Mild KC-ABCD from Normal

IVA, KISA, D final, HOA, IHD, Da, ARTmax, Db, PPI-max, Df, Dp, IHA, PPI-avg, B.Ele.Th, F.Ele.Th, TP pachymetry, Dt, ISV, PPI-min, and KI had AUC values ranging from 0.998 to 0.722 (listed from highest to lowest, p<0.05) in distinguishing mild KC-ABCD from normal.

Discussion

The current study provides a comprehensive re-evaluation of Pentacam parameters in the diagnosis of mild and subclinical KC, also comparing with the earlier publications that utilized a variety of patient selection criteria and definitions. This study demonstrated that the efficacy of Pentacam parameters in diagnosing mild KC was influenced by differences in the "mild KC" criteria between the AK and CLEK classification systems. The present study also demonstrated the performance of Pentacam metrics in identifying eyes with KC that were classified as stage 0 by the Belin ABCD progression display.[14,17,18]

This study showed that among the Pentacam parameters, ARTmax had the highest individual performance in

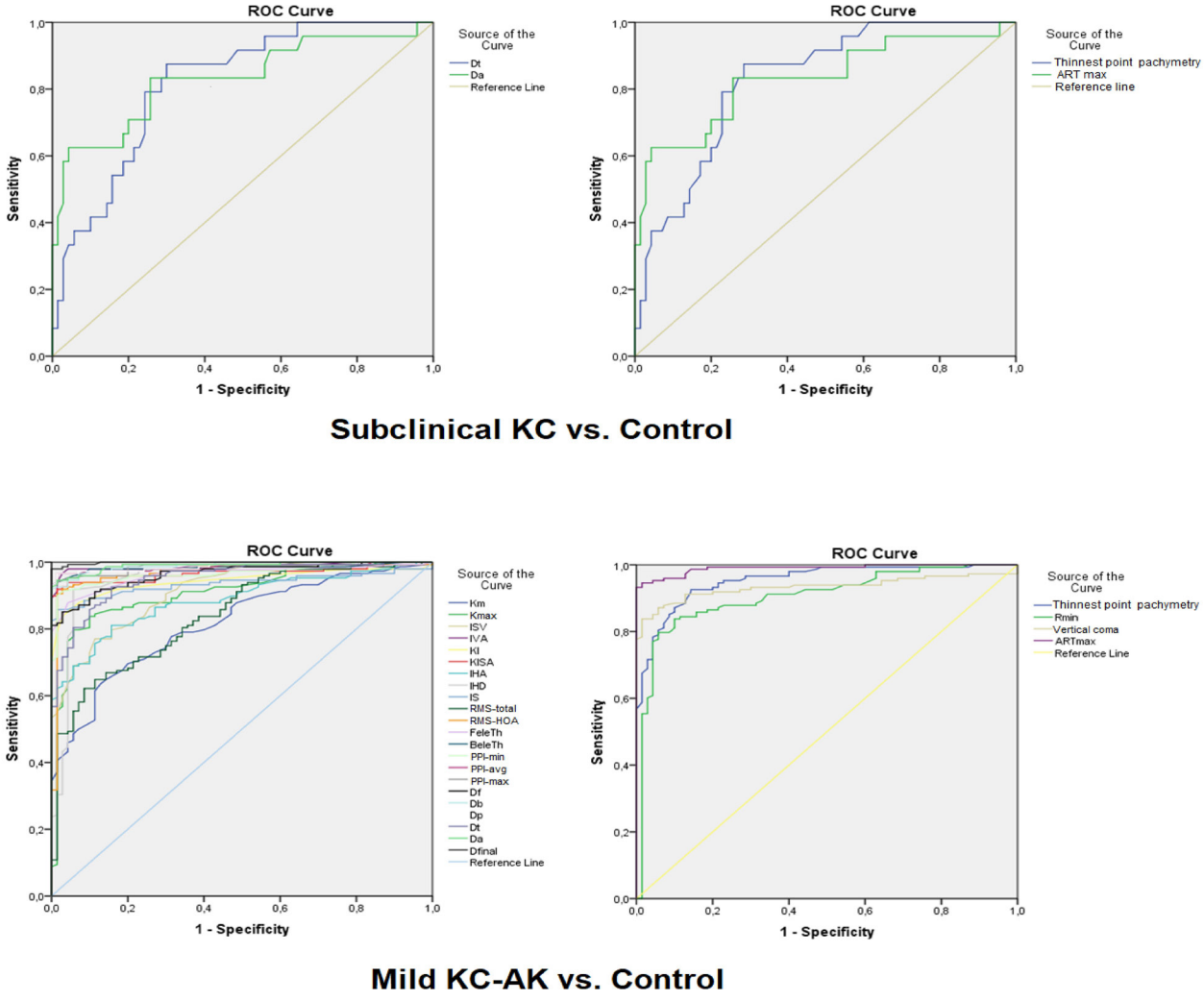

Figure 2. AUC presenting sensitivity and 1-specificity values for Pentacam parameters that had an AUC value over 0.800 in the diagnosis of subclinical KC (top), and mild KC based on the AK classification system (bottom)

distinguishing eyes with subclinical KC from normal (83.3% sensitivity and 74.3% specificity), followed by TP pachymetry, Dt, and Da. However, D final, KISA, I-S, topometric indices, corneal aberrations, and elevation values had no or poor utility in the detection of subclinical KC. In contrast, most of the Pentacam parameters showed highly satisfying performance in the diagnosis of mild KC, although the most powerful Pentacam parameters and their sensitivity/specificity differed depending on the definition of "mild KC" used. For instance, final D score showed excellent performance in the detection of mild KC based on both the AK (98% sensitivity and 100% specificity) and CLEK (97.4% sensitivity and 100% specificity) definitions when the threshold value was ≥1.985. However, when the ABCD stage 0 descriptors were used, IVA accurately detected all eyes (100%) with KC and 97.1% of the normal eyes, for which D final had 93.3% sensitivity and 95.7% specificity. It should also be noted that ARTmax, KISA, IVA, IHD, RMS-HOA, Da, Db, and PPI-max were the common (for all mild KC-AK, -CLEK and -ABCD groups) powerful Pentacam parameters that showed very high performance (AUC>0.900) in the diagnosis of mild KC.

In agreement with the most common definitions of subclinical KC, all eyes in our subclinical KC group had 20/20 corrected vision and normal biomicroscopy, keratometry, and pachymetry but subtle tomographical alterations not reaching the threshold for KC diagnosis.[7,8,9] Therefore, the subclinical KC group in the current study was able to represent real-world risky cases for laser refractive surgery.

In the present study, ARTmax, TP pachymetry, Dt, and Da had the best sensitivity and specificity values (range: 82.6%-87% and 70%-74.3%, respectively) in subclinical KC detection. Interestingly, these parameters were all associated with corneal thickness and its distribution, suggesting that corneal thickness-related Pentacam data might be particularly useful in the diagnosis of subclinical KC. The sensitivity and specificity values for the Pentacam parameters found in this study were

Table 4. Diagnostic value of Pentacam parameters in diagnosis of mild keratoconus based on the CLEK and Belin ABCD classification systems based on the receiver operating characteristic analysis

	Mild KC-CLEK vs. control				Mild KC-ABCD vs. control			
	AUC	Sensitivity and specificity (%)	Cut-off value	p*	AUC	Sensitivity and specificity (%)	Cut-off value	p*
Kmean (D)	<0.700	NA	NA	p>0.05	<0.700	NA	NA	p>0.05
TP pachymetry (µm)	0.904	87.2/85.7	≤518	<0.0001	0.815	80/78.6	≤530.5	<0.0001
Kmax (D)	0.721	NA	NA	<0.0001	<0.700	NA	NA	p>0.05
ISV	0.854	87.2/70	≥27.50	<0.0001	0.786	NA	NA	0.001
IVA	0.978	92.3/100	≥0.220	<0.0001	0.998	100/97.1	≥0.175	<0.0001
KI	0.875	82.1/82.9	≥1.035	<0.0001	0.722	NA	NA	0.007
Center KI	<0.700	NA	NA	p>0.05	<0.700	NA	NA	p>0.05
IHA	0.833	82.1/73.9	≥6.65	<0.0001	0.868	86.7/72.9	≥6.75	<0.0001
IHD	0.930	89.7/94.3	≥0.019	<0.0001	0.931	86.7/92.9	≥0.017	<0.0001
Rmin (mm)	0.715	NA	NA	<0.0001	<0.700	NA	NA	p>0.05
I-S asymmetry (D)	0.886	74.4/100	≥1.025	<0.0001	<0.700	NA	NA	p>0.05
KISA (%)	0.993	94.9/100	≥8.83	<0.0001	0.997	100/95.7	≥6.95	<0.0001
RMS-Total (µm)	<0.700	NA	NA	p>0.05	<0.700	NA	NA	p>0.05
RMS-HOA (µm)	<0.700	NA	NA	p>0.05	<0.700	NA	NA	p>0.05
Spherical aberration (µm)	0.940	87.2/98.6	≥0.474	<0.0001	0.974	93.3/94.3	≥0.365	<0.0001
Vertical coma (µm)	<0.700	NA	NA	p>0.05	<0.700	NA	NA	p>0.05
F.Ele.Th (µm)	0.862	79.5/95.7	≤ -0.226	<0.0001	<0.700	NA	NA	p>0.05
B.Ele.Th (µm)	0.929	76.9/95.7	≥5.50	<0.0001	0.827	86.7/70	≥3.50	<0.0001
PPI-min	0.963	84.6/100	≥14.00	<0.0001	0.829	73.3/90	≥9.50	<0.0001
PPI-avg	0.906	76.9/97.1	≥0.835	<0.0001	0.735	NA	NA	0.004
PPI-max	0.968	92.3/90	≥1.075	<0.0001	0.860	73.3/90	≥1.075	<0.0001
ARTmax	0.980	89.7/95.7	≥1.455	<0.0001	0.907	86.7/80	≥1.330	<0.0001
BAD-Df	0.983	87.2/98.6	≤344.5	<0.0001	0.921	93.3/81.4	≤396	<0.0001
BAD-Db	0.944	84.6/87.1	≥0.925	<0.0001	0.892	86.7/82.9	≥0.775	<0.0001
BAD-Dp	0.988	94.9/92.9	≥0.700	<0.0001	0.909	86.7/81.4	≥0.280	<0.0001
BAD-Dt	0.970	92.3/92.9	≥1.195	<0.0001	0.869	73.3/92.9	≥1.195	<0.0001
BAD-Da	0.897	87.2/84.3	≥0.585	<0.0001	0.801	80/77.1	≥0.210	<0.0001
BAD-D final	0.983	89.7/95.7	≥1.185	<0.0001	0.921	86.7/85.7	≥0.935	<0.0001

*p<0.05 indicates statistical significance. NA: Not analyzed (sensitivity and specificity values not presented for variables with a p value >0.05 and AUC <0.800)

similar to those reported in published studies on subclinical KC diagnosis, which were summarized as follows: 82%-90.5% and 70%-86.5% for ARTmax, 89.2% and 90.3% for Da, and 52.6%-95.5% and 32.4%-94.1% for D final (Supplementary Table S1).[3,10,11,12,13,19,20,21,22,23,24,25] On the other hand, it can be seen in Supplementary Table S1 that there were overlaps among the criteria for "subclinical KC" and "mild KC". For instance, Heidari et al.[3] included clinically normal eyes with anterior elevation >12 µm, posterior elevation >17 µm, SRAX <20, Kmax >47.2 D (but <48.7 D) and I-S value >1.4 D (but <1.9 D) at the 3-mm radii as subclinical KC, whereas these criteria practically describe mild KC without biomicroscopic findings.

However, it should be pointed out that the present study and related literature review mainly focused on the individual performance of Pentacam parameters in the diagnosis of subclinical and mild KC. Therefore, we do not discuss the topography-based multifactorial regression formulas introduced in previous studies or parameters from other imaging modalities. Nevertheless, it is obvious from the published literature that corneal epithelial imaging, corneal biomechanical measurements (i.e., CorVis ST, Oculus Inc.® and Ocular Response Analyzer, ORA, Reicherts®) and 3-D morphovolumetric analysis have significant value in diagnosing subclinical KC in addition to corneal topography/tomography.[4,5,6,23,26,27,28,29,30]

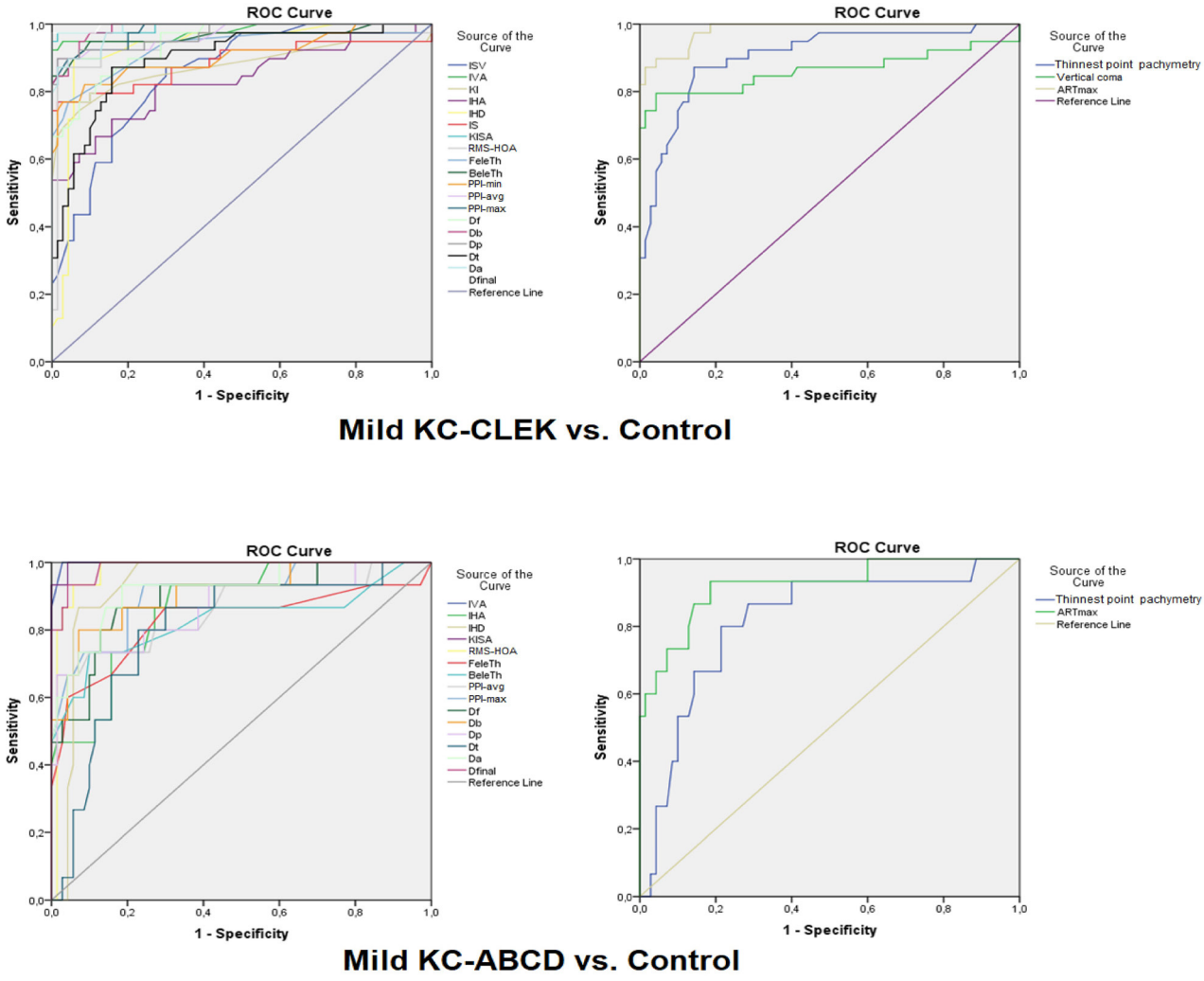

Figure 3. Sensitivity and 1-specificity values for Pentacam parameters that had an AUC value over 0.800 in distinguishing mild KC based on the CLEK (top) and Belin ABCD (bottom) classification system criteria

In terms of mild KC diagnosis, the majority of studies in the current literature used the AK stage 1 KC criteria, and excellent to good sensitivity/specificity was observed for F.Ele.Th (sensitivity/specificity: 97.8%/94.8%), B.Ele.Th (100%/99.4%), D final (98%-100%/95.9%-100%), IVA (97.8%/95.8%), KI (93.3%/97.9%), and PPI values (Supplementary Table S2).[14,22,27,29,31] These values were very similar to those found in the present study in the diagnosis of mild KC based on the AK, CLEK, and ABCD criteria (sensitivity/specificity ranged from 87.2% to 97.4%/92.9% to 100%). These results might indicate that Pentacam parameters are able to detect an eye with mild KC with high sensitivity regardless of the presence of biomicroscopic signs.

Regarding the "mild" KC-ABCD group in the current study, the ABCD system was actually developed by Belin and Duncan[18] to track KC progression, and stage 0 theoretically describes "normal" eyes. However, we detected 15 eyes with mild KC (all had typical topographical map patterns for KC and the contralateral eye had manifest KC) that were labelled as "stage 0" by the Pentacam ABCD system in our database. These eyes were also included in the present study as the "mildest" KC stage for the ABCD grading system instead of using cases with ABCD stage 1 KC, since Belin and Duncan[18] already reported that ABCD stages 1-4 were closely matched with the AK stages 1-4 in terms of anterior curvature. However, assuming "stage 0" as the mildest grade in the ABCD system may have led to the selection of milder KC cases compared to the mild KC-AK and -CLEK groups. Therefore, the diagnostic performance of the Pentacam parameters might have been underestimated in the mild KC-ABCD group. On the other hand, although the size of the mild KC-ABCD group was relatively small due to its rarity, to our knowledge there is no other study testing the diagnostic performance of Pentacam parameters in the detection of keratoconic eyes categorized as "ABCD stage 0." One exception is a study by Zhang et al.,[28] who used the ABCD stage 0 criteria as "topographic normality" for their forme fruste

KC group, which could have led to the inaccurate classification of keratoconic eyes as normal.

Study Limitations

The relatively small number of cases in the subclinical KC group might be considered a limitation of the current study. This study also did not include eyes with "forme fruste KC," which in the majority of the existing literature describes "a clinically and topographically normal eye with manifest KC in the contralateral eye." The term "forme fruste" was first proposed by Amsler[9] to define unilateral cases as an incomplete, abortive, or atypical form of KC. This conclusion was made mostly due to the fact that unilateral KC is genetically described as a form of autosomal dominant transmission with complete penetrance but partial expression, and if individuals are followed for long enough, the opposite eye may eventually show evidence of KC. In 2015, the Global Consensus on Keratoconus and Ectatic Diseases agreed that environmental, biomechanical, genetic, and biochemical anomalies all contribute to the pathogenesis of KC and true unilateral KC does not exist. However, a recent report by Saad et al.[32] presented a case of stable "unilateral KC" with the longest follow-up period of 14 years.

Conclusion

As a result of the non-uniform definitions and selection criteria employed in the literature, sensitivity and specificity values show substantial variation in the diagnosis of "subclinical KC." The current study revealed that corneal thickness-related Pentacam parameters might have value for detecting subclinical KC. However, even with this sophistication, Pentacam has modest capability in the diagnosis of subclinical KC, and further approaches such as corneal biomechanical assessment, epithelial mapping, and 3-D morphovolumetric analysis, which provide robust data on subclinical alterations in the cornea, appear to be necessary.[4,5,6,23,26,27,28,29,30]

On the other hand, this study also confirmed that Pentacam is able to detect eyes with mild KC with high accuracy, despite the fact that the most powerful parameters have varying specificities and sensitivities depending on the "mild KC" criteria used. Nevertheless, definitive and objective criteria for grading subclinical and clinical KC are essential to attain a global consensus regarding the early diagnosis and management of KC, and clinicians should follow a multi-diagnostic strategy rather than relying solely on Pentacam data prior to corneal refractive surgery.

Abbreviations

ABCD: Belin ABCD classification system, AK: Amsler-Krumeich classification, ARC: Anterior average radii of curvature, ART: Ambrósio Relational Thickness, AUC: Area under the receiver operating characteristic curve, BAD-D: Belin/Ambrósio Enhanced Ectasia Display scores (Df, Db, Dp, Dt, Da, and D final), B.Ele.Diff: Back elevation difference, B.Ele.Th: Back elevation at the thinnest point, CDVA: Corrected distance visual acuity, CLEK: Collaborative Longitudinal Evaluation of Keratoconus study, D: Diopters, F.Ele.Diff: Front elevation difference, F.Ele.Th: Front elevation at the thinnest point, HOA: Higher-order aberrations, IHA: Index of height asymmetry, IHD: Index of height decentration, I-S: Inferior-superior keratometric difference at 3-mm radii, ISV: Index of surface variance, IVA: Index of vertical asymmetry, K: Keratometry, KC: Keratoconus, KI: Keratoconus index, KISA: KC percentage index, LogMAR: Logarithm of the minimal angle of resolution, PPI: pachymetric progression index, PRC: Posterior average radii of curvature, Rmin: Minimum radius of curvature, RMS: Root mean square, SRAX: Skewed radial axes, ST-IN: Superotemporal-inferonasal asymmetry, TKC: Topographical Keratoconus Classification, TP: Thinnest point, Kmax: Maximum keratometry, SD: Standard deviation

Ethics

Ethics Committee Approval: The Pamukkale University Non-Interventional Clinical Research Ethics Committee approved the study protocol (decision no: 23, date: 08.12.2020).

Informed Consent: Retrospective study. Consent for all procedures was obtained in advance.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: İ.T., J.A., C.E.G., Design: İ.T., J.A., Data Collection or Processing: İ.T., C.M., C.E.G., Analysis or Interpretation: İ.T., J.A., Literature Search:. İ.T., C.M., C.E.G., Writing: İ.T.

Conflict of Interest: The authors declare no financial or proprietary interest in any product or company associated with any device, instrument or drug mentioned in this article.

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Original Article

The Effect of Autografts from the Inferior and Superior Bulbar Conjunctiva on the Ocular Surface in Primary Pterygium Surgery: A Cytology Study

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Abstract

Objectives: This study aimed to evaluate the effect of using an inferior or superior conjunctival autograft in primary pterygium surgery on the postoperative ocular surface.

Materials and Methods: Forty eyes of 40 patients who underwent pterygium surgery with autograft were included in the study. Cytological cell counts were performed on samples taken from the bulbar conjunctiva by impression cytology before and 1 year after the operation. Schirmer 1 test score, lissamine green conjunctival staining score, tear film break-up time (TBUT), and fluorescein corneal staining scores were evaluated. The pain levels of the patients were evaluated with visual analog scale at postoperative 1 day and 1 week.

Results: Corneal and conjunctival staining, TBUT, and Schirmer test results demonstrated significant improvement in all patient groups after surgery, but there was no difference between groups ($p>0.05$). In both preoperative and postoperative impression cytology, the number of goblet cells in the inferior bulbar conjunctiva was higher than in the superior bulbar conjunctiva ($p<0.001$), while there was no such difference in epithelial cell or mucin staining. There were no significant cytological changes postoperatively in either group ($p>0.05$).

Conclusion: Pterygium surgery with autografting improved tear function tests regardless of graft location. Goblet cell count was higher in the inferior bulbar conjunctiva than in the superior bulbar conjunctiva in both postoperative and preoperative impression cytology. However, there was no significant difference in postoperative epithelial and goblet cell counts or mucin staining between the groups before and after surgery. We think that using the inferior bulbar conjunctiva is an appropriate choice in cases where the superior conjunctiva cannot be used as a graft or when future glaucoma surgery is possible.

Keywords: Pterygium surgery, autograft, superior bulbar conjunctiva, inferior bulbar conjunctiva, impression cytology

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Introduction

Pterygium is a common corneal ocular surface disorder caused by fibrovascular tissue that spreads through the limbus from the bulbar conjunctiva to the cornea. Although it is often located in the nasal interpalpebral space, it can also occur on the temporal side.[1] Genetic predisposition plays a role in the etiology, but epidemiological studies support that ultraviolet exposure is the most important environmental factor.[2,3] Studies have shown that exposure to ultraviolet light during the first years of life has a causal relationship with pterygium development.[4,5] It is more common in occupations involving outdoor work, such as fishing and farming. Dry, hot air and a dusty environment are also accepted as having a role in the etiology due to chronic irritation.[2,3,4,5,6]

Pterygium is thought to contribute to the symptoms of irritation, mucoid discharge, and dryness often experienced.[3] Abnormal tear film and meibomian gland dysfunction cause dry eye symptoms in patients with pterygium and improve with successful surgical treatment.[7]

Impression cytology of the conjunctival surface is a relatively non-invasive and repeatable procedure. It provides information on cell morphology, cell types, and the topographic cell-cell relationship and is widely used in studies of ocular surface disorders, including dry eye.[8] Therefore, we planned to use this technique to determine the effect of obtaining grafts from the superior or inferior conjunctiva on cellular changes at the donor site.

The first record of pterygium surgery is by the Indian ophthalmologist Sustura in 1000 B.C. Since then, surgery has been the primary treatment.[9] To date, many different methods and surgical techniques have been used, including radiation.[1] The naked sclera technique is among the surgical techniques that has waned in popularity in recent years due to the high recurrence rate, but successful results can be obtained when combined with conjunctival autografting. Although mitomycin C, 5-fluorouracil, and other agents are used as adjuvant therapy to lower recurrence rates, close follow-up is still required for complications.[10] Application of a limbal autograft to the scleral bed after pterygium excision is currently the method that yields the lowest reported recurrence rates.[11,12,13,14] In studies comparing recurrence between conjunctival autograft and amniotic membrane, the results have been similar or better with conjunctival autograft.[15,16] However, there is no clear consensus regarding the use of inferior or superior limbal autografts. A few studies in the literature evaluated the effect of obtaining an inferior or superior autograft on surgical success and tear function tests.[17,18,19] However, these studies have not investigated impression samples. To the best of our knowledge, this is the first cytologic study to evaluate the effect of obtaining autografts from the inferior or superior bulbar conjunctiva on the postoperative ocular surface and the success of primary pterygium surgery.

Materials and Methods

Patients with pterygium who were treated at the University of Health Science Ulucanlar Eye Training and Research Hospital and consented to the planned surgery were included in the study between May 2018 and May 2019. The protocol was approved by the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (decision number E-18-2449, dated 18/04/2018). The study was conducted in accordance with the rules of the Declaration of Helsinki.

The study included patients with no systemic or ocular disease that could cause secondary pterygium by disrupting the ocular surface. Patients who had previous ocular surgery, pseudo-pterygium due to ocular trauma or chemical burn, used topical drugs for conditions such as glaucoma or uveitis, or used topical/systemic steroids or non-steroidal anti-inflammatory drugs were not included in the study. All patients in the study voluntarily signed an informed consent form.

Ocular Surface Examination

All participants underwent best corrected visual acuity (BCVA) test with Snellen chart and slit-lamp examination of the cornea, conjunctiva, and eyelids preoperatively and at 1 year postoperatively (Figure 1). Translucency of the pterygium tissue was classified according to the study by Prabhasawat et al.[20] Grade 1 (atrophic) is more transparent and the episcleral vessels below can be distinguished, while grade 3 is thick and opaque, and the underlying vessels are not visible. Grade 2 is between these two groups. The preoperative and postoperative tear amounts of the patients were measured by using the Schirmer 1 test. Tear film break-up time (TBUT) and corneal epitheliopathy were evaluated with fluorescein staining, and conjunctival staining was performed with lissamine green.

The Schirmer 1 test was performed without topical anesthetic drops. Standardized Schirmer strips were bent in the notch and carefully placed on the lower lid edge. During the test, the patient was instructed to keep the eyelids closed. The strips remained in place for 5 min or until they were completely saturated with tears. After 5 min, the degree of moistening of the strips was measured using a millimeter scale on each strip. To evaluate TBUT, a fluorescent strip (fluorescein paper, Haag-Streit AG, Köniz, Switzerland) was applied to the inferior conjunctival fornix following a drop of balanced salt solution (Alcon Laboratories, Inc., Fort Worth, TX, USA). After normal blinking for a few seconds to spread the fluorescein, the ocular surface was examined under the cobalt blue-filtered light of the slit-lamp biomicroscope and TBUT was recorded as the time (in seconds) from the last blink to the appearance of the first break in the tear film. The procedure was repeated three times for each eye. After TBUT measurement, corneal staining with fluorescein was evaluated according to the Oxford scheme, which consists of a sequence classified as A-E in order of increasing severity.[21] Then, a strip with 1.5 mg of lissamine green (Ophtechnics, Haryana, India) was placed in the lower lid margin as far temporally as possible, and conjunctival staining was evaluated. Staining scores of the cornea, temporal conjunctiva, and nasal conjunctiva according to the Oxford scheme were recorded for each case.[21] Staining was graded by comparing the dots on the Oxford chart to the exposed interpalpebral conjunctiva and cornea of the patient (Figure 2).[21,22] Mean Oxford staining scores were compared between the groups. All ocular surface assessments and impression sampling were performed by the same ophthalmologist.

All surgeries were performed by the same experienced surgeon in the same operating room. Autografts were randomly taken from the superior or inferior conjunctiva, adjacent to the limbus, from an area more than 90° away from the pterygium area.

Impression Cytology Method

Samples for impression cytology were obtained by instilling a single drop of local anesthetic, waiting with the eye closed for

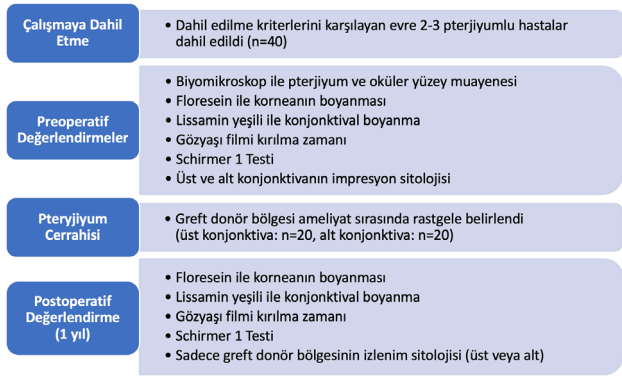

Figure 1. Flow chart of study methodology

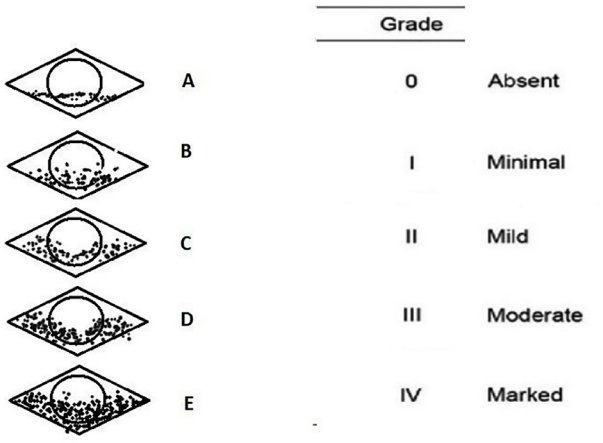

Figure 2. Grading of corneal and conjunctival staining (Oxford scheme)

15-20 seconds, then applying a piece of cellulose acetate filter paper to the conjunctival surface. The samples were placed in 96% ethanol and transferred to the cytology laboratory.

The impression samples were stained and visualized as described by Rivas et al.,[23] with a few modifications. In brief, the procedure was as follows: 1) fixation in 96% ethanol; 2) washing in distilled water for 5 min; 3) applying periodic acid for 5 min; 4) washing in distilled water for 5 min; 5) applying Schiff reagent for 5-10 min; 6) rinsing in tap water, followed by staining with Harris hematoxylin for 1 min; 7) rinsing in distilled water, followed by dehydration in increasing alcohol series; 8) clearing the filter paper with xylol; and 9) covering the sample with Entellan new rapid mounting medium (107961; Merck, Germany). Images were obtained using a Zeiss Axio Scope A1 microscope (Carl Zeiss, Oberkochen, Germany). Samples were evaluated for epithelial and goblet cells by a researcher who was blinded to which group the samples belonged to. Impression cytology specimens were graded as normal or abnormal for epithelial cell density, goblet cell density, and mucin spots (goblet cell secretions).[23,24]

Cells were classified as type 1 epithelial cells with eosinophilic cytoplasm, type 2 goblet cells with basophilic cytoplasm, and type 3 mucin spots that stained eosinophilic.[24,25,26,27] After the samples were digitally recorded using the Image J processing program (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018), the nuclear-to-cytoplasmic (N/C) ratio and cell density (cells/mm^2) were calculated.

Since the graft placement decision was made randomly during the operation, preoperative impression cytology samples were obtained from both the inferior and superior conjunctiva from all patients. At postoperative 1-year follow-up, cytology samples were taken only from the graft site.

Postoperative treatment was the same for both groups. Topical 0.5% moxifloxacin (Moxai, Abdi İbrahim, Türkiye) and 0.5% loteprednol etabonate (Dolte, Abdi İbrahim, Türkiye) were administered 6 times a day for 2 weeks, followed by 1 drop 4 times a day for the next 2 weeks. The patients were advised to refrain from scratching their eyes after surgery, use sunglasses outdoors, and avoid air-conditioning, dusty, and dirty environments. Follow-up examinations were performed at postoperative 1 day, 1 and 6 months, and 1 year. The patients' ocular pain levels were evaluated using the visual analog scale (VAS) at postoperative 1 day and 1 week.[19] The VAS is a pain measurement tool consisting of a linear scale between 0 and 10 cm, where 0 indicates no pain and 10 indicates the worst pain imaginable. Patients were asked to mark the line with an "X" to indicate pain intensity and the score was determined using a 10.0-point scale. The mean VAS scores of the patients in the inferior and superior graft groups were compared. A flow chart of study methodology is shown in Figure 1.

Surgical Procedure

The surgical procedure was performed under subconjunctival local anesthesia. The pterygium head was lifted and dissected from the corneal surface. The pterygium head and the body tissue were then resected from the underlying sclera 4 to 5 mm from the limbus and after dissection of subconjunctival fibrous tissue, a bare scleral bed was left. The defect area was covered with a free limboconjunctival autograft moved from the superior or inferior bulbar conjunctiva and free from the Tenon capsule. The graft was secured at the limbus and peripherally to the surrounding conjunctiva and episclera using 8-0 Vicryl sutures. Mitomycin C was not used in the surgeries.

Statistical Analysis

The sample size was determined based on a type 1 error rate (α) of 0.05, power of 80%, and effect size (Cohen's d) of 0.8. We determined that at least 20 participants would need to be assigned to each group for a two-tailed t-test analysis.

SPSS software (version 25.0, IBM, Armonk, NY, USA) was used for the statistical analysis. Descriptive statistics, including mean, standard deviation, and range, were calculated for different variables. In all patient groups, TBUT, Schirmer's 1 test, and corneal and conjunctival staining were evaluated using the paired samples t-test. Statistical significance was set at $p<0.05$. Compliance of the preoperative and postoperative cytology data to normal distribution was investigated with the Kolmogorov-Smirnov test. The results indicated non-normal distribution for all cell types. Therefore, the Wilcoxon test was used to compare pre- and postoperative type 1, type 2, and type 3 cells in the same patient. The Mann-Whitney U test was performed to determine if there was a statistically significant difference between the two groups.

Results

In our study group, the mean age was 53.6±11.2 years (range: 35-74 years) and 65% (n=26) of the patients were male. Pterygium affected the right eye in 18 patients (45%) and the left eye in 22 patients (55%). Twelve patients were grade 2 (30%) and 28 were grade 3 (70%). The distributions of age, gender, and pterygium severity were equal in both groups (Table 1). The mean preoperative BCVA (in Snellen decimal) was 0.89±0.17 (range: 0.5-1) in the superior graft group and 0.88±0.16 (range: 0.6-1) in the inferior graft group. There was no significant

difference between the two groups (p=0.8). Postoperative BCVA was 0.98±0.04 (range: 0.9-1) in the superior graft group and 0.97±0.06 (range: 0.8-1) in the inferior graft group (p=0.9). The difference between preoperative and postoperative BCVA in the groups was 0.09±0.13 (range: 0-0.4) and 0.05±0.08 (range: 0-0.2), respectively. There was no statistically significant difference between the groups (p=0.56). Mean VAS pain scores on day 1 were 6.2±1.3 (range: 4-8) in the superior graft group and 4.7±1.88 (range: 4-8) in the inferior graft group (p=0.01). On day 7, the scores were 1.5±0.8 (range: 0-3) and 1.3±0.9 (range: 0-3), respectively (p=0.6).

Corneal and conjunctival staining, TBUT, and the Schirmer 1 test showed significant improvement after surgery in both patient groups (Table 2). When the pre-to postoperative changes in these parameters were compared between the superior and inferior graft groups using the Mann-Whitney U test, no significant differences were observed (p>0.05).

Three types of cells were observed in the stained samples. These were epithelial cells with eosinophilic cytoplasm (type 1), goblet cells with basophilic cytoplasm (type 2), and mucin spots with stained eosinophilic cytoplasm (type 3). In between-group comparisons, the preoperative epithelial cell count was 4.13±4.56 in the superior bulbar conjunctiva and 3.53±3.96 in the inferior bulbar conjunctiva, but this difference was not statistically significant (p=0.719). Postoperative values were 4.25±4.79 and 3.06±3.44, respectively (p=0.557). There were significantly more goblet cells in the inferior bulbar conjunctiva than the superior bulbar conjunctiva both postoperatively and preoperatively (Figure 3, Table 3). No significant differences were observed between the groups in terms of preoperative and postoperative epithelial cell numbers and eosinophilic mucin spots (Table 3). In within-group comparisons, neither group showed any significant cytological changes between the pre- and postoperative 1-year assessments (p>0.05) (Table 4).

Complications such as bleeding and graft necrosis were not observed during the operation or postoperatively. Pterygium size was not measured; autograft size varied in each case but was approximately 4x5 mm. Graft healing occurred in all patients with no redness and discomfort at 1-month follow-up. Pterygium recurrence and unsatisfactory cosmesis was not observed in any of the patients, and no negative feedback was received from the patients at postoperative 6-month follow-up.

Table 1. Demographic distribution of patients in the superior and inferior graft groups

	Superior graft group (n=20)	Inferior graft group (n=20)	p value
Age (years) (mean ± SD)	54.68±10.3	52.52±13.5	0.7*
Gender (male/female) (n)	12/8	14/6	0.410**
Grade 2 pterygium (n)	6	6	0.465**
Grade 3 pterygium (n)	14	14	

*Independent-samples t-test, **Chi-square test, SD: Standard deviation, n: Number of patients

Discussion

In this study, the pronounced corneal and conjunctival staining observed before pterygium surgery significantly regressed and the low TBUT and no-anesthesia Schirmer test values significantly increased postoperatively, independent of whether the autograft was taken from the inferior or superior conjunctiva. This may be attributed to the tear instability and dry eye findings caused by the ocular surface irregularity associated with pterygium and the subsequent improvement in the ocular surface after pterygium surgery with autografting.

A variety of results have been reported in the literature regarding the effect of pterygium on tear function tests. Ergin and Bozdoğan[28] indicated that pterygium had no abnormal effect on tear function tests in their study of 56 patients. In contrast, a study by Ozsutcu et al.[29] including 65 unilateral pterygium patients and their fellow eyes as a control group revealed significant differences in TBUT, Schirmer test, and corneal staining. They concluded that these differences between the two eyes of the same patients were related to pterygium.[29] In our study, we demonstrated that pterygium causes deterioration in tear function tests. These discrepancies in the results of ocular surface tests between different studies may be a result of genetic and environmental factors.

Table 2. Comparison of preoperative and postoperative Oxford staining score (corneal and conjunctival), TBUT, and Schirmer I values in the superior and inferior graft groups

		Preoperative mean ± SD	Postoperative mean ± SD	p*
Superior graft group (n=20)	Corneal Oxford staining score	1.0±0.7	0.24±0.4	**0.0001**
	Conjunctival Oxford staining score	1.7±0.7	0.18±0.4	**0.0001**
	TBUT (s)	4.2±1.4	7.0±1.6	**0.0001**
	Schirmer I (mm)	15.5±5.2	17.0±4.5	**0.003**
Inferior graft group (n=20)	Corneal Oxford staining score	0.7±0.7	0.3±0.5	**0.02**
	Conjunctival Oxford staining score	1.5±0.7	0.06±0.3	**0.0001**
	TBUT (s)	4.0±1.6	6.8±1.9	**0.0001**
	Schirmer I (mm)	15.1±4.1	17.5±2.7	**0.018**

*Wilcoxon test. Significant p values (<0.05) shown in bold. TBUT: Tear film break-up time, n: Number of patients, SD: Standard deviation

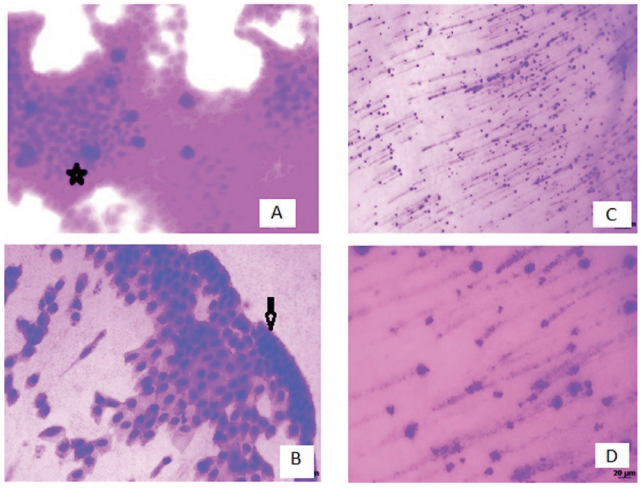

Figure 3. Impression cytology samples obtained preoperatively (A) and postoperatively (B), stained with periodic acid-Schiff and hematoxylin. The arrow (↓) indicates epithelial cells, the star (★) indicates goblet cells. C, D) Goblet cell secretion without goblet and epithelial cells (400X magnification in panels A, B, and D, 100X in panel C)

Table 3. Comparison of impression cytology values between the superior and inferior graft groups

Cell density (count/mm²)	Superior graft group (n=20) mean ± SD (range)	Inferior graft group (n=20) mean ± SD (range)	p*
Preop type 1 cells	4.13±4.56 (0-15)	3.53±3.96 (0-12)	0.719
Preop type 2 cells	354.9±101.6 (278-698)	476.6±151.6 (310-867)	**0.001**
Preop type 3 cells	125.9±138.4 (14-450)	79.9±56.5 (11-225)	0.705
Postop type 1 cells	4.25±4.79 (0-14)	3.06±3.44 (0-10)	0.557
Postop type 2 cells	329.3±52.4 (247-440)	480.2±183.3 (312-1015)	**0.0001**
Postop type 3 cells	119.6±147.1 (3-455)	98.1±86.1 (12-300)	0.801

*Mann-Whitney U test. Significant p values (<0.05) are shown in bold. Preop: Preoperative, Post op: Postoperative, SD: Standard deviation

Li et al.[7] examined tear film instability, and tear function parameters after pterygium surgery and demonstrated that tear film abnormality and meibomian gland dysfunction improved significantly after surgery. They emphasized that the thickness and size of the pterygium layer were significant in preoperative symptom severity.[7] Most of our patients (67.7%) had grade 3 pterygium, which is thicker and wider. The marked recovery of tear function at 6-month follow-up was associated with improvement of the ocular surface. A recent systematic review by Linaburg et al.[6] analyzing 59 studies indicated that abnormal tear function tests improve after pterygium surgery. However, the effect of autograft donor site on tear function and recurrence was not examined in this review. The results of our study showed

Table 4. Comparison of pre- and postoperative impression cytology values within the superior and inferior graft groups

Cell density (count/mm²)	Cell type	Preoperative mean ± SD (range)	Postoperative mean ± SD (range)	p*
Superior graft group (n=20)	Type 1	4.13±4.56 (0-15)	4.25±4.79 (0-14)	0.672
	Type 2	354.9±101.6 (278-698)	329.3±52.4 (247-440)	0.178
	Type 3	125.9±138.4 (14-450)	119.6±147.1 (3-455)	0.187
Inferior graft group (n=20)	Type 1	3.53±3.96 (0-12)	3.06±3.44 (0-10)	0.06
	Type 2	476.6±151.6 (310-867)	480.2±183.3 (312-1015)	0.46
	Type 3	79.9±56.5 (11-225)	98.1±86.1 (12-300)	0.47

*Wilcoxon test, n: Number of patients, SD: Standard deviation

that tear function tests improved after surgery, but autograft location had no effect on these parameters. However, Linaburg et al.[6] suggested in their meta-analysis study that the use of an inferior conjunctival autograft may be more advantageous in people with ocular surface disease.

In our study, we did not detect any abnormality in goblet cell density or epithelial morphology at the autograft donor site pre- or postoperatively on impression cytology examination. In addition, we detected extensive mucin spots, as described by Egbert et al.[26] These are considered secretions from goblet cells that adhere to the impression paper. No significant difference was demonstrated in these factors pre- or postoperatively according to graft site. This indicates that graft removal does not cause any changes at the donor site. Moreover, no pterygium recurrence was observed in either the inferior or superior autograft group. However, we observed higher goblet cell density the inferior bulbar conjunctiva compared to the superior bulbar conjunctiva. Rivas et al.[23] investigated the topographic distribution of goblet cells with impression cytology and reported densities of 331±148/mm² in the superior bulbar conjunctiva and 427±112/mm² in the inferior bulbar conjunctiva. Although Chan et al.[8] demonstrated that goblet cell density increased with squamous metaplasia in pterygium tissue, a decrease in goblet cell density was observed in studies by Safarzadeh et al.[24] and Julio et al.[30] Labbé et al.[31] explained that the change in the number of goblet cells is related to pterygium activity.

Mucin is secreted by goblet cells and plays an important role in lubrication, ocular surface wetness, and the prevention of microbial infections. Mucin is known to play a role not only in the integrity of the tear film layer but also in the epithelial homeostasis of the ocular surface through its anti-inflammatory and antimicrobial activity.[32] Conjunctival autografts are primarily preferred for ocular surface reconstruction. However, nasal mucosal grafts, which also contain goblet cells, can be

used in appropriate cases and have been shown to maintain their effectiveness even in the long term.[33,34]

Li et al.[17] reported that there was no significant difference in pterygium recurrence with inferior and superior autografts in their recent meta-analysis of randomized controlled studies with a follow-up period of more than 6 months. Our results are consistent with this. However, the inferior bulbar conjunctiva has been preferred over the superior bulbar conjunctiva in patients with superior conjunctival scars, a history of glaucoma surgery, or the possibility of undergoing glaucoma surgery.[35,36] Similar to the results of our study, other researchers have also reported that less early postoperative pain and discomfort were seen in patients who received inferior autografts.[18,19] Zloto et al.[37] attributed this to the greater range of motion in the upper eyelid than the lower eyelid, which might produce more ocular surface inflammation and delay healing of the corneal epithelium. The superior bulbar conjunctiva cannot be used in glaucoma patients who are candidates for glaucoma filtration surgery or patients who have scarring in the superior bulbar conjunctiva.[32] In patients who have already undergone glaucoma filter surgery, the graft donor site should be a suitable distance from the surgical site to avoid impairing bleb function. Undiagnosed and late-recognized glaucoma cases are common worldwide, especially in Africa and Asia.[38] Therefore, preserving the superior conjunctiva seems more beneficial to patients in both the short and long term.

Strong points of our study are all surgeries were performed by the same surgeon and in the same environment, and the preoperative and postoperative parameters were evaluated by blinded researchers who did not know which patient was in which group. In addition, to the best of our knowledge, this study is the first cytology study published in the literature to evaluate the effect of using inferior or superior conjunctival autografts on the ocular surface and surgical success in primary pterygium surgery.

Study Limitations

The most important limitation of our study is the small number of patients. We preferred the first year for the last control of patients because pterygium recurrence is frequently observed at around 6 months postoperatively.[17] Although we planned to perform impression cytology at the 1-year visit, some of the patients from whom we took initial samples did not come for follow-up. As a result, the number of patients was lower than we originally planned.

Conclusion

In this study, preoperative impression cytology demonstrated a higher goblet cell density in the inferior conjunctiva than in the superior, and the conjunctiva retained its goblet cell content regardless of whether a superior or inferior conjunctival autograft was used in pterygium surgery. As a result, we think that inferior limboconjunctival grafts should be preferred because they have the goblet cell density to promote surface reconstruction, and this approach preserves the superior conjunctiva for future glaucoma surgery or avoids impairing bleb function in patients with a history of filtration surgery. Studies with larger patient numbers and longer follow-up may provide more detailed information.

Ethics

Ethics Committee Approval: The protocol was approved by the Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (decision number E-18-2449, dated 18/04/2018).

Informed Consent: Obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K., F.Ç.E., Concept: B.K., E.Ş., Design: B.K., F.Ç.E., İ.İ., B.S., Data Collection or Processing: B.K., F.Ç.E., İ.İ., Analysis or Interpretation: B.K., F.Ç.E., İ.İ., E.Ş., B.S., Literature Search: B.K., F.Ç.E., İ.İ., Writing: B.K., F.Ç.E., İ.İ.

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Original Article

Expression Analysis of the Small GTP-Binding Protein Rac in Pterygium

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Abstract

Objectives: To determine the roles of small GTP-binding proteins Rac1, Rac2, and Rac3 expression in pterygial tissue and to compare these expressions with normal conjunctival tissue.

Materials and Methods: Seventy-eight patients with primary pterygium were enrolled. Healthy conjunctival graft specimens obtained during pterygium surgery were used as control tissue. The real-time polymerase chain reaction method on the BioMark HD dynamic array system was utilized in genomic mRNA for the gene expression analysis. Protein expressions were analyzed using western blot and immunohistochemical methods.

Results: *RAC1, RAC2,* and *RAC3* gene expressions in pterygial tissues were not markedly elevated when compared to the control specimens ($p>0.05$). As a very low level of *RAC1* gene expression was observed, further protein expression analysis was performed for the Rac2 and Rac3 proteins. Western blot and immunohistochemical analysis of Rac2 and Rac3 protein expression revealed no significant differences between pterygial and healthy tissues ($p>0.05$).

Conclusion: This is the first study to identify the contribution of Rac proteins in pterygium. Our results indicate that the small GTP-binding protein Rac may not be involved in pterygium pathogenesis.

Keywords: Pterygium, Rac, gene expression, protein expression, immunohistochemical analysis

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Introduction

Pterygium is a benign and common ocular surface disease characterized by abnormal conjunctival fibrovascular tissue growth on the cornea. Pathologically, pterygium is a proliferative, invasive, and highly vascularized tissue. It is generally accepted that pterygium is a conjunctival degenerative and proliferative disorder. Due to the presence of various common features between pterygium and neoplasia, pterygium is also considered as a neoplastic-like growth lesion.[1] Many environmental factors such as inflammation, ultraviolet irradiation, and chronic irritation have been postulated to be causative factors.[2] Genetic factors are also important in the etiology of pterygium.[3] However, the exact molecular mechanisms of pterygium development are not fully understood. Accumulating evidence indicates that many growth factors may contribute directly or indirectly to the pathogenesis of pterygium.[4] Some studies have reported that cyclo-oxygenase (COX), vascular endothelial growth factor (VEGF), and various proinflammatory cytokines are associated with the development and formation of pterygium.[5,6,7]

Low molecular weight (20-30 kDa) small GTPases are monomeric G-proteins that bind guanine nucleotides and hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate. The human Ras superfamily of small GTPases consists of 166 members, which is subdivided into five major subfamilies (Rho, Ras, Arf/Sar, Rab, Ran) and "unclassified" proteins based on their functional and sequence similarities.[8] The Rho family of small GTPases contains 20 members including Rac1, Rac2, and Rac3. The three distinct mammalian Rac isoforms, mapped by different genes, share between 89% and 92% identity in their respective amino acid sequences.[9] It has been reported that Rho GTPase activates proteins involved in VEGF-induced cell migration and that VEGF signaling

requires Rac activation during chemotaxis.[10,11] Rac activation also induces an increase in endothelial cell focal adhesion and stress fiber formation.[11] Rac activity is crucial for efficient cell movement and migration.[12,13] These effects may contribute to the development of the wing-like or triangular-shaped tissue growth of the conjunctiva tissue in pterygium. The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme present in non-phagocytic and phagocytic cells is a Rac-regulated complex that generates reactive oxygen species (ROS) for the purposes of intracellular signaling and innate immunity.[14] Since Rac proteins can regulate the oxidative burst in phagocytic cells and are involved in ROS production, Rac may contribute to the inflammatory processes of pterygium development.[15,16] Although Rac family members may have several roles in certain cellular functions, their roles in pterygium pathophysiology remain uncharacterized. Therefore, the goal of this research was to assess the expression of the small GTP-binding proteins Rac1, Rac2, and Rac3 in pterygial tissue.

Materials and Methods

Participants

This prospective study was performed in the Ophthalmology Department of Gaziantep University Hospital and the Ophthalmology Clinic of the Gaziantep Dr. Ersin Arslan Training and Research Hospital. The study was approved by the Gaziantep University Local Clinical Ethics Committee (decision no: 2017/312, date: 11.09.2017), and the tenets of the Declaration of Helsinki were adhered to throughout this study. Pterygium specimens were collected during surgery from 78 consecutive patients with primary pterygium (40 male and 38 female). Normal conjunctival tissue samples from the superior temporal bulbar conjunctiva were taken during pterygium excision surgery with conjunctival autograft transfer.[17] Each patient underwent routine eye examinations. Inclusion criteria were as follows: (1) age 18 years or above; (2) presence of primary grade 2 or 3 pterygium; and (3) enrolling in the study voluntarily and providing informed written consent.

Exclusion criteria were as follows: (1) history of any previous ocular surgery such as pterygium excision, vitrectomy, trabeculectomy, cataract extraction, and squint surgery; (2) history of trauma such as chemical injury or conjunctival laceration within the last three months; (3) presence of other conjunctival pathology or corneal pathology; (4) presence of other ocular surface disease such as Sjogren syndrome; (5) presence of infection such as conjunctivitis; (6) history of topical medication use such as immunosuppressants, mitomycin C, or corticosteroids; and (7) current or previous contact lens use.

Gene Expression Analysis

Total RNA was isolated from the tissues using the miRNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's instructions. The purity and concentration of RNA were measured by spectrophotometrically (Epoch, BioTek, Winooski, VT, USA). cDNA synthesis from RNA was performed using the Ipsogen RT Kit (Qiagen, Hilden, Germany) and the protocol recommended by the manufacturer. Polymerase chain reaction measurements with *RAC* primers were done using the BioMark HD system (Fluidigm, South San Francisco, CA, USA). Expression of each gene was measured and messenger RNA (mRNA) expression was determined. β-actin *(ACTB)* was used as a housekeeping gene for internal control. Relative mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method, according to the formula: $\Delta\Delta C_t = \Delta C_{tRAC} - \Delta C_{tACTB}$, where C_t = threshold cycle.[17]

Western Blot Analysis

Frozen tissue samples were homogenized in HEPES buffer using a tissue homogenizer (Tissue Lyser LT, Qiagen, Hilden, Germany), and stored at -80 °C until analysis. Protein concentrations were determined using the Bradford method (Thermo Fisher Scientific, IL, USA). The protein samples were incubated with a sample buffer (5 µL) and HEPES buffer and heated for 5 min at 95 °C. Then, 20 µg of proteins from the tissue specimens were run in 10% sodium dodecyl sulfate polyacrylamide gels and electroblotted onto polyvinylidene difluoride (PVDF) membranes at 4 °C overnight. After proper blocking with non-fat dry milk and washing, the membranes were treated overnight with primary antibodies to Rac2 (sc-517424, SantaCruz Biotechnology, Dallas, TX, USA, 1/300) or Rac3 (ab124943, Abcam, Cambridge, UK, 1/1000) at 4 °C. β-actin (sc-47778, SantaCruz Biotechnology, Dallas, TX, USA, 1/1000) antibody was utilized as a loading control. Then secondary antibodies were incubated with the PVDF membranes for 90 minutes at room temperature (anti-mouse immunoglobulin G [IgG] for anti-Rac2, sc-516102, SantaCruz Biotechnology, Dallas, TX, USA, 1/1000, or goat anti-rabbit horseradish peroxidase for anti-Rac3, ab6721, Abcam, UK, 1/3000). The antibody-reactive bands were visualized using enhanced chemiluminescence signals (Super Signal West Pico, cat. no. 34080, Thermo Fisher Scientific, IL, USA). The densities of the bands were recorded using a gel image analysis system (ChemiDoc XRS+ Imager, Bio-Rad, Hercules, CA, USA), then normalized according to β-actin levels.[17]

Immunohistochemical Analysis

To perform immunohistochemical studies, formalin-fixed and paraffin-embedded tissue blocks were sectioned into 5-µm slides using a microtome. Rac2 (PA5-29281, polyclonal rabbit IgG, Thermo Fisher Scientific, Rockford, USA, 1/100) and Rac3 (EPR6679B, rabbit monoclonal anti-Rac3 antibody, Abcam, Cambridge, USA, 1/100) antibodies were applied using an automated immunohistochemistry-staining device (Ventana, Bench Mark Ultra Auto-Stainer, Roche Diagnostics, IN, USA). Intensity of Rac2 and Rac3 immunoreactivities were scored on a 0-3 rating scale. A single researcher (Ö.E.) scored all samples for consistency. Intensity of staining was graded as follows: 0, <10% and accepted as negative; 1+, 11 to 20%; 2+, 21 to 75%; 3+, >75%.[17]

Statistical Analysis

Data were presented as mean ± standard error of the mean or percentage. The Kolmogorov-Smirnov test was applied to assess for data normality. The unpaired Student's t-test or Mann-Whitney U test was utilized to compare the means of two groups as appropriate. QIAGEN GeneGlobe online program (http://www.qiagen.com/geneglobe) was used for gene expression analysis. All results were presented as fold changes, with values between 0.001 and 0.5 considered significant downregulation and values above 2.0 considered significant upregulation.[18] The Mann-Whitney U test was utilized to identify marked differences between immunohistochemical scores. Correlations were determined using the Spearman rank correlation test. Statistics were performed using GraphPad Instat (version 3.05, GraphPad Software Inc., San Diego, CA, USA). Differences with a p value less than 0.05 were considered statistically significant.

Results

Among the 78 pterygium patients, there were 40 men (51.3%) and 38 women (48.7%), demonstrating approximately equal gender distribution. The mean age of the patients was 52.4±1.4 years (range, 29-72 years). There were no marked differences in *RAC1, RAC2,* and *RAC3* gene expressions in pterygial tissues when compared to the controls (n=30, Figure 1, p values were 0.819, 0.326, and 0.112 for *RAC1, RAC2,* and *RAC3*, respectively). Since a very low level of *RAC1* expression was observed, further analyses were performed with the Rac2 and Rac3 proteins. In the western blot analysis, there were no marked differences in Rac2 and Rac3 protein expression in pterygial tissues when compared to controls (n=30, p values were 0.330 and 0.309 for Rac2 and Rac3, respectively, Figure 2).

The immunohistochemical data revealed weak staining for Rac2 (Figure 3A, B) and Rac3 (Figure 3C, D) mainly localized to the pterygium epithelial and capillary endothelial cells. Almost no stromal cell staining was observed in pterygial tissue. However, these cells were also stained with the Rac2 or Rac3 antibodies in the control samples (Figure 3). Although there was a trend for Rac2 or Rac3 upregulation in pterygial tissue, these increases did not show statistical significance (p values were 0.2113 and 0.2524 for Rac2 and Rac3, respectively, Figure 4).

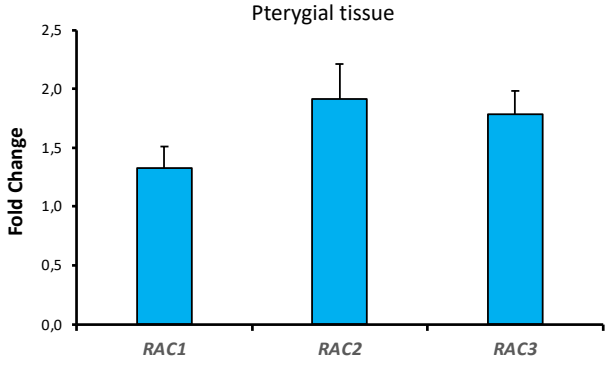

Figure 1. Comparison of the *RAC1, RAC2*, and *RAC3* gene expressions in pterygial tissues (n=30). Values are given as mean ± SEM
SEM: Standard error of the mean

Correlation analysis revealed positive correlation between *RAC1* and *RAC2* gene expressions (r=0.606, r^2=0.367, p<0.001) and between *RAC2* and *RAC3* gene expressions (r=0.367, r^2=0.135, p=0.046). No significant correlations were detected between gene and protein expressions (p=0.239 for Rac2, p=0.609 for Rac3). There was also no marked correlation between Rac2 and Rac3 protein expressions (r^2=0.012, p=0.531).

Discussion

In the present study, we observed that *RAC1, RAC2,* and *RAC3* gene expression was not markedly modified in primary pterygium specimens when compared to normal conjunctival tissues. Additionally, no elevation in protein expression was observed. These findings suggests that gene expression and posttranslational Rac protein formation are not involved in pterygium development or the regulation of pterygium growth across the ocular surface. Although several studies have reported that cellular immunity and inflammatory response play a crucial role in pterygium formation,[19,20] our results do not support the idea that Rac proteins participate in the inflammatory process of pterygium development.

Rac activity is essential for cell movement.[12] Rac is generally accepted to be a regulator of initial cell-cell contact,

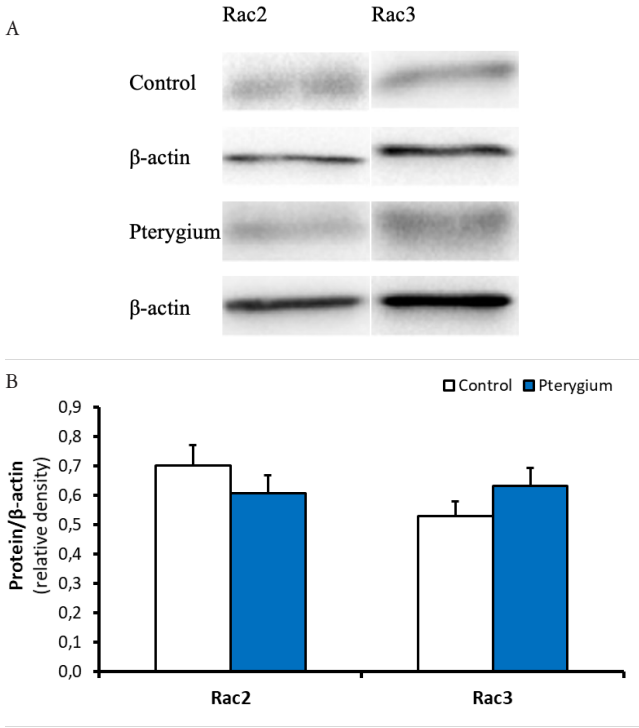

Figure 2. Representative western blotting bands (A) and comparison of Rac2 and Rac3 protein expression (B) in conjunctival autograft (control, n=36) and pterygial tissues (n=38). Values are given as mean ± SEM
SEM: Standard error of the mean

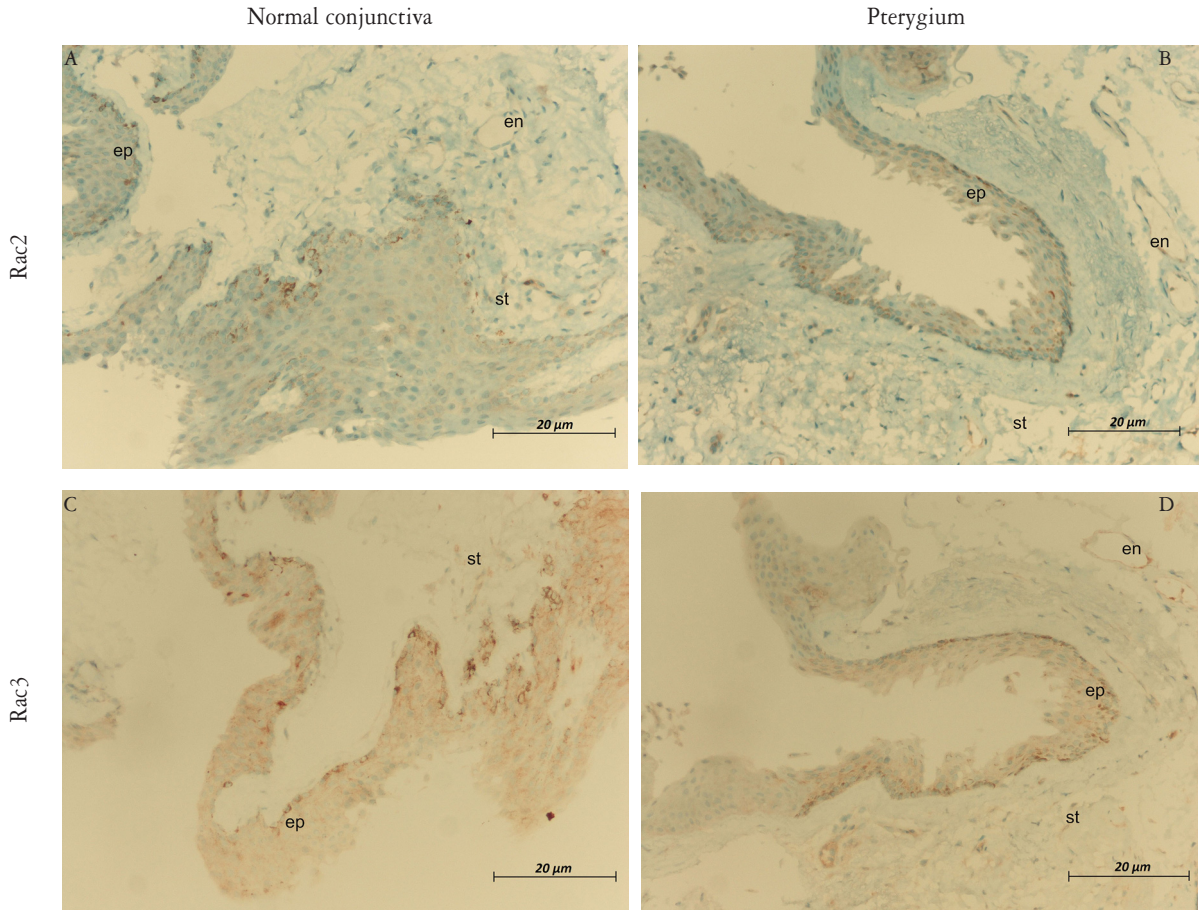

Figure 3. Immunohistochemical images of Rac2 and Rac3 staining. Immunostaining of Rac2 (top) and Rac3 (bottom) in human normal conjunctiva (left) and pterygium (right)
ep: Epithelium, en: Endothelium, st: Stroma

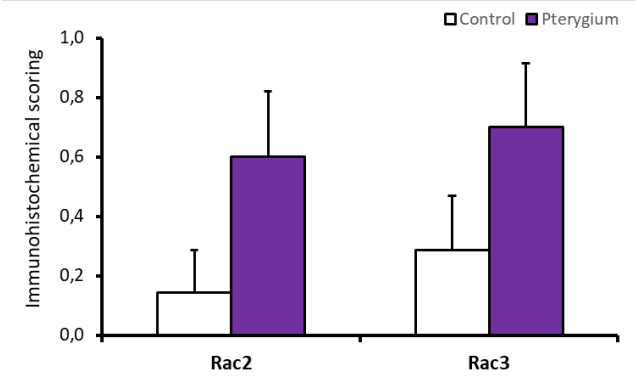

Figure 4. Comparison of the immunohistochemical scores for Rac2 and Rac3 protein expression in conjunctival autograft (control, n=7) and pterygial tissues (n=10). Values are given as mean ± SEM
SEM: Standard error of the mean

cell-matrix adhesions, and cellular transformation.[21,22,23] Rac is known to stimulate lamellipodia and membrane ruffles in fibroblasts, and Rac signaling is essential for efficient cell migration.[13] It is known that fibroblasts involved in the scarring and fibrosis processes may contribute to the progression of pterygium.[24] Rac is thought to modulate the development of lamellipodial extensions in epithelial cells and facilitate interactions between adjacent cells.[21,22] Rac GTPases are also important to the maintenance or establishment of polarity in chemotactic migration.[25] Thus, Rac might control cell polarization and migration.[12] Rac is required for lamellipodium extension triggered by cytokines, extracellular matrix components, and growth factors.[12] All these features of Rac could contribute to the wing-shaped growth of conjunctival tissue onto the cornea seen in pterygium.

Rac1, the best-investigated Rac isoform, modulates gene expression, intercellular adhesion, cell cycle progression, the organization of the actin cytoskeleton to aid cell spreading and membrane ruffling.[26] The expression of Rac proteins differs considerably in terms of level and tissue distribution. Rac1 and Rac3 are ubiquitously expressed in different tissues and therefore modulate a wide variety

of cellular functions, whereas Rac2 is largely expressed in the hematopoietic cells.[27,28,29] Further, Rac2 is required for phagocytosis in macrophages.[30] Rac2 appears to specifically regulate chemotaxis, cellular differentiation, proliferation, actin remodeling, and generation of superoxide and kinase activation in neutrophils.[29,31,32] Rac2 plays a significant role in COX2 expression in macrophages,[33] and COX2 expression is also associated with the pathogenesis of pterygium.[5] Rac1 and Rac2 act as a regulatory component of the superoxide-producing NADPH oxidase and regulate the oxidative burst in phagocytic cells.[15] Rac directly contributes to ROS production.[16] There is evidence that ICAM-2 and ICAM-3 gene and protein expressions were significantly upregulated in pterygial tissues,[17] and ICAM-2 can regulate N-cadherin localization and vascular permeability through Rac1 signalling.[34] However, our findings in the present study showed that *RAC1* gene expression was not markedly modified in pterygial tissues.

Our data demonstrated that the *RAC2* gene and Rac2 protein were expressed in pterygial tissue, indicating that Rac2 is not restricted to hematopoietic cells. In support of this view, two reports describe Rac2 expression in vascular smooth muscle cells (VSMC).[35,36] Although Rac2 expression is undetectable under quiescent or normal conditions, its expression is stimulated upon induction of VSMC with inflammatory cytokines. Overexpression of Rac2 significantly increases VSMC migration and intracellular superoxide production.[36] It has been shown that tumor necrosis factor-α and transforming growth factor-β are able to increase Rac2 expression in VSMC.[36] These growth factors are also present in the pterygial tissue.[4] Rac2 is expressed in endothelial cells and is also a requisite signaling component for endothelial cell migration.[37] Rac2 is also present in human bronchial epithelial cells and upregulation of Rac2 leads to increased NADPH oxidase activity and increased intracellular ROS generation.[38] Moreover, Rac2 is expressed in tumor cells. Although reduced expression of Rac2 was reported in malignant brain tumors, its overexpression was demonstrated in head and neck squamous cell carcinoma.[39,40] Pterygium can display tumor-like features and has been proposed to be a neoplastic-like growth disorder.[41]

Rac3 is the least studied Rac isoform. Our findings implicate for the first time that Rac3 expression is detectable in pterygial tissue. Although Rac3 may be involved in the stress activation pathway and tumor growth, its contribution to the pathogenesis of pterygium is currently unknown.[9,42]

Study Limitations

The main limitation of our study is the small sample size. Further studies with larger sample sizes may help shed more light on the cellular characteristics of this disease.

Conclusion

This study is the first to demonstrate gene expression of the small GTP-binding proteins Rac1, Rac2, and Rac3 in pterygium. However, gene and protein expressions were not modified compared to normal conjunctival tissue, suggesting that Rac proteins may not play a role in pterygium development. The signaling pathways involved in Rac-mediated functions remain unknown in pterygium, and clarification of the participation of each of these proteins in pterygium requires further study.

Ethics

Ethics Committee Approval: The study was approved by the Gaziantep University Local Clinical Ethics Committee (decision no: 2017/312, date: 11.09.2017).

Informed Consent: Obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.G., B.D., Concept: A.T.D., Design: A.S., A.T.D., Data Collection or Processing: K.G., B.D., Ş.D., Analysis or Interpretation: A.S., Ö.E., E.T., Literature Search: K.G., Ş.D., A.T.D., Writing: A.T.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

Evaluation of Central and Peripheral Retinal Vascular Changes in the Fellow Eyes of Patients with Unilateral Retinal Vein Occlusions

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Abstract

Objectives: To evaluate the subtle peripheral retinal and macular vascular changes in the fellow eyes of patients with unilateral retinal vein occlusion (RVO).

Materials and Methods: This retrospective study included 53 patients with unilateral RVO and 44 age-matched controls. The frequency of peripheral retinal vascular pathologies in both eyes was evaluated using high quality ultra-wide field fluorescein angiography (UWFFA). Macular vascular density, flow area, and foveal avascular zone measurements from optical coherence tomography angiography (OCTA) were analyzed together with laser flare photometry values in patients and controls.

Results: Peripheral retinal vascular pathologies were detected on UWFFA in the fellow eyes of 36 (67.9%) patients. No significant central vascular pathologies were detected on OCTA and there was no significant difference in OCTA parameters between the fellow eyes and the controls. Flare values did not differ significantly between the control and the fellow eyes.

Conclusion: Two thirds of the fellow eyes of unilateral RVO patients had subtle peripheral retinal vascular changes, while there was no significant microvascular change detected with OCTA in the macula. This suggests that vascular changes caused by systemic vascular disorders probably first start in the peripheral retina of the fellow eyes of patients with RVO.

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Introduction

One of the most common and severe retinal vascular diseases is retinal vein occlusion (RVO).[1] Based on the location of the lesion, RVOs are classified as branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO). Although the exact pathogenesis of both BRVO and CRVO has not yet been determined, there are multiple suggested mechanisms and identified risk factors. Systemic conditions such as atherosclerosis, hypertension (HT), diabetes mellitus (DM), and thrombophilia are among the leading risk factors for the development of RVO.[2,3,4,5,6] Patients with RVO also have an increased risk of cardiovascular diseases.[7,8] It was shown that RVO is associated with increased arterial stiffness and significant endothelial dysfunction. This strengthens the theory that systemic arteriosclerosis with endothelial dysfunction has a significant role in the development of RVO.[9] Considering all these systemic etiologies and risk factors in RVO, the fellow eyes of unilateral RVO patients may carry subtle vascular changes and should be at risk of developing RVO. Some epidemiologic studies have confirmed this expectation and reported that the fellow eyes of RVO patients have a significantly increased risk of RVO when compared with the general population and the disease becomes bilateral in 15% of patients over time.[2,10]

Subtle vascular changes in the fellow eyes cannot be detected during routine ophthalmological examination or imaging with routine optical coherence tomography (OCT) and fundus fluorescein angiography (FFA). However, they may be revealed using novel examination techniques like optical coherence

tomography angiography (OCTA) for macular vascular changes and ultra-widefield fluorescein angiography (UWFFA) for the peripheral retina. OCTA is a newer, non-invasive method for the visualization of retinal vascular layers and may detect subtle changes in the deep capillary plexus at an early stage.[11,12] UWFFA, which shows almost the entire retina (up to 200°) and captures the peripheral retina simultaneously without the need for patient refixation, may reveal subtle vascular changes in the extreme periphery of fellow eyes.[13,14] Lastly, it is well known that blood-retinal and blood-aqueous barriers are disrupted in eyes with RVO. Therefore, there may also be barrier disruption in the fellow eyes of RVO patients during the asymptomatic early period that could be detected via laser flare photometry.[15,16,17] In literature, there are studies evaluating the fellow eyes of patients with unilateral RVO with electrophysiologic tests, microperimetry, and retina layer thickness measurements.[10,18,19,20,21] Additionally, a limited number of angiographic studies were done to reveal retinal or choroidal vascular changes in the fellow eye using UWFFA in BRVO patients, adaptive optics scanning light ophthalmoscope fluorescein angiography in CRVO patients, and OCTA in RVO patients.[22,23,24,25,26] However there is no study evaluating both peripheral and central subtle vascular changes in the fellow eyes of unilateral RVO patients at the same time. Therefore, this study aimed to search for possible blood-eye barrier disruption and subtle peripheral and central retinal vascular changes affecting the fellow eyes of unilateral RVO patients using laser flare photometry, UWFFA, and OCTA.

Materials and Methods

Study Design

This retrospective study included adult patients who underwent standard clinical evaluation and treatment for eye diseases. The research protocol was approved by the Gazi University Institutional Ethical Review Board (decision no: 2019-375/11, date: 04.11.2019). The study was designed in compliance with the Declaration of Helsinki.

Participants

The medical records and images of unilateral RVO patients who applied to the Ophthalmology Clinic of Gazi University Hospital between January 2018 and March 2020 were reviewed. Those with good quality UWFFA, OCTA, and laser flare photometry measurements from both eyes were included. Of 87 patients, 34 were excluded because of diabetic retinopathy, age-related macular degeneration, other retinal vascular diseases, high myopia (spherical equivalent >6 diopters), previous retinal surgery, or any media opacity which prevented high-quality OCTA and UWFFA acquisition from both eyes. Age-matched subjects who presented to the clinic for refraction examination without any significant ocular diseases were included as controls. Eligibility criteria for participants in the control group were having undergone a comprehensive ophthalmic examination with OCTA imaging and laser flare photometry measurements. Best corrected visual acuity (BCVA) <20/25, high myopia (spherical equivalent >6 diopters), significant media opacity, and any retinal or choroidal pathology were the exclusion criteria for the controls and the fellow eyes.

Demographic data, history of ocular and systemic diseases, drugs used, and RVO duration and previous treatment (for the RVO group) were collected from all participants. BCVA and intraocular pressure (IOP) were noted for both eyes. All participants had bilateral Heidelberg SD-OCT (Heidelberg Engineering Inc., Heidelberg, Germany), OCTA (AngioVue; Optovue Inc., Fremont California, USA), and laser flare photometry measurements (Kowa Company Ltd, Nagoya, Japan). Only participants in the RVO group had Optos 200 Tx (Optos, Dunfermline, Scotland) wide-angle color fundus photography and angiography.

Ultra-widefield Fluorescein Angiography

UWFFA images were taken by Optos 200 Tx (Optos, Dunfermline, Scotland) after intravenous fluorescein administration and evaluated independently by two physicians (Ş.Ö., M.E.). The presence and extent of capillary non-perfusion, neovascularization, collaterals, hyperfluorescent dots, and late peripheral vascular leakage were recorded for RVO eyes. Patients with a capillary non-perfusion area greater than 10 disc diameters were considered ischemic cases and the others as non-ischemic. In cases of BRVO, the affected area was noted. Images of the fellow eyes were evaluated for the presence of any vascular pathology such as capillary non-perfusion, late leakage, vascular loops or anastomosis, hyperfluorescent dots, and pathological vessels.

Optical Coherence Tomography Angiography

OCTA images were analyzed based on 6×6 mm images using the RTVue XR Avanti device (ReVue software, version 2015.100.0.35; Optovue Inc., Fremont California, USA). The software automatically divides the tissue into different layers: superficial capillary plexus, deep capillary plexus, outer retinal layer, and choriocapillaris layer. Before any measurements, two ophthalmologists (Ş.Ö., M.E.) independently evaluated the images and checked the segmentation of the retinal layers. For each layer (superficial or deep), vascular density (VD) measurements from six areas (whole, superior, inferior, fovea, parafovea, perifovea) were calculated separately. Superficial and deep capillary plexus foveal avascular zone (FAZ) area, outer retinal flow area (FA), choriocapillaris FA, and central macular thickness (CMT) were provided automatically by the device. Measurements of the FAZ area in the images of RVO eyes could not be standardized due to cystoid macular edema or retinal thinning.

Statistical Analysis

The SPSS statistical package program (version 22.0 for Windows; IBM Corp., Armonk, NY, USA) was used for statistical analysis. Chi-square test was used for comparisons of categorical variables. Continuous variables were compared with the independent variables t-test, One-Way ANOVA, Kruskal-Wallis test, and Mann-Whitney U test, depending on conformity to normal distribution. The significance level was accepted as $p<0.05$.

Results

A total of 53 patients with unilateral RVO (18 patients with CRVO and 35 patients with BRVO) and 44 controls were included in this study. The demographic data of the controls and patients are shown in Table 1. The mean age of the patients was 61.28±11.87 years (range, 26-84 years). Twenty-eight patients (52.8%) were female and 25 (47.2%) were male. HT was observed in 31 patients (58.5%) and DM in 17 patients (32.1%). Seventeen patients (32.1%) were on antiplatelet therapy and 10 (18.8%) of them started antiplatelet therapy before RVO diagnosis. Glaucoma was observed in 13 patients (24.5%). There was no history of additional ocular disease except mild cataract in the patients' fellow eyes.

The control group included 44 eyes of 44 participants. The male/female ratio was 29/15 and the mean age was 59.4±9.1 years (range, 36-73 years). Nineteen (43.2%) of them had HT, 13 (29.5%) had DM, and 6 (13.6%) were on antiplatelet therapy (Table 1). None of the controls had a history of ocular disease except mild cataract. There was no statistically significant difference between the control group and the study group in terms of mean age, sex, or systemic comorbidities (Table 1).

The ophthalmologic findings of the groups are shown in Table 2. The mean LogMAR BCVA of the patients was 0.55±0.54 in the RVO eyes and 0.03±0.11 in the fellow eyes (Mann-Whitney U test, p<0.001). There was no significant difference in BCVA or IOP between the fellow eyes and the control group (p>0.05) and no significant difference in IOP between the RVO and fellow eyes (p>0.05). The frequency of pseudoexfoliation and glaucoma did not differ significantly between RVO and fellow eyes (chi-square test, p=0.696 and p=0.143, respectively). The mean duration of RVO was 56.3±50.84 months (range, 4-200 months). Of the 53 patients with unilateral RVO, 12 (22.6%) were treatment-naive, 15 (28.3%) had undergone laser photocoagulation and anti-Vascular endothelial growth factor (VEGF) injections, and 26 (49.1%) had received only anti-VEGF injections.

Laser flare photometry measurements were done at least one month after the anti-VEGF injections or at least two months after laser photocoagulation. The mean laser flare photometry values were 11.94±8.47 photons per millisecond (ph/ms) in RVO eyes, 7.47±5.64 ph/ms in the fellow eyes, and 6.68±3.51 ph/ms in the control group (Table 3). The flare photometry values were significantly higher in RVO eyes compared to both fellow eyes and controls (p=0.001). Although flare values were slightly higher in the fellow eyes than the control eyes, the difference was not significant (p=0.935). Flare values in the RVO eyes did not differ significantly based on the type of RVO, extent of ischemia, or previous treatment for RVO (Table 3).

The UWFFA findings in RVO eyes and fellow eyes are shown in Table 4. There was ischemic RVO in 16 eyes (30.2%), collateral shunting vessels in 14 eyes (31.1%), retinal neovascularization in 3 eyes (5.7%), and panretinal laser photocoagulation scars in 15 eyes (28.3%) with RVO. Four of the 35 BRVO cases were macular (7.5%) and 31 were extramacular (92.5%). Of the extramacular BRVOs, 21 (39.6%) were superotemporal, 8 (15.1%) were inferotemporal, 1 (1.9%) was superior hemispheric, and 1 (1.9%) was inferior hemispheric. On UWFFA of the fellow eyes of RVO patients, the peripheral retina was normal in 17 eyes (32.1%; 7 CRVO, 10 BRVO), whereas some pathological findings could be identified in 36 eyes (67.9%; 11 CRVO, 25 BRVO). Peripheral

Table 1. Demographic properties and systemic conditions of patients and controls

	RVO (n=53)	Control (n=44)	p value
Age (years)	Mean ± SD (range)		
	61.28±11.87 (26-84)	59.41±9.06 (36-73)	0.483
Gender Male/female	25/28	29/15	0.07
Systemic comorbidities	n (%)		
None	12 (22.6%)	10 (22.7%)	0.99
Hypertension	31 (58.5%)	19 (43.2%)	0.156
Diabetes mellitus	17 (32.1%)	13 (29.5%)	0.828
Anti-aggregant/anti-coagulant use	10 (18.8%)	6 (13.63%)	0.489

RVO: Retinal vein occlusion, SD: Standard deviation

Table 2. Ophthalmological findings of the study eyes

	RVO eyes (n=53)	Fellow eyes (n=53)	Control eyes (n=44)
Mean ± SD			
BCVA (logMAR)	0.55±0.54	0.03±0.11	0.03±0.06
IOP (mmHg)	17.31±4.42	16.49±3.33	15.32±2.41
n (%)			
Glaucoma	13 (24.5%)	7 (13.2%)	0 (0%)
Pseudoexfoliation	4 (7.6%)	3 (5.7%)	0 (0%)
Pseudophakia	14 (26.4%)	6 (11.3%)	3 (6.8%)
Type of RVO: CRVO/BRVO	18 (34%)/35 (66%)	-	-

BCVA: Best corrected visual acuity, logMAR: Logarithm of the minimum angle of resolution, IOP: Intraocular pressure, RVO: Retinal vein occlusion, CRVO: Central retinal vein occlusion, BRVO: Branch retinal vein occlusion, SD: Standard deviation

Table 3. Laser flare photometry values of the study groups

Compared study groups	Flare (ph/ms)	p value*
BRVO/CRVO	10.23/15.04	0.240
Ischemic RVO/non-ischemic RVO	10.71/12.55	0.366
Previous treatment (+)/(-)		
Anti-VEGF	11.58/13.06	0.441
Laser + anti-VEGF	14.54/10.77	0.119
RVO/control	11.94/6.68	**0.001**
RVO/fellow eyes	11.94/7.47	**0.001**
Fellow eyes/controls	7.47/6.68	0.935
Fellow eyes of CRVO/fellow eyes of BRVO	8.35/6.97	0.704

*Mann-Whitney U test was used for comparisons, BRVO: Branch retinal vein occlusion, CRVO: Central retinal vein occlusion, RVO: Retinal vein occlusion, VEGF: Vascular endothelial growth factor, ph/ms: photons per millisecond

hyperfluorescent dots and varying degrees of disruption in the peripheral capillary bed were the most common pathological findings, observed in 29 (54.7%) and 22 (41.5%) of the fellow eyes, respectively. There were vascular anastomosis or loop-like shunt vessels in 8 (15.1%) eyes and late peripheral vascular leakage in 4 (7.5%) of the fellow eyes (Figure 1). There was no significant difference between the types of RVO in terms of peripheral retinal vascular pathologies observed in the fellow eyes with UWFFA (p=0.759) when the BRVO and CRVO patients were evaluated in two groups.

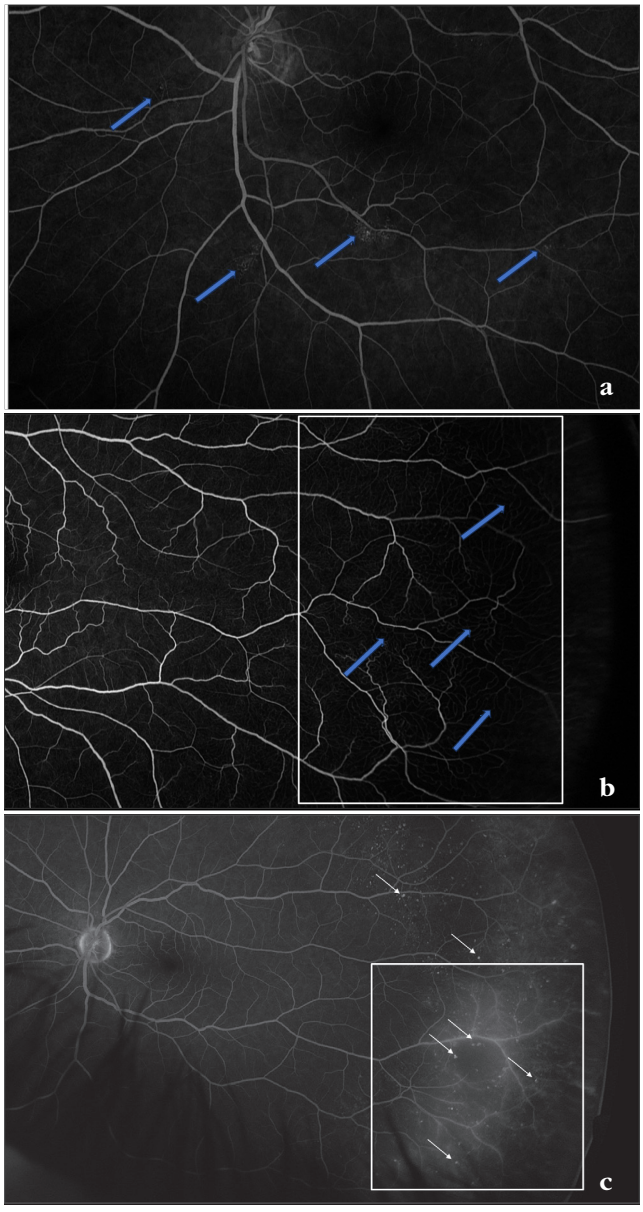

Figure 1. Ultra-widefield fluorescein angiography images from the unaffected fellow eyes of patients with retinal vein occlusion: (a) localized microvascular changes; capillary drop-out and microaneurysms (arrows). (b) Vascular anastomoses (arrows) and peripheral capillary drop-out (rectangle). (c) Multiple hyperfluorescent dots (arrows) and late peripheral leakage (rectangle)

Concerning systemic comorbidities in the 36 patients with fellow eye pathologies, 10 patients (27.8%) had both HT and DM, 13 (36.1%) had HT only, 2 (5.5%) had DM only, and 11 patients (30.6%) had no known systemic diseases. Of the 17 patients without any UWFFA findings in the fellow eyes, 5 (31.3%) of them had no systemic comorbidities. There was no statistically significant difference between the groups with respect to systemic comorbidities (p=0.366)

OCTA findings are shown in Table 5. The VD values were significantly lower in RVO eyes compared to fellow eyes in almost all areas except foveal VD in the deep capillary plexus. Choriocapillaris FA was lower in RVO eyes and CMT was higher in RVO eyes as compared to the fellow eyes, as expected (p<0.001) (Table 5). However, there was no significant difference between VD, FA, FAZ, and CMT values in the control and fellow eyes (Table 5).

Discussion

This study primarily focused on the peripheral retinal and macular vascular changes in the fellow eyes of patients with unilateral RVO using UWFFA and OCTA. We demonstrated peripheral retinal vascular changes in 67.9% of the fellow eyes of RVO patients using UWFFA. The most commonly detected vascular changes were peripheral hyperfluorescent dots (54.7%) and disruption of the peripheral capillary bed (41.5%). In the literature, there is only one study investigating UWFFA findings

Table 4. Ultra-widefield fundus fluorescein angiography characteristics of RVO patients

	n	%
RVO eyes		
CRVO	18	34
BRVO	35	66
Inferior hemispheric BRVO	1	1.9
Superior hemispheric BRVO	1	1.9
Inferotemporal BRVO	8	15.1
Superotemporal BRVO	21	39.6
Macular BRVO	4	7.5
Ischemic	16	30.2
Non-ischemic	37	69.8
Neovascularization	3	5.7
Collateral formation	28	52.8
Fellow eyes		
Positive angiography findings	36	67.9
Peripheral hyperfluorescent dots	29	54.7
Peripheral capillary plexus disruption	22	41.5
Peripheral shunt vessels (vascular loops)	8	15.1
Late peripheral leakage	4	7.5
No angiographic findings	17	32.1

BRVO: Branch retinal vein occlusion, CRVO: Central retinal vein occlusion, RVO: Retinal vein occlusion

Table 5. Optical coherence tomography angiography findings in RVO eyes, fellow eyes, and healthy control eyes					
	RVO group (n=53)	**Fellow eyes (n=53)**	**Control group (n=44)**	**p***	**p****
Superficial capillary plexus vascular density (%)					
Whole	41.2±6.4	47.5±4.8	48.5±3.2	**<0.001**	0.260
Superior	41.0±6.3	47.0±5.0	48.5±3.2	**<0.001**	0.111
Inferior	41.4±7.2	47.8±4.9	48.5±3.2	**<0.001**	0.437
Fovea	22.1±11.5	18.2±8.2	18.6±7.6	**0.047**	0.831
Parafovea	41.9±7.9	50.3±5.7	51.2±4.3	**<0.001**	0.416
Perifovea	41.6±7.1	48.1±4.8	49.1±3.3	**<0.001**	0.245
Deep capillary plexus vascular density (%)					
Whole	40.1±6.6	44.7±5.6	46.7±6.2	**<0.001**	0.092
Superior	39.5±7.0	44.5±5.9	46.5±6.3	**<0.001**	0.107
Inferior	40.3±7.2	44.8±5.8	46.9±6.3	**<0.001**	0.091
Fovea	34.8±12.2	33.4±9.5	35.1±7.8	**0.509**	0.35
Parafovea	43.8±8.2	51.0±5.3	52.3±4.5	**<0.001**	0.204
Perifovea	40.7±7.1	45.7±6.3	47.8±6.9	**<0.001**	0.122
FAZ area (mm²)	0.337±0.243	0.295±0.130	0.289±0.101	0.789	0.826
Outer retinal flow area (mm²)	0.688±0.453	0.770±0.399	0.666±0.369	0.234	0.120
Choriocapillaris flow area (mm²)	1.86±0.35	2.05±0.18	2.02±0.13	**<0.001**	0.295
Central macular thickness (µm)	333.43±184.14	248.51±23.84	251.91±26.88	**0.007**	0.511
*RVO eyes vs. fellow eyes, **Fellow eyes vs. control eyes, RVO: Retinal vein occlusion, FAZ: Foveal avascular zone					

in the fellow eye of RVO patients, but it included only patients with BRVO.[22] They detected peripheral vascular leakage in 9 of the 81 eyes (11.1%) but did not investigate other peripheral vascular changes in the fellow eyes.[22] In the present study, peripheral late vascular leakage was detected in 7.5% of the fellow eyes, similar to the previously mentioned study.

Systemic vascular diseases like DM and HT may cause microcirculation problems like endothelial dysfunction and peripheral vasoconstriction due to increased vascular resistance, decreased blood flow, and increased plasma viscosity.[27] As the duration of the disease increases, severe atherosclerotic vascular changes occur in the peripheral arteries of hypertensive patients.[28] This information is consistent with our findings of the disrupted peripheral capillary bed and the resultant shunt vessels observed in UWFFA. The presence of peripheral hyperfluorescent spots (probably representing microaneurysms) also suggests localized ischemia caused by HT and DM. Although in this study we observed no statistically significant difference in systemic comorbidities between patients with and without UWFFA findings in the fellow eye, this could be due to the small patient population. Further studies with larger patient populations may show differences between these two groups. Nearly 6% of the patients had HT and 32.5% had DM in the present cohort and this explains why the retinal vascular structures of fellow eyes were also affected to some degree. However, local factors (e.g., crowded disc, arterial compression, increased IOP) remain the main determinants of asymmetric involvement in RVO.[26,29]

Macular microcirculation is better assessed with the recent technology of OCTA as compared to conventional FFA.[12,13] In the present study we assessed macular microcirculation changes in RVO eyes, fellow eyes, and control eyes with 6x6 mm OCTA images. VD values were significantly lower in RVO eyes as compared to fellow eyes in almost all areas except foveal VD in the deep capillary plexus. This was expected and consistent with the literature.[21,24,30] Koulisis et al.[30] examined the fellow eyes of unilateral RVO patients and demonstrated that VD was lower in the superficial and deep capillary plexus in fellow eyes than in the control group. However, we failed to demonstrate any significant difference in the VD, FA, FAZ, and CMT measurements between the fellow eyes and the control eyes. This discordance with the literature may be caused by the similar distribution of systemic diseases like HT and DM in the control group in our study. Supporting this idea is the fact that the control groups did not have similar systemic diseases to the RVO groups in the previously mentioned studies.[24,30] Patients with HT have been shown to have decreased perifoveal capillary density and decreased capillary blood flow velocity compared to healthy subjects.[19] We believe that OCTA measurements in a 6×6 mm area (which is average macula size) are more helpful to show the vascular status of the entire macula rather than 3x3 mm measurements.

In the present study, we found that aqueous flare values were significantly higher in RVO eyes than fellow eyes (p=0.001). Increased aqueous flare mainly reflects the disruption of the blood-aqueous barrier, which was shown to be damaged in RVO

eyes.[15,16] Flare values have been reported to be even higher in ischemic CRVO than non-ischemic CRVO.[17] However, we failed to demonstrate any statistically significant difference in aqueous flare values of RVO eyes based on the presence of ischemia and the type of RVO (Table 3). There was no statistically significant difference between the control eyes and the fellow eyes, which suggests that the subtle microvascular changes caused by systemic vascular diseases do not reach a level that could affect the blood-eye barriers to a detectable degree.

Study Limitations

First of all, peripheral retinal vascular changes could not be compared with control eyes because we did not perform UWFFA, which is an invasive test, in the control group. Secondly, the duration of RVO and treatment status of the eyes were variable in the patients due to the retrospective nature of the study, and this may have affected OCTA and flare measurements to some extent. Thirdly, the sample size was small.

Conclusion

This is the first study to investigate all aspects of retinal microvascular changes in the fellow eyes of patients with RVO as determined with UWFFA (peripheral retina), OCTA (central retina), and laser flare photometry (blood-retina and blood-aqueous barriers). The fellow eyes of unilateral RVO patients demonstrated some peripheral retinal vascular changes such as capillary disruption, hyperreflective dots, peripheral vascular anastomosis and loops, and late leakage in the UWFFA, indicating that systemic factors affect both eyes to some extent. However, microvascular changes in the macula could not be demonstrated with OCTA analysis, suggesting that early subtle vascular changes start to occur first in the peripheral retina in these patients. Aqueous flare values demonstrated that the blood-eye barriers were disrupted in the RVO eyes but not the fellow eyes. Future prospective studies could help to determine whether these subtle findings in the fellow eyes of unilateral RVO patients could be associated with a higher risk of developing bilateral disease.

Ethics

Ethics Committee Approval: Gazi University Institutional Ethical Review Board (decision no: 2019-375/11, date: 04.11.2019).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Ö., G.G., M.E., H.B.Ö., Concept: Ş.Ö., F.Y.T., Design: M.E., F.Y.T., Data Collection or Processing: M.E., F.Y.T., M.E.A., Analysis or Interpretation: M.E., F.Y.T., Y.K.A., Literature Search: M.E., F.Y.T., Writing: M.E., F.Y.T., Ş.Ö.

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Original Article

Real-World Outcomes of Intravitreal Anti-Vascular Endothelial Growth Factor Treatment for Diabetic Macular Edema in Türkiye: MARMASIA Study Group Report No. 1

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Abstract

Objectives: This study aimed to report the demographic and clinical characteristics of diabetic macular edema (DME) patients treated with intravitreal injection (IVI) of anti-vascular endothelial growth factors (anti-VEGF) and provide an overview of outcomes during routine clinical practice in Türkiye.

Materials and Methods: This retrospective, real-world study included 1,372 eyes (854 patients) treated with a pro re nata protocol by 21 ophthalmologists from 8 tertiary clinics on the Asian side of the Marmara region of Türkiye (MARMASIA Study Group). Five cohort groups were established by collecting the patients' baseline and 3, 6, 12, 24, and 36-month follow-up data, where each subsequent cohort may include the previous. Changes in best-corrected visual acuity (BCVA, approximate ETDRS letters) and central macular thickness (CMT, µm), number of visits and IVI, and rates of anti-VEGF switch and intravitreal dexamethasone implant (IDI) combination were evaluated.

Results: The 3, 6, 12, 24, and 36-month cohorts included 1372 (854), 1352 (838), 1185 (722), 972 (581), and 623 (361) eyes (patients), respectively. The mean baseline BCVA and CMT were 51.4±21.4 letters and 482.6±180.3 µm. The mean changes from baseline in BCVA were +7.6, +9.1, +8.0, +8.6, and +8.4 letters, and in CMT were -115.4, -140.0, -147.9, -167.3, and -215.4 µm at the 3, 6, 12, 24, and 36-month visits ($p<0.001$ for all). The median cumulative number of anti-VEGF IVI was 3.0, 3.0, 5.0, 7.0, and 9.0, respectively. The overall anti-VEGF switch and IDI combination rates were 18.5% (253/1372 eyes) and 35.0% (480/1372 eyes), respectively.

Conclusion: This largest real-life study of DME from Türkiye demonstrated BCVA gains inferior to randomized controlled trials, mainly due to the lower number of IVI. However, with the lower baseline BCVA and higher IDI combination rates in our cohorts, these gains were relatively superior to other real-life study counterparts.

Keywords: Anti-VEGF, diabetic macular edema, intravitreal injection, real-life study, routine clinical practice

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Introduction

Traditionally, the data considered in evidence-based retinal disease management guidelines have been primarily, if not exclusively, dependent on the gold standard, randomized controlled trial (RCT)-based "efficacy" studies.[1] However, the design of RCTs, which utilizes restrictive eligibility criteria to control data variability while ensuring quality, limits their replicability and reproducibility in clinical practice.[2] Therefore, real-world evidence (RWE) from diversified routine clinical practice has recently received significant attention worldwide, particularly in diseases that require more individualized treatment, such as diabetic macular edema (DME).[3,4]

DME is the leading vision-threatening complication of diabetic retinopathy (DR). It has been shown to be anatomically and functionally responsive to intravitreal anti-vascular endothelial growth factors (anti-VEGF) and corticosteroids in numerous milestone RCTs.[5,6,7,8,9,10,11,12,13,14,15] However, even considering two well-designed RCTs, RISE/RIDE and VIVID/VISTA, the former evaluating intravitreal ranibizumab (IVR; Lucentis®, Genentech, CA, USA) and the latter intravitreal aflibercept (IVA; Eylea®, Regeneron, NY, USA) in the treatment of DME, similar results could not be obtained in their respective study arms, even though they both included patients with similar demographics and disease characteristics.[9,12] These two examples alone demonstrate the need for complementary studies of DME treatment in real-life settings.

Furthermore, the 5-year extension study of Diabetic Retinopathy Clinical Research Network (DRCR.net) Protocol T, the first RCT to compare IVR, IVA, and intravitreal bevacizumab (IVB; Avastin®, Genentech, CA, USA) in treating DME, showed that after 2 years of protocol-defined follow-up and re-treatment, DME patients may receive different modalities at clinician discretion in routine clinical practice.[14,15,16] Those patients were shown to lose best-corrected visual acuity (BCVA) between 2 and 5 years, even though they preserved central macular thickness (CMT) with a protocol chosen at clinician discretion.[16] Also, several RWE studies, even systematic reviews and meta-analyses, report anatomical and functional effectiveness of anti-VEGF agents in DME but with less impressive results than in RCTs, mainly due to undertreatment, less frequent monitoring, and lower patient compliance.[17,18,19,20,21,22,23,24,25,26,27]

Recently, Durukan et al.[27] published the first large-scale RWE study of DME treatment from the Central Anatolia region of Türkiye, reporting a similar lower number of injections and gains like other RWE studies on DME. Therefore, we established a multicenter collaboration to further evaluate the real-world outcomes of intravitreal anti-VEGF treatment of DME in 8 tertiary reference centers located on the ASIAn side of the MARMara region of Türkiye (MARMASIA Study Group). This first report by the MARMASIA Study Group aims to demonstrate the demographic and clinical features of the evaluated DME patients and provide an overview of the treatment outcomes.

Materials and Methods

This descriptive, retrospective, observational, multicenter, real-world study was conducted by the MARMASIA Study Group, which includes 22 ophthalmologists experienced in retinal diseases from 8 tertiary clinics in 3 cities (İstanbul, Kocaeli, and Sakarya) on the Asian side of the Marmara region of Türkiye. The Institutional Review Board of Kocaeli University Faculty of Medicine approved the study protocol (no: GOKAEK-2022/07.19, date: 14.04.2022). The study followed the ethical principles of the 1964 Declaration of Helsinki and later amendments. In addition, written informed consent for the use of their medical data for research purposes was routinely obtained from all patients at their first presentation to the participating clinics. The study is registered on ClinicalTrials.gov (number: NCT05472376).

Study Population

Patients who had received at least one intravitreal injection (IVI) of any anti-VEGF agent (IVR, IVA, or IVB) for DME between January 2015 and December 2018 and was followed up for at least 3 months were retrospectively screened and included in the study. In Türkiye, for treatment-naive DME patients to receive reimbursement from the Turkish Social Security Institution, it was made mandatory as of December 28, 2018 to start treatment with three loading doses of IVB injections.[28] Accordingly, the reimbursement of anti-VEGFs approved for intraocular use (i.e., IVR and IVA) could only be obtained by patients in case of failure of treatment with IVB.[28] Therefore, patients whose treatment started after this date were excluded from the study. The patients' demographics, clinical characteristics, and follow-up information were collected retrospectively from electronic or traditional patient records.

The study inclusion criteria were established as being 18 years of age or older, having received at least one IVB (1.25 mg/0.05 mL), IVR (0.5 mg/0.05 mL), or IVA (2 mg/0.05 mL) injection as initial treatment for DME during the specified dates, having at least 3 months of follow-up, and having at least four or more visits per year for patients who were followed up for more than one year. Patients who underwent phacoemulsification surgery within the previous month and panretinal, focal, or grid laser photocoagulation or micropulse laser treatment in the previous 4 months before study enrollment, as well as patients who had any intraocular surgery other than phacoemulsification and pars plana vitrectomy (PPV) during the study period were excluded from the study. If eligible, both eyes of the patients were included in the study analysis separately. There were no restrictions on previous intravitreal therapy with anti-VEGFs or corticosteroids, presenting BCVA, whether loading doses of intravitreal anti-VEGFs were administered, the use of intravitreal dexamethasone implant (IDI; Ozurdex®, Abbvie-Allergan, CA, USA), micropulse laser, panretinal, focal, or grid laser photocoagulation, and undergoing phacoemulsification or PPV at any point during follow-up.

Baseline and Follow-up Data

The baseline demographics and medical information of the patients included age, gender, duration of diabetes mellitus, treatment of diabetes mellitus (none, oral antidiabetic drugs, insulin, or combination of oral antidiabetic drugs and insulin), comorbidities (none, hypertension, coronary artery disease, cerebrovascular accident, and chronic kidney disease leading to hemodialysis), history of glaucoma, antiglaucoma drug use (classified as prostaglandin analogs and others), previous anti-VEGF IVI (number of injections and agents), previous panretinal photocoagulation, and previous PPV history.

Five retrospective cohort groups were formed so that subsequent cohorts may also include patients from the previous cohorts by using examinations performed at 3, 6, 12, 24, and 36 months (±2 weeks) as follow-up data. All patients underwent comprehensive ophthalmic examination at baseline and follow-up visits, including BCVA assessment with an electronic Snellen chart, Goldmann applanation tonometry, slit-lamp biomicroscopy, dilated fundus examination, and optical coherence tomography (OCT) scans obtained by either Spectralis (Heidelberg Eng., Heidelberg, Germany), RS-3000 (Nidek, Gamagori, Japan), or RTVue-100 (Optovue Inc., CA, USA) OCT devices, depending on the availability in each clinic. We used the follow-up software feature of these devices to ensure the accuracy of the measurement positions. In addition, fundus fluorescein angiography was performed at clinicians' discretion if there was suspicion of new neovascularization or persistent peripheral retinal ischemia.

BCVA, lens status (pseudophakic or phakic), DR grading (non-proliferative or proliferative), and OCT parameters from the specified follow-up visits were collected. The OCT parameters of particular importance were as follows:

1. CMT (µm), automatically calculated by the software of the corresponding OCT device after foveal alignment was ensured by the clinician;

2. DME pattern, classified as diffuse/spongious, cystoid, diffuse/spongious plus subretinal fluid (SRF), and cystoid plus SRF;

3. Cystic pattern according to the European School for Advanced Studies in Ophthalmology classification:[29] absent (0), mild (1), moderate (2), or severe (3);

4. Largest cyst diameter (µm), measured manually by the corresponding OCT device software;

5. SRF height (µm), measured manually by the corresponding OCT device software from the outer surface of the photoreceptor layer to the inner surface of the retinal pigment epithelium;

6. Presence of disorganization of the retinal inner layers (DRIL), defined as the horizontal distance (µm) in which it was not possible to identify the boundaries between the inner nuclear layer, outer plexiform layer, and ganglion cell-inner plexiform layer complex;[30]

7. Continuity of the ellipsoid zone and external limiting membrane, classified as interrupted, partially preserved, totally preserved, or indiscernible;

8. Presence of epiretinal membrane;

9. Status of the posterior hyaloid, classified as attached, detached, or indiscernible.

Additional information collected at each follow-up visit included the intravitreal anti-VEGF agent used; treatment protocol (defined as 3+ pro re nata [PRN] if three loading doses were given and 1+PRN if not); cumulative number of injections; cumulative number of visits; stabilization time of the macula (defined as the first visit [in months] that injection was deferred according to the PRN protocol); the application and timing (months) of phacoemulsification, PPV, panretinal, focal, and grid laser photocoagulation, and micropulse laser, and presence of intravitreal hemorrhage, neovascular glaucoma, and any other complications and adverse events.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software for Windows version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical data analysis. Data distribution was determined by histogram plots and the Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous data were presented as mean ± standard deviation or median (interquartile range [IQR], expressed as 25th and 75th quartile values), and categorical data were presented as frequency (n) and percentage (%). Snellen BCVA values were converted to logarithm of the minimum angle of resolution (logMAR) values for statistical analysis, and the logMAR equivalent value for "counting fingers" and "hand motion" were assumed to be 2.10 and 3.10, respectively. LogMAR values were also converted to approximate Early Treatment Diabetic Retinopathy Study (ETDRS) letter scores using the formula "ETDRS letter score = 1.7 - logMAR) / 0.02" as suggested by Beck et al.[31] As logMAR values of 1.7 and higher give a negative value, the ETDRS letter scores of eyes higher than 1.6 logMAR were accepted as 0 (zero). Dependent variables were evaluated with paired samples t-test or repeated measures analysis of variance (ANOVA), and Wilcoxon signed rank test or Friedman test, depending on the data distribution and variable counts. Post-hoc analyses of more than two dependent variables were conducted with Dunn-Bonferroni post-hoc test and pairwise comparisons provided by the SPSS software for repeated measures ANOVA and Friedman test, respectively. The p values for post-hoc analysis were adjusted with Bonferroni correction and given as "adj. p" value where appropriate. A two-sided p value of less than 0.05 was considered statistically significant.

Results

Baseline Characteristics

The study included 1,372 eyes of 854 patients with a mean age of 62.7±8.7 (range, 30-94) years (455 [53.3%] females). All patients (eyes) had at least 3 months of follow-up and were included in the 3-month cohort, and there were 838 (1352), 722 (1185), 581 (972), and 361 (623) patients (eyes) in the 6-, 12-, 24-, and 36-month cohorts, respectively.

Of the 1,372 eyes in the study, 818 (59.6%) were treatment-naïve and 554 (40.4%) had previously been treated with a

mean of 4.3±3.0 (range, 1-24) anti-VEGF injections. Only 28 eyes (2.0%) were previously treated with intravitreal steroid injections (dexamethasone implant or triamcinolone acetonide) in combination with anti-VEGF agents. Also, 377 eyes (27.5%) had a history of panretinal laser photocoagulation, and 35 (2.6%) had a history of PPV.

The treatment protocol was 1+PRN in 525 eyes (38.3%) and and 3+PRN in 847 eyes (61.7%). The initial anti-VEGF agent used during the study period was bevacizumab in 60 eyes (4.4%), ranibizumab in 893 eyes (65.1%), and aflibercept in 419 eyes (30.5%).

The baseline characteristics of the patients and eyes in each cohort are given in Table 1.

Functional and Anatomical Outcomes

The mean BCVA and CMT of the eyes in the whole cohort during the study period are given in Figure 1. While BCVA increased and CMT decreased in the first 6-month period, BCVA gradually declined after 6 months despite the progressive decrease in CMT.

The mean baseline and final approximate ETDRS letter scores of the eyes were 51.4±21.4 and 57.6±21.5, with a mean change of 8.4±25.6 letters in 3 years. The mean change in letter scores from baseline was 7.6±17.3 at 3 months (p<0.001), 9.1±19.0 at 6 months (adj. p<0.001), 8.0±21.2 at 12 months (adj. p<0.001), 8.6±23.0 at 24 months (adj. p<0.001), and 8.4±85.4 letters at 36 months (adj. p<0.001). The mean letter score change from the previous visit was 7.6±17.3 (p<0.001), 1.5±11.9 (adj. p<0.001), -0.6±14.0 (adj. p=1.000), 0.3±14.8 (adj.p=1.000), and 0.2±0.4 (adj. p=1.000) letters at the 3-, 6-, 12-, 24-, and 36-month visits, respectively.

The mean baseline CMT of 482.6±180.3 µm was decreased to 267.4±87.3 µm at the last follow-up visit, with a mean change of -215.4±221.7 µm. The mean CMT changes from the baseline visit were -115.4±150.1 at 3 months (p<0.001), -140.0±181.1 at 6 months (adj. p<0.001), -147.9±211.6 at 12 months (adj. p<0.001), -167.3±196.4 at 24 months (adj. p<0.001), and -215.4±221.7 µm at 36 months (adj. p<0.001). The mean change in CMT from the previous visit was -115.4±150.1 (p<0.001), -24.6±123.1 (adj. p<0.001), -15.1±141.5 (adj. p=0.003), -15.5±147.6 (p<0.001), and -44.6±127.0 (p<0.001) µm at the 3-, 6-, 12-, 24-, and 36-month visits, respectively.

The most common baseline DME type was cystoid (n=617, 45%), followed by cystoid plus SRS (n=317, 23.1%), diffuse/spongious (n=261, 19%), and diffuse/spongious plus SRF (n=177, 12.9%). At the last follow-up visit, 42.9% (267/623) of the eyes had dry macula. DME pattern and dry macula rates during the study period are given in Figure 2.

Number of Visits and Intravitreal Anti-VEGF Injections

Table 2 displays the median number of visits and intravitreal anti-VEGF injections in each cohort stratified by study visits. In 3-, 6-, 12-, 24-, and 36-month cohorts, the median (IQR) cumulative number of visits was 2 (2-2), 4 (4-5), 7 (6-10), 11 (9-14), and 16 (14-18), and the median number of anti-VEGF IVIs was 3 (2-3), 3 (3-4), 5 (4-6), 7 (5-8), and 9 (7-10), respectively. The median number of injections per year decreased from 5 (4-6) in the first year to 2 (1-3) in the second (p<0.001) and 2 (1-3) in the third year (adj. p<0.001 for first vs. second and third years and adj. p=1.000 for second vs. third year).

Anti-VEGF Switch and Additional Treatments

The anti-VEGF agent was switched in a total of 254 eyes (18.5%) during the study period, of which 229 (90.2%) of the switches were intentional at the clinician's discretion. Fifty-one (20.1%) of the anti-VEGF agent switches occurred between 3 and 6 months, 97 (38.2%) between 6 and 12 months, 66 (26.0%) between 12 and 24 months, and 40 (15.7%) between 24 and 36 months of follow-up. The most frequent anti-VEGF agent switch was from ranibizumab to aflibercept (n=193, 76%). The rates of switches between anti-VEGF agents are given in Figure 3.

Of the 1372 eyes, 480 (35.0%) in the entire cohort had combination therapy with at least one IDI injection (mean: 2.4±1.4 injections, range, 1-9). While none of the eyes in the 3-month cohort had IDI injection, the cumulative rates of combination with IDI injection were 9.5% (129/1352), 26.0% (308/1185), 41.2% (400/972), and 44.8% (279/623) in the 6-, 12-, 24-, and 36-month cohorts, respectively. Combination with IDI resulted in significantly more BCVA letter gains and CMT reductions in all cohorts (Table 3).

Additional treatments employed at any time during the study period included panretinal laser photocoagulation in 444 eyes (32.4%), phacoemulsification in 315 eyes (23.0%), only focal or grid laser photocoagulation in 267 eyes (19.5%), focal and grid laser photocoagulation in 192 eyes (14.0%), PPV in 68 eyes (5.0%), and micropulse laser in 44 eyes (3.2%).

Adverse Events

Ocular adverse events encountered during the study period were intravitreal hemorrhage in 98 eyes (7.1%), neovascular glaucoma in 22 eyes (1.6%), increased intraocular pressure in 2 eyes (0.15%), rhegmatogenous retinal detachment in 2 eyes (0.15%), and endophthalmitis in 1 eye (0.07%).

Systemic adverse events that could be associated with anti-VEGFs were acute myocardial infarction in 5 patients (0.6%) and cerebrovascular accident in 1 patient (0.1%).

Discussion

This first report of the largest-scale RWE study of DME treatment from Türkiye demonstrates lower overall number of injections and visual gains than in RCTs (Table 4), supporting the findings from various other countries. Moreover, it provides insight into the rates of macular laser, anti-VEGF agent switch, and steroid combination in the treatment of DME at clinician discretion in real life.

One of the earliest RCTs comparing the efficacy of an anti-VEGF agent (ranibizumab) against macular focal/grid laser photocoagulation (READ-2) had results similar to those at 6 and 24 months in our IVR-only group (+7.2 and +7.7 letters, respectively).[32,33] However, its small sample size and the established treatment protocol obligating IVR at a frequency of

Table 1. Baseline characteristics of the patients and eyes in each cohort

	3-Month cohort (whole group)	6-month cohort	12-month cohort	24-month cohort	36-month cohort
Patients (eyes), n	854 (1372)	838 (1352)	722 (1185)	581 (972)	361 (623)
Age, years, mean ± SD	62.7±8.7	62.8±8.7	62.9±8.8	63.3±8.8	63.8±8.2
Sex, n (%)					
Female	455 (53.3)	447 (53.3)	385 (53.3)	325 (55.9)	203 (56.2)
Male	399 (46.7)	391 (46.7)	337 (46.7)	256 (44.1)	158 (43.8)
DM duration, years, mean ± SD	16.3±6.6	16.3±6.6	16.5±6.6	16.7±6.5	16.8±6.2
DM treatment, n (%)					
None	3 (0.4)	3 (0.4)	3 (0.4)	2 (0.3)	0 (0.0)
OAD	306 (35.8)	302 (36.0)	257 (35.6)	215 (37.0)	123 (34.1)
Insulin	483 (56.6)	471 (56.2)	404 (56.0)	327 (56.3)	288 (63.2)
Combination	62 (7.3)	62 (7.4)	58 (8.0)	37 (6.4)	10 (2.8)
Accompanying disorders, n (%)					
None	347 (40.6)	343 (40.9)	296 (41.0)	245 (42.2)	146 (40.4)
HT	481 (56.3)	469 (56.0)	402 (55.7)	315 (54.2)	198 (54.8)
CAD	115 (13.5)	113 (13.5)	98 (13.6)	71 (12.2)	51 (14.1)
CVA	7 (0.8)	6 (0.7)	5 (0.7)	4 (0.7)	2 (0.6)
CKD	37 (4.3)	36 (4.3)	31 (4.3)	22 (3.8)	19 (5.3)
BCVA, logMAR, mean ± SD	0.68±0.46	0.68±0.46	0.68±0.46	0.71±0.47	0.72±0.45
Glaucoma history, n (%)	148 (10.8)	146 (10.8)	127 (10.7)	114 (11.7)	65 (10.4)
PGA use, n (%)	49 (3.6)	49 (3.6)	41 (3.5)	37 (3.8)	23 (3.7)
Lens status, n (%)					
Phakic	1056 (77.0)	1040 (76.9)	911 (76.9)	742 (76.3)	467 (75.0)
Pseudophakic	316 (23.0)	312 (23.1)	274 (23.1)	230 (23.7)	156 (25.0)
DR grade, n (%)					
NPDR	999 (72.8)	985 (72.9)	865 (73.0)	709 (72.9)	486 (78.0)
PDR	373 (27.2)	367 (27.1)	320 (27.0)	263 (27.1)	137 (22.0)
CMT, µm, mean ± SD	482.61±180.32	482.70±180.83	475.88±178.62	479.68±185.47	482.79±196.13
Previous DME treatment, n (%)					
Treatment-naive	818 (59.6)	805 (59.5)	694 (58.6)	537 (55.2)	339 (54.4)
Previously treated	554 (40.4)	547 (40.5)	491 (41.4)	435 (44.8)	284 (45.6)
Treatment protocol, n (%)					
1+PRN	525 (38.3)	522 (38.6)	470 (39.7)	409 (42.1)	213 (34.2)
3+PRN	847 (61.7)	830 (61.4)	715 (60.3)	563 (57.9)	410 (65.8)
Initial anti-VEGF agent, n (%)					
Bevacizumab	60 (4.4)	60 (4.4)	59 (5.0)	58 (6.0)	57 (9.1)
Ranibizumab	893 (65.1)	876 (64.8)	787 (66.4)	631 (64.9)	359 (57.6)
Aflibercept	419 (30.5)	416 (30.8)	339 (28.6)	283 (29.1)	207 (33.2)

Anti-VEGF: Anti-vascular growth factor, BCVA: Best corrected visual acuity, CAD: Coronary artery disease, CKD: Chronic kidney disease, CVA: Cerebrovascular accident, DM: Diabetes mellitus, DME: Diabetic macular edema, DR: Diabetic retinopathy, HT: Hypertension, logMAR: Logarithm of the minimum angle of resolution, NPDR: Non-proliferative diabetic retinopathy, OAD: Oral antidiabetic, PDR: Proliferative diabetic retinopathy, PGA: Prostaglandin analogs, PRN: Pro re nata, SD: Standard deviation

more than 2 months on a PRN basis differentiates READ-2 from other RCTs regarding the risk of possible undertreatment.[32,33] Moreover, the 3-year extension period of the trial allowing monthly follow-up and PRN IVR injections resulted in a +10.3 mean letter gain from baseline with a mean of 5.4 IVIs during the third year (cumulative mean of 14.7 IVIs), further supporting undertreatment in the earlier study period.[34] The subsequent RESTORE study adopted a treatment protocol of monthly PRN IVR injections after starting with three loading doses.[35,36,37] However, the reported 12-, 24-, and 36-month functional and anatomical results of the RESTORE study were even worse than our results, with a much higher number of IVIs throughout the study period (Table 4).[35,36,37] These results can be explained by the fact that the proportion of eyes with an initial BCVA of 60 or fewer letters was relatively lower in the RESTORE study (33.0% and 27.7% in 12- and 24- to 36-month results, respectively) compared to our study (61.4%). Those ratios could have resulted in a so-called ceiling effect due to the higher proportion of better-seeing eyes in the RESTORE study.[35,36,37] However, the mean visual gains in the worse-seeing eyes (≤60 letters) were reported to be +8.2 and +10.5 letters in the 12- and 24-month results.[35,36]

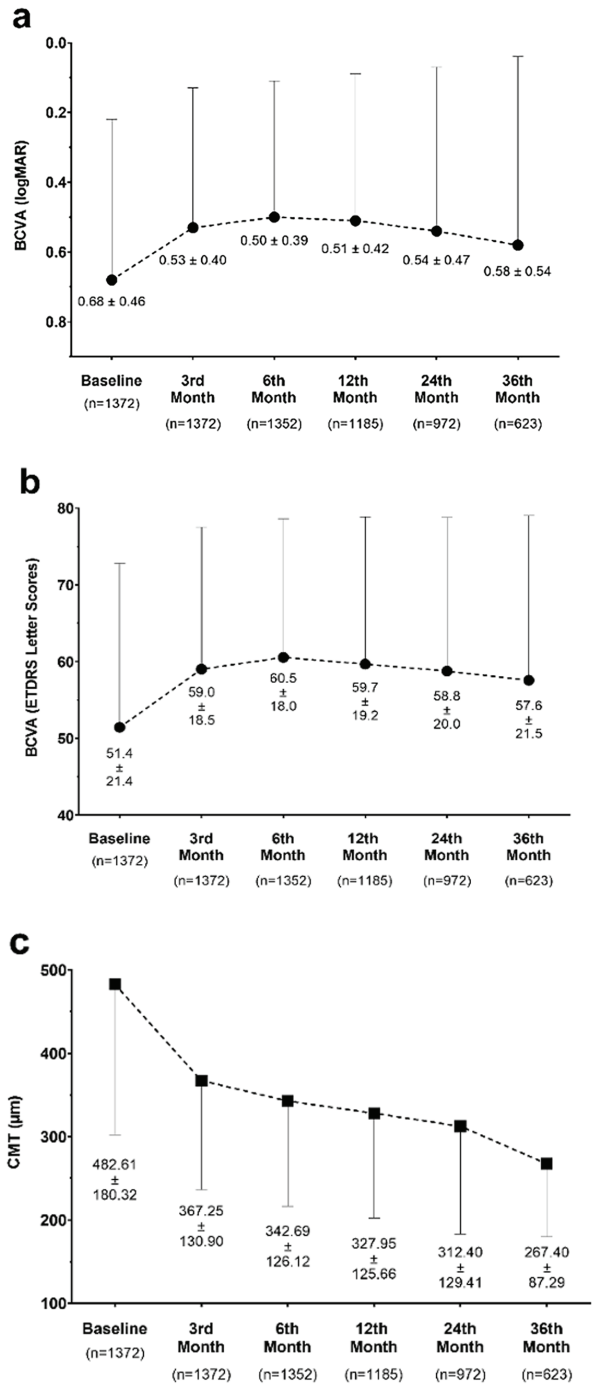

Figure 1. Best corrected visual acuity in logMAR (a) and ETDRS letter scores (b) and central macular thickness (c) of the eyes during the study period. Error bars indicate standard deviation

BCVA: Best corrected visual acuity, CMT: Central macular thickness, ETDRS: Early treatment diabetic retinopathy study, logMAR: Logarithm of the minimum angle of resolution

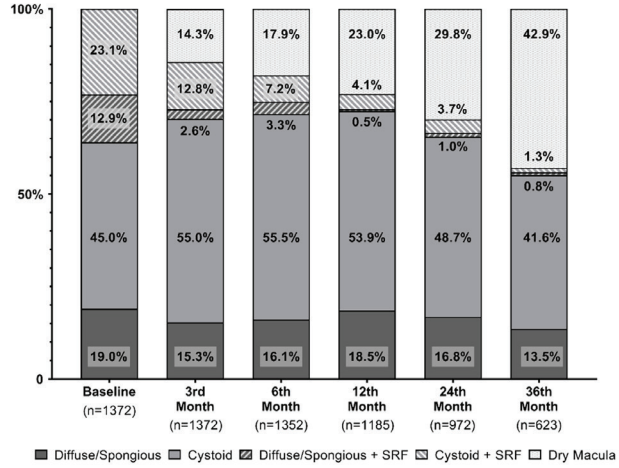

Figure 2. Diabetic macular edema patterns and dry macula rates during the study period

SRF: Subretinal fluid

The DRCR.net Protocol I study was a 5-year multicenter RCT comparing four treatments for DME (IVR plus deferred [after 24 weeks] vs. IVR plus prompt [within 1 week] macular laser photocoagulation vs. intravitreal triamcinolone plus prompt vs. intravitreal sham injections plus prompt macular laser photocoagulation) with protocol-defined re-treatment and follow-up criteria.[5,38,39,40] It was the first study providing level-1 evidence on the efficacy of an anti-VEGF agent (i.e., ranibizumab) for DME treatment, demonstrating improved and sustained BCVA for up to 5 years.[5,38,39,40] Although the injection frequencies per year gradually decreased during the study period, the number of cumulative injections, as well as letter gains, were also higher than in RWE studies like ours.[5,38,39,40] Further milestone RCTs comparing intravitreal anti-VEGF agents to sham and laser treatments also resulted in similar outcomes (Table 4).[8,9,10,11,12,41] Another DRCR.net study, Protocol T, was a 2-year RCT comparing the efficacies of PRN IVB, IVR, and IVA in DME, with protocol-defined re-treatment criteria, a salvage regimen, and scheduled visits (every 4 weeks in the first year and every 4 to 16 weeks in the second year depending on treatment response).[14,15] The 1- and 2-year results of Protocol T also demonstrated greater visual gains with a higher number of IVIs than in RWE studies and our report (Table 4).[14,15] However, the 5-year extension study of Protocol T after the randomized trial ended at the end of the second year showed that between 2 and 5 years, the median number of anti-VEGF IVIs was 4 (0-12), with only 68% of patients receiving at least one injection.[16] Moreover, although BCVA improved by 7.4 letters from baseline, patients were shown to have lost 4.7 letters from year 2 to 5.[16] On the other hand, the Protocol I study showed that when protocol-defined re-treatment with IVR continued, the mean visual gain at 1 year could be maintained for 5 years with a progressively diminishing number of injections.[40] The open-label extension study of RISE/RIDE trials also showed that the visual and anatomical gains achieved after monthly IVR were maintained

Table 2. Number of visits and intravitreal anti-vascular endothelial growth factor injections in each cohort

	3-month cohort (n=1372)	6-month cohort (n=1352)	12-month cohort (n=1185)	24-month cohort (n=972)	36-month cohort (n=623)
At 3 months					
Visits, median (IQR)					
Per year	-	-	-	-	-
Cumulative	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)	2 (2-2)
Injections*, median (IQR)					
Per year	-	-	-	-	-
Cumulative	3 (2-3)	3 (2-3)	3 (2-3)	3 (1-3)	3 (2-3)
At 6 months					
Visits, median (IQR)					
Per year	-	-	-	-	-
Cumulative	-	4 (4-5)	4 (4-5)	4 (4-5)	4 (4-5)
Injections*, median (IQR)					
Per year	-	-	-	-	-
Cumulative	-	3 (3-4)	3 (3-4)	3 (3-4)	3 (3-4)
At 12 months					
Visits, median (IQR)					
Per year	-	-	7 (6-10)	7 (6-9)	7 (6-9)
Cumulative	-	-	7 (6-10)	7 (6-9)	7 (6-9)
Injections*, median (IQR)					
Per year	-	-	5 (4-6)	5 (4-6)	5 (4-6)
Cumulative	-	-	5 (4-6)	5 (4-6)	5 (4-6)
At 24 months					
Visits, median (IQR)					
Per year	-	-	-	4 (4-5)	4 (4-5)
Cumulative	-	-	-	11 (9-14)	10 (9-13)
Injections*, median (IQR)					
Per year	-	-	-	2 (1-3)	2 (1-3)
Cumulative	-	-	-	7 (5-8)	7 (6-8)
At 36 months					
Visits, median (IQR)					
Per year	-	-	-	-	5 (4-7)
Cumulative	-	-	-	-	16 (14-18)
Injections*, median (IQR)					
Per year	-	-	-	-	2 (1-3)
Cumulative	-	-	-	-	9 (7-10)

*Injections include only intravitreal anti-vascular endothelial growth factors. IQR: Interquartile range

with protocol-defined PRN re-treatment and follow-up criteria up to a mean of 14.1 months of follow-up.[42] Likewise, the open-label extension study of VISTA (i.e., the ENDURANCE study), showed similar visual gains maintained by IVA through 12 and 24 months with an individualized PRN treatment protocol with reduced IVI frequency.[43,44] The differences between extension studies with and without protocol-defined re-treatment and follow-up criteria support the findings of undertreatment and lower visual gains in RWE studies.

During their treatment course in routine clinical practice, DME patients were shown to be affected more by patient-related non-adherence than other macular pathologies, as they usually have multiple comorbidities and a disease requiring individualized treatment patterns.[45,46,47,48] Numerous prospective and retrospective RWE studies involving these patients have provided complementary information about the effectiveness of intravitreal anti-VEGF agents on DME, particularly emphasizing the importance of number of follow-ups and injections to avoid undertreatment.[17,18,19,20,21,22,23,24,25,26,27,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63]

The prospective, non-interventional RWE of the OCEAN Study Group from Germany reported a mean of 4.4 and 5.5 IVR injections in 12 and 24 months, leading to mean BCVA gains of +4.0 and +5.2 letters from baseline, respectively.[49] They stated that BCVA changes from baseline were slightly greater in those receiving 7 or more injections (+6.3 and +6.1 letters in 12 and 24 months, respectively).[49] The relatively lower number of IVIs and visual gains than in our study could be attributed to the fewer OCT evaluations at follow-up visits in the OCEAN study due to reimbursement issues in Germany.[49] In contrast, OCT was employed in all follow-up visits in our study as a main contributor to the IVI decision (mean cumulative evaluations of 4.1 and 7.5 vs. 7.8 and 12.3 at 12 and 24 months, respectively). The prospective BOREAL-DME study from France reported mean

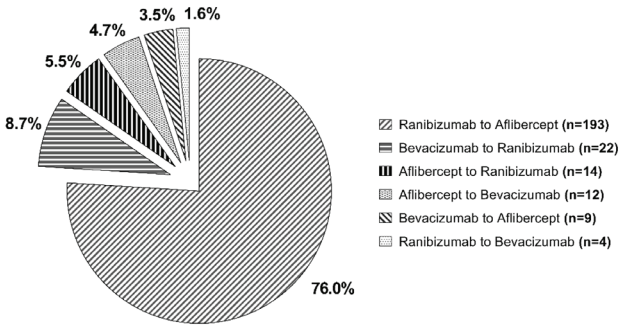

Figure 3. Rates of switches between intravitreal anti-vascular endothelial growth factor agents during the study period

BCVA gains of +7.4 and +4.1 with a cumulative mean of 5.1 and 7.6 anti-VEGF IVIs in 12 and 36 months, respectively.[20,50] Recently, the 2-year prospective APOLLON study from France reported a higher mean cumulative number of IVA injections (7.6 and 11.6) in 12 and 24 months, leading to +6.5 and +3.9 mean letter gains, respectively.[51,52] The authors attributed the relatively smaller visual improvements despite a higher number of IVIs at 2 years in the APOLLON study to structural changes related to the long-standing DME in previously treated patients.[52] One-year results of the global LUMINOUS study, which prospectively evaluated the effectiveness of IVR for any indications in real-life settings, showed that BCVA change from baseline in DME patients differs between -0.3 to +6.9 letters with mean numbers of IVR injections ranging from 2.2 to 6.0 among countries.[53] Additionally, better visual gains were observed in patients receiving 5 or more IVR injections (including loading doses) in the first year.[53]

In a 4-year retrospective RWE study from Denmark including 566 eyes with DME, the mean changes in BCVA and CMT from baseline to 12, 24, 36, and 48 months were reported as +3.9, +3.5, +2.7, +1.8, and +2.3 letters and -102.6, -106.9, -105.9, and -131.6 µm, respectively.[54] The mean number of IVIs per year gradually decreased from 6.1 in the first year to 3.0, 2.6, and 1.8 in the second, third, and fourth years, respectively.[54] The authors also reported an increase of 1.01 letters for every extra anti-VEGF IVI when adjusted for age and baseline BCVA, further emphasizing the importance of number of IVIs in visual prognosis.[54] Another 4-year retrospective RWE study from Sweden with a much smaller sample size of 102 eyes reported an improvement of +7.0 and +6.6 letters at 2 and 4 years with a mean of 4.7, 1.4, 0.7, and 0.9 IVIs per year in the first, second, third, and fourth years of the study, respectively.[55] A retrospective RWE study from Moorfields reported mean BCVA changes of +5.2, +4.8, +3.4, and +2.5 letters with mean cumulative IVI rates of 6.4, 8.9, 11.1, and 14.0 during 12, 24, 36, and 48 months of follow-up.[56] Other studies from different countries reported mean cumulative BCVA gains of +3.0-11.2 letters at 1 year with a mean of 3.1-8.0 IVIs,[17,18,19,21,22,23,26,57,58,59,60,61,63] +2.3-10.0 letters at 2 years with a mean of 5.0-12.8 IVIs,[18,19,21,22,58,60,62,63] and +3.0-6.9 letters at 3 years with a mean of 9.0-12.5 IVIs.[19,21,58]

Apart from demonstrating lower visual gains from RCTs due to lower injection frequencies and undertreatment, we observed relatively better BCVA letter gains than most RWE studies mentioned above. The probable reason is the so-called ceiling effect resulting from fewer gainable letters because of the better baseline BCVAs in those studies compared to ours (51.4 letters). For example, prospective RWEs such as the OCEAN,

Table 3. Outcomes of combination therapy with intravitreal dexamethasone implant in study cohorts

	Eyes n (%)	BCVA mean ± SD letters			CMT mean ± SD µm			Number of anti-VEGF IVIs n (IQR)	Number of visits n (IQR)
		Baseline	Final	Change	Baseline	Final	Change		
6-month cohort									
IDI (+)	1352 (100)	41.2±23.0	58.2±18.2	17.0±25.1	602.1±216.0	343.8±116.4	-258.3±251.7	3 (3-4)	4 (4-5)
IDI (-)	129 (9.5)	52.5±21.0	60.8±18.0	8.3±18.1	470.1±172.1	342.6±127.4	-127.5±167.2	3 (3-4)	4 (3-5)
p[a]	1223 (90.5)	**<0.001**	0.073	**0.001**	**<0.001**	0.891	**<0.001**	0.131	**0.005**
12-month cohort									
IDI (+)	1185 (100)	41.6±21.4	55.5±18.3	13.9±25.7	579.0±210.0	330.5±119.1	-248.5±252.4	5 (4-6)	7 (6-9)
IDI (-)	308 (26.0)	55.2±20.4	61.2±19.3	6.0±19.0	439.7±150.4	327.1±128.0	-112.6±182.8	5 (4-6)	8 (6-10)
p[a]	877 (74.0)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.356	**<0.001**	**0.001**	**<0.001**
24-month cohort									
IDI (+)	972 (100)	43.0±21.1	56.0±19.0	13.0±25.9	554.2±210.8	339.5±152.7	-214.7±236.2	7 (6-8)	10 (9-13)
IDI (-)	400 (41.2)	55.2±20.7	60.7±20.6	5.5±20.1	472.6±144.4	293.5±106.4	-134.1±154.7	6 (5-8)	11 (9-15)
p[a]	572 (58.8)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
36-month cohort									
IDI (+)	623 (100)	43.0±21.4	54.4±22.6	11.6±27.5	549.5±229.4	274.3±92.0	-275.2±261.4	9 (8-11)	16 (14-17)
IDI (-)	279 (44.8)	54.2±20.5	60.1±20.2	5.9±23.4	428.7±143.3	261.8±83.0	-166.9±168.6	9 (7-10)	16 (14-18)
p[a]	344 (55.2)	**<0.001**	**0.002**	**0.018**	**<0.001**	0.136	**<0.001**	**<0.001**	0.977

[a]Mann-Whitney U test. Bold values indicate statistical significance. Anti-VEGF: Anti-vascular endothelial growth factor, BCVA: Best corrected visual acuity, CMT: Central macular thickness, IDI: Intravitreal dexamethasone implant, IQR: Interquartile range, SD: Standard deviation

Table 4. Functional and anatomical gains, number of intravitreal injections, and macular laser rates in selected milestone randomized controlled trials

	Eyes (n)	BCVA change from baseline (ETDRS letters)	CMT change from baseline (µm)	Number of cumulative intravitreal injections (n)	Macular laser rates (%)
Our study					33.5 (overall)
3 months	1372	+7.6[a]	-115.4[a]	3.0[b]	
6 months	1352	+9.1[a]	-140.0[a]	3.0[b]	
12 months	1185	+8.0[a]	-147.9[a]	5.0[b]	
24 months	972	+8.6[a]	-167.3[a]	7.0[b]	
36 months	623	+8.4[a]	-215.4[a]	9.0[b]	
BOLT					
12 months[6]	42	+8.0[b]	-130.0[a]	9.0[b]	-
24 months[7]	37	+8.6[a]	-146.0[a]	13.0[b]	-
READ-2[c]					
6 months[32]	37	+7.2[a]	-106.7[a]	4.0[a]	-
24 months[33]	33	+7.7[a]	-78.9[a,d]	9.3[a]	-
36 months[34]	28	+10.3[a]	-132.0[a]	14.7[a]	-
RESTORE[c]					
12 months[35]	115	+6.8[a]	-118.7[a]	7.0[b]/7.0[a]	-
24 months[36]	83	+7.9[a]	-140.6[a]	10.0[b]/11.3[a]	16.9
36 months[37]	83	+8.0[a]	-142.9[a]	14.2[a]	24.1
RISE[e]					
24 months[8]	125	+11.9[a]	-253.1[a]	24.0[b]/20.9[a]	35.2
36 months[9]	125	+11.0[a]	-269.1[a]	34.0[b]/28.5[a]	37.6
RIDE[e]					
24 months[8]	127	+12.0[a]	-270.7[a]	24.0[b]/21.9[a]	19.7
36 months[9]	127	+11.4[a]	-266.7[a]	34.0[b]/30.4[a]	21.3
DRCR.net Protocol I[f]					
12 months[5]	188	+9.0[a]	-137.0[a]	9.0[a]	30.0
24 months[38]	139	+9.0[a]	-150.0[a]	12.0[a]	42.0
36 months[39]	147	+10.0[a]	-155.0[a]	15.0[a]	46.0
60 months[40]	111	+10.0[a]	-165.0[a]	17.0[a]	44.0
DRCR.net Protocol T					
12 months[14]					
IVB	206	+9.7[a]	-101.0[a]	10.0[b]	56.0
IVR	206	+11.2[a]	-147.0[a]	10.0[b]	46.0
IVA	208	+13.3[a]	-169.0[a]	9.0[b]	37.0
24 months[15]					
IVB	185	+10.0[a]	-126.0[a]	16.0[b]	64.0
IVR	191	+12.3[a]	-149.0[a]	15.0[b]	52.0
IVA	201	+12.8[a]	-171.0[a]	15.0[b]	41.0
VIVID					
52 weeks[10]	136[g]/135[h]	+10.5[g]/+10.7[h]	-195.0[g]/-192.4[h]	12.2[a,g]/ 8.7[a,h]	4.4[g]/8.1[h]
100 weeks[11]	136[g]/135[h]	+11.4[g]/+9.4[h]	-211.8[g]/195.8[h]	22.6[a,g]/13.6[a,h]	7.4[g]/11.1[h]
148 weeks[12]	136[g]/135[h]	+10.3[g]/+11.7[h]	-221.3[g]/-222.4[h]	32.0[a,g]/18.1[a,h]	7.4[g]/11.9[h]
VISTA					
52 weeks[10]	154[g]/151[h]	+12.5[g]/+10.7[h]	-185.9[g]/-183.1[h]	11.8[a,g]/8.4[a,h]	2.6[g]/0.7[h]
100 weeks[11]	155[g]/152[h]	+11.5[g]/+11.1[h]	-191.4[g]/-191.1[h]	21.3[a,g]/13.5[a,h]	3.2[g]/8.6[h]
148 weeks[12]	155[g]/152[h]	+10.4[g]/+10.5[h]	-204.6[g]/-212.7[h]	29.6[a,g]/18.1[a,h]	4.5[g]/10.5[h]
VIVID-east					
52 weeks[41]	122[g]/116[h]	+13.6[g]/+13.1[h]	-231.1[g]/-232.0[h]	12.6[g]/8.7[h]	7.1[g,i]/6.2[h,i]

[a]Mean value, [b]Median value, [c]Ranibizumab only group, [d]Manually calculated from Supplementary Table 2B of the original article by Nguyen et al.[33], [e]Ranibizumab 0.5 mg group, [f]Ranibizumab plus deferred laser group, [g]Aflibercept 2 mg intravitreal injections every 4 weeks, [h]Aflibercept 2 mg intravitreal injections every 8 weeks after 5 initial monthly dosing, [i]Proportion of eyes meeting the criteria for additional treatment, regardless of whether they received the treatment. ETDRS: Early treatment diabetic retinopathy study, CMT: Central macular thickness, IVA: Intravitreal aflibercept, IVB: Intravitreal bevacizumab, IVR: Intravitreal ranibizumab, DRCR.net: Diabetic Retinopathy Clinical Research Network

BOREAL-DME, APOLLON, and global LUMINOUS studies had patients with mean baseline BCVAs of 60.6, 59.2, 62.7, and 57.7 letters, respectively, even if they did not have any related exclusion criteria.[20,49,50,51,52,53] Similar differences also can be seen in relatively large-scale retrospective RWE from Denmark, Sweden, and Moorfields with baseline BCVAs of 64.9, 60.8, and 61.0, respectively.[54,55,56]

Recently, Durukan et al.[27] reported +8.3, +5.3, and +4.4 mean letter gains and -105.5, -107.7, and -114.3 µm CMT reductions compared to baseline with a mean of 4.6±2.0, 2.3±1.9, and 1.8±1.8 anti-VEGF IVIs per year in mutually exclusive groups of DME patients from Türkiye followed up for 12, 24, and 36 months, respectively. Those findings align with our results regarding IVI numbers of all cohorts and mean letter gains in the first year (8.0). However, better mean letter gains were observed in our 24- and 36-month cohorts (8.6 and 8.4, respectively), as well as better CMT reductions in all our cohorts. This discrepancy in BCVA gains could have resulted from Durukan et al.[27] excluding the eyes with visual acuity worse than 20/400 Snellen, resulting in a mean overall baseline BCVA of 55.6 letters, which is higher than ours. Also, although they stated that there were no significant differences in BCVA gains of the cohorts at any time, another reason could be the mutually exclusive nature of the cohort groups and adjunctive therapies the patients received, since there were also smaller reductions in CMT from baseline, especially at 24 and 36 months.[27] Furthermore, although they did not stratify according to cohort, the overall IDI combination rate (23.6%) was also lower than the corresponding cumulative IDI combination rates in our study (26.0%, 41.2%, and 44.8% for the 12-, 24-, and 36-month cohorts, respectively), which might explain our better BCVA letter gains and CMT reductions.[27] In another study recently published in Türkiye, the number of mean visits in both groups at 12 months (6.8±2.1 and 6.7±1.9) was similar to that in our study.[64]

While not allowed in RCTs evaluating anti-VEGFs in DME treatment, anti-VEGF switch and IDI combination rates and their effects on study outcomes are often ignored in RWE, or if they are not already an exclusion criterion, those eyes are removed from the outcome analysis.[19,51,52,53,54,56,57,60] Of the DME RWE studies reporting treatment switch rates, the rates of switching the index agent to any other anti-VEGF ranges from 8.5 to 20.9%[20,23,50,60] and rates of switching to IDI range from 3.9 to 26.7%[20,23,27,50,55] depending on the follow-up time. The overall anti-VEGF switch rate in our study is comparable to those reported studies, but the IDI combination rates are relatively higher. An RWE study of IDI for DME comparing treatment-naive and refractory eyes (i.e., the IRGREL-DEX Study) showed that the BCVA of the refractory eyes was improved by a mean of +7.3 letters and the mean CMT decreased from 565 to 313 µm in 24 months with a mean of 3.1 IDIs (range, 1-4), while 16.9% of the patients also received IVIs of anti-VEGFs.[65] Although we did not explicitly investigate the reason for IDI combination in our cohort, if these patients are considered resistant to anti-VEGFs, the results can be regarded as comparable to the IRGREL-DEX study.

The variable macular laser rescue treatment criteria of RCTs have resulted in different studies with several intravitreal agents reporting macular laser rates at various time intervals and during specific study dates (Table 4).[5,8,9,10,11,12,14,15,36,37,38,39,40,41] Nevertheless, the overall macular laser treatment rate in our study (33.5%) appears comparable to the rates of salvage therapy in RCTs. The TURK-DEM real-life registry study demonstrated that between the years of 2013 and 2014, the most common DME treatment preferences among Turkish retina specialists were laser photocoagulation (32.1%) and intravitreal anti-VEGF injection (31.8%), followed by their combination (30.8%).[66] As can be appreciated from our current study, those preferences seem to change with the growing literature supporting the superior outcomes of anti-VEGF agents and the risk of limiting visual gain potential by laser-induced iatrogenic structural damage.[40] Recently, subthreshold micropulse laser was shown to be non-inferior to macular laser in treating DME, with a slightly higher treatment rate.[67] There are also numerous reports of its additive effects as a combination therapy with anti-VEGFs, such as reducing the need for re-injection.[68,69] Therefore, although the gains in such a subgroup of patients are beyond the scope of this report, the use of micropulse laser as adjunctive therapy in this real-life DME treatment study (n=44, 3.2%) is worth mentioning.

Study Limitations

Several limitations should be considered while interpreting the results of this study. First of all, its retrospective, observational nature prevented randomization and intervention, reducing the reliability of effectiveness parameters. Similarly, the selected time intervals for assessing treatment outcomes were arbitrary rather than scheduled as in RCTs and may not have coincided with an actual effect. Also, the possibility of under-reporting any complication cannot be eliminated due to the retrospective data collection from patient files. Similarly, unstandardized re-treatment indications from different clinics would have affected the number of overall treatments and visits. Visual acuity evaluated in routine clinical practice may not reflect actual BCVA. Finally, the study population included patients who were treated before 2018 and according to drug reimbursement rules at that time. The reimbursement rules changed after 2018, and patients with DME in Türkiye have been treated according to the new reimbursement rules since that time. This may have altered the real-world data in Türkiye. However, strengths of the study are the relatively large sample size from a diverse DME patient population, the inclusion of different treatment modalities as a whole, the absence of exclusion criteria related to visual acuity (mirroring routine clinical practice), and the provision of complete data without using any imputation method for missing data.

Conclusion

This largest-scale RWE study from Türkiye provides further insights into the treatment of DME initiated with anti-VEGF agents, supporting the observations of less satisfactory anatomical

and functional real-life outcomes than in RCTs. Furthermore, our results also suggest that the lower number of IVIs is the probable reason, as in other RWE studies. Future reports from the MARMASIA Study Group will focus on specific groups of patients with particular disease characteristics, which is expected to increase the literature data on real-life DME treatment.

Ethics

Ethics Committee Approval: Kocaeli University Faculty of Medicine approved the study protocol (no: GOKAEK-2022/07.19, date: 14.04.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: MARMASIA Study Group, Concept: V.L.K., Design: V.L.K., A.Ö., Ö.Ş., Data Collection or Processing: MARMASIA Study Group, Analysis or Interpretation: MARMASIA Study Group, Literature Search: MARMASIA Study Group, Writing: U.Y., M.O.S.

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Original Article

Anterior Segment Changes and Refractive Outcomes after Cataract Surgery Combined with Gonioscopy-Assisted Transluminal Trabeculotomy in Open-Angle Glaucoma

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Abstract

Objectives: To compare the accuracy of intraocular lens (IOL) calculation formulas in patients undergoing phacoemulsification combined with gonioscopy-assisted transluminal trabeculotomy (phaco-GATT) and to determine the predictive factors for refractive errors.

Materials and Methods: Fifty-three eyes of 53 patients undergoing phaco-GATT were retrospectively reviewed. The preoperative and postoperative 3-month anterior segment (AS) parameters were measured by Scheimpflug camera. The mean prediction error (PE), mean absolute error (MAE) in the Sanders-Retzlaff-Kraft/theoretical (SRK/T), Barrett-Universal II, Hill-radial basis function (Hill-RBF) and Kane formulas were compared. The influence of biometric parameters on PE were analyzed by correlation analysis.

Results: Postoperatively, there was a statistically significant decrease in axial length (AL) and significant enlargement in anterior chamber depth (ACD), anterior chamber angle (ACA), and anterior chamber volume ($p<0.001$). The mean PE using SRK/T (-0.08 diopters [D]) was more myopic than in the Barret (0.01 D) and Hill-RBF (0.01 D). The PE closest to zero was in the Kane formula (0.001 D). The Kane formula provided a lower MAE (0.30±0.28 D) than the SRK/T (0.38±0.32 D) and Barrett (0.36±0.30 D) ($p<0.001$). The MAE in Hill-RBF (0.32±0.28) was comparable with that in Kane ($p=0.02$). Preoperative AL was significantly associated with PE in all formulas except Kane. Barrett was the only formula that did not have a significant correlation between PE and postoperative ACD and ACA.

Conclusion: The Kane formula may provide higher predictability of the IOL power calculation than the SRK/T and Barrett-Universal II formulas in phaco-GATT surgery, which can cause significant changes in the AS and AL.

Keywords: Gonioscopy-assisted transluminal trabeculotomy, intraocular lens formula, phacoemulsification, refractive error

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Introduction

Combining glaucoma surgery with cataract surgery is widely accepted as an appropriate procedure for the management of coexisting cataract and glaucoma.[1,2] Despite advances in surgical techniques, ocular biometry, and intraocular lens (IOL) calculation formulas, calculating IOL power remains a challenge in certain clinical cases such as glaucomatous eyes and combined cataract and glaucoma surgery.[3,4,5,6,7] In these special circumstances, the obstacles to accurate IOL calculation include the intraocular pressure (IOP)-lowering effect of surgery and instability of axial length (AL), keratometry (K), and anterior chamber depth (ACD).[8,9,10]

In eyes with glaucoma, micro-invasive glaucoma surgery (MIGS) has gained popularity as an adjunct procedure during cataract surgery. The reduced risk of a significant refractive surprise compared to traditional filtering surgery is one potential advantage of these less invasive approaches.[11,12,13,14] Gonioscopy-assisted transluminal trabeculotomy (GATT) is a newly described, minimally invasive, sutureless, and blebless procedure for the treatment of glaucoma.[15] The IOP-lowering effect of cataract surgery combined with GATT has been substantiated by several studies.[16,17,18] However, to our knowledge, no studies have investigated the refractive outcomes and predictive factors for refractive error after cataract surgery combined with GATT. Therefore, the present study aimed to evaluate the refractive

results of combined phacoemulsification and GATT (phaco-GATT) and determine the factors that can predict unstable refractive outcomes. In this study, we compared the postoperative refractive outcomes in the Sanders-Retzlaff-Kraft/theoretical (SRK/T), Barrett-Universal II, Hill-radial basis function (Hill-RBF), and Kane IOL calculation formulas. We also analyzed the change in IOP and anterior segment (AS) parameters after combined surgery to investigate the influence of these parameters on refractive results.

Materials and Methods

We retrospectively reviewed the medical records of patients with open-angle glaucoma (OAG) who had underwent uncomplicated phaco-GATT at a single center between September 2020 and July 2022. All research and measurements followed the tenets of the Declaration of Helsinki, and the Haydarpaşa Numune Training and Research Hospital Ethics Committee of the same hospital approved the protocol (decision no: HNEAH-KAK-KK-2022-210, date: 07.11.2022). The need for informed consent was waived.

The diagnostic criteria for OAG included gonioscopically-confirmed open angle, glaucomatous optic nerve head changes, and glaucomatous visual field defects with computerized visual field test (24-2 test, SITA Standard, Humphrey Visual Field Analyzer II; Carl Zeiss Meditec, Jena, Germany). Primary open-angle glaucoma (POAG) was defined as OAG with no secondary cause of glaucoma, and pseudoexfoliation glaucoma (PXG) as OAG with visible exfoliation material in the AS. Phaco-GATT surgery was performed in patients with visually disabling cataract whose IOP could not be controlled despite maximum medical treatment or who could not tolerate medical treatment.

The exclusion criteria included any history of ocular surgery or ocular trauma, coexisting eye diseases that could affect the refractive results (corneal or retinal diseases), intraoperative complications (capsular tear, zonule dialysis), and postoperative complications (prolonged corneal edema, macular edema, retinal detachment, additional glaucoma surgery). In addition, cases who had trabeculotomy of less than 180-degrees were defined as "failed GATT surgery" and were excluded. Eyes with dense cataracts requiring ultrasound biometry were not included in the study. As refractive outcomes could be affected, patients with a postoperative corrected distance visual acuity (CDVA) ≤40/200 and with a postoperative corneal astigmatism ≥2.0 diopters (D) were excluded.

If both eyes of a patient met the study criteria, the first operated eye was included.

All surgical procedures were performed under sub-Tenon anesthesia by one experienced glaucoma surgeon (S.İ.). Corneal incisions were formed in the superior and temporal quadrants with a 20-gauge knife. Ocular viscoelastic substance was injected into the anterior chamber. The patient's head and microscope were tilted to visualize the nasal angle, and a 1- to 1.5-mm goniotomy was made on the nasal iridocorneal angle using a direct gonioscopy lens through a temporal incision. A 6-0 Prolene suture (Kent Medical, Ankara, Türkiye), the end of which was blunted with cautery, was directed to the nasal angle through the superior incision. The suture was inserted into the goniotomy and advanced through the Schlemm canal. The distal edge of the suture protruding from the goniotomy was held, and trabeculotomy was performed by pulling both ends of the suture out of the temporal incision. In 20 cases, 180 to 270-degree trabeculotomy could be achieved. In all patients, a 2.8-mm clear corneal incision in the upper corneal limbus and phacoemulsification with the Infiniti Vision System (Alcon Laboratories, Fort Worth, TX, USA) were performed after GATT. An acrylic hydrophobic, foldable, one-piece IOL (Eyecryl Plus ASHFY600; Biotech Vision Care Pvt. Ltd., Ahmedabad, India) was implanted in the capsular bag. Cefuroxime axetil (1 mg/0.1 mL; Aprokam; Thea Pharma, Clermont-Ferrand, France) was administered into the anterior chamber at the end of surgery. After surgery, patients were treated with 0.5% moxifloxacin eye drops (Vigamox; Alcon Laboratories, Fort Worth, TX, USA) and 1% prednisolone acetate ophthalmic suspension (Pred Forte; AbbVie Biopharma, North Chicago, USA) 4 times per day during the postoperative first month.

Data Collection

The patient's sex, age, glaucoma type, and preoperative data including IOP measured by Goldmann applanation tonometer, CDVA as the logarithm of the minimum angle of resolution, AS parameters measured by Scheimpflug imaging (Sirius topography; Schwind eye-tech-solutions, Kleinostheim, Germany), AL, IOL power (D), and predicted refraction were recorded. AL and IOL power were calculated using partial coherence interferometry (IOL Master 500; Carl Zeiss Meditec, Jena, Germany). The SRK/T formula was used for selecting the IOL power for implantation, with an A-constant of 118.4.

Sirius topography was performed on non-dilated pupils in a standard dimly lit room, with 25 images per scan at the automatic release mode. The patient fixated on a far wall target to prevent accommodation. Scheimpflug camera measurements were exported only when the quality of the measurement showed "OK". ACD was determined as the distance from the central corneal endothelium to the anterior pole of the lens. Anterior chamber volume (ACV), ACD, anterior chamber angle (ACA), and central corneal thickness (CCT) were measured automatically by the Sirius device. Flat and steep K were also measured by the Sirius, and the mean K was calculated. Lens thickness (LT) was measured as the distance between the anterior and posterior surfaces of the crystalline lens. The mean of three values was used for statistical analysis.

Refraction measurements were obtained using an automatic refractor, then the manifest refraction that provided the best-corrected visual acuity from 6 meters was recorded. Manifest refraction was used for statistical analysis after converting to spherical equivalents (SEQ=spherical power+½ cylinder power). The prediction error (PE) was calculated by subtracting the expected refraction from the postoperative SEQ. The mean absolute error (MAE) was defined as the absolute deviation

between the postoperative SEQ and predicted refraction. The percentages of eyes with a PE greater than ±1.0 D, greater than ±0.75 D, and greater than ±0.50 D were calculated.

K, ACD, and AL measurements were manually entered into the online Barrett-Universal II calculator (https://calc.apacrs.org/barrett_universal2105/, accessed 28 February 2021), Hill-RBF calculator (Hill-RBF calculator version 3.0. https://rbfcalculator.com/, accessed 4 September 2020), and Kane formula calculator (https://www.iolformula.com/, accessed 16 February 2020) by one investigator (H.T.), and another investigator (S.İ.) checked the results. The lens factor for the Barrett-Universal II was 1.57. A-constants for Hill-RBF and Kane were 118.3 and 118.5, respectively. The predicted refraction in the Hill-RBF, Barrett II, and Kane formulas according to the implanted IOL power were recorded from the online calculation systems.

Postoperative 3-month examination findings, including refractive results, CDVA, IOP, AL, ACD, ACV, ACA, CCT, and K measurements, were recorded as postoperative outcomes. The changes in IOP, AL, mean K, and AS parameters were also calculated by subtracting the postoperative value from the preoperative value.

The primary outcome was to compare refractive results following phaco-GATT in four IOL calculation formulas. The secondary outcome was to determine the effect of preoperative and postoperative factors on the refractive results.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (v.20.0, IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov test was used to determine whether the continuous variables were normally distributed. To compare the accuracies of the four formulas, general linear model repeated measures test of the PE (with post-hoc Bonferroni analysis) and nonparametric Friedman test of the MAE (with post-hoc Wilcoxon signed-rank test) were used. Cochran Q test (with post-hoc McNemar test) was performed to compare the percentage of eyes within a certain range of PE between the four formulas. The preoperative and postoperative measurements were analyzed using paired-samples t-test and Wilcoxon test. The independent-samples t-test and Mann-Whitney U test were used for the comparisons of parameters between the 360-degree GATT and 180- to 270-degree GATT subgroups. To determine the association between the pre- and postoperative parameters and PE, Pearson and Spearman correlation analyses were performed. Continuous variables were presented as mean ± standard deviation and categorical variables as percentages (%). A p value <0.05 was considered statistically significant.

Results

Fifty-three eyes of 53 patients with a mean age of 69.26±5.96 years were included in this study. There were 23 (43.4%) men and 30 (56.6%) women, as well as 39 (73.6%) eyes with PXG and 14 (26.4%) eyes with POAG.

Comparisons of the ocular characteristics and AS measurements before and after phaco-GATT are shown in Table 1. Visual acuity improvement and decrease in IOP after surgery were statistically significant (p<0.001). There was a statistically significant decrease in AL (p<0.001) and significant increases in ACD, ACA, ACV (p<0.001), and CCT (p=0.02).

The mean IOL power was 20.27±3.53 D. The postoperative mean spherical power, cylinder power, and SEQ were -0.25±0.58 D, -0.90±0.45 D, and -0.68±0.53 D, respectively. There were statistically significant differences in PE, MAE, and percentages of myopic PE lower than -0.50 D among the four IOL formulas (p<0.05). Post-hoc analysis for PE showed statistically significant differences between the SRK/T and the Barrett and Hill-RBF formulas (p<0.05). There were statistically significant differences

Table 1. Comparison of the ocular characteristics and anterior segment measurements before and after combined cataract surgery and GATT

Parameter mean ± SD (range)	Preoperative	Postoperative	Mean change	p value
CDVA (logMAR)	0.80±0.63 (0.30-3.10)	0.09±0.11 (0.0-0.40)	-0.71±0.60 (-3.10--0.08)	<0.001*
IOP (mmHg)	21.09±5.79 (11.0-40.0)	14.16±3.38 (8.0-21.0)	-6.92±6.18 (-29.0-1.0)	<0.001*
Flat K (D)	43.37±1.50 (40.41-46.53)	43.30±1.60 (40.07-47.25)	-0.07±0.50 (-0.96-1.31)	0.09
Steep K (D)	44.19±1.42 (41.65-47.32)	44.13±1.51 (41.33-48.25)	-0.05±0.48 (-0.89-1.51)	0.42
Mean K (D)	43.78±1.44 (41.11-46.93)	43.72±1.54 (40.75-47.75)	-0.06±0.44 (-0.82-1.41)	0.05
Corneal astigmatism (D)	0.81±0.49 (0.05-1.94)	0.83±0.42 (0.05-1.68)	0.02±0.42 (-1.22-1.10)	0.71
AL (mm)	23.80±1.32 (22.02-28.07)	23.60±1.32 (21.70-27.85)	-0.19±0.12 (-0.43-0.37)	<0.001*
ACD (mm)	2.70±0.37 (1.92-3.44)	3.54±0.37 (2.15-4.51)	0.83±0.39 (0.13-1.89)	<0.001*
ACV (mm^3)	136.1±26.41 (86.0-210.0)	176.5±23.35 (121.0-221.0)	40.35±19.43 (9.0-88.0)	<0.001*
ACA (°)	40.79±6.91 (29.0-53.0)	53.52±5.45 (38.0-65.0)	12.73±5.69 (4.0-28.0)	<0.001*
CCT (µm)	531.4±34.15 (450.0-590.0)	536.3±39.88 (455.0-624.0)	4.94±15.10 (-18.0-68.0)	0.02*
LT (mm)	1.48±0.46 (0.50-2.22)			

*p<0.05. GATT: Gonioscopy-assisted transluminal trabeculotomy, SD: Standard deviation, logMAR: Logarithm of the minimum angle of resolution, CDVA: Corrected distance visual acuity, IOP: Intaocular pressure, K: Keratometry D: Diopters, AL: Axial length, ACD: Anterior chamber depth, ACV: Anterior chamber volume, ACA: Anterior chamber angle, CCT: Central corneal thickness, LT: Lens thickness

between Kane and SRK/T, Kane and Barrett, and also Hill-RBF and Barrett in pairwise comparisons for MAE (p<0.008). The only statistically significant difference in myopic surprise frequency was between SRK/T and Kane (p<0.008) (Table 2).

Comparisons of preoperative and postoperative CDVA, IOP, AL, and AS between eyes with 360-degree GATT and those with 180- to 270-degree GATT are presented in Table 3. The only statistically significant difference between groups was in preoperative CDVA (p=0.01), and this difference became insignificant after surgery (p=0.80). There were no statistically significant differences between the two subgroups in MAE or PE with any of the investigated formulas (p>0.05) (Table 4).

Correlation analysis for PE in SRK/T revealed that there was a statistically significant negative correlation with preoperative AL (p=0.04) and significant positive correlation with postoperative ACD (p=0.04) and postoperative ACA (p=0.008) (Figure 1). The only statistically significant association for PE was with preoperative AL in the Barrett-Universal II (p=0.03) (Figure 2). For PE in Hill-RBF, there was a statistically significant negative correlation with preoperative AL (p=0.04) and a significant positive correlation with postoperative ACA (p=0.01) (Figure 3). In the results with the Kane formula, PE was significantly positively associated with postoperative ACD (p=0.02) and postoperative ACA (p=0.005) (Figure 4). The PE did not show any significant association with age, CDVA, LT, ACV, CCT, or keratometric values in all four IOL formulas. There was also no statistically significant correlation between PE in any formula and preoperative IOP, postoperative IOP, or reduction in IOP (p>0.05).

Discussion

Combined cataract surgery and trabeculectomy was recently shown to cause changes in AS configuration and AL.[19,20] Even if newer angle-based procedures provide less dramatic IOP-lowering than trabeculectomy, significant changes in AS following combined cataract surgery and MIGS have been reported.[21,22,23,24] Changes in IOP and AS may cause unexpected results in refractive findings following combined surgery, so the chosen IOL calculation formula may become more critical in these cases. To our knowledge, our study is the first analysis of refractive outcomes in different IOL formulas and changes in AS parameters after phaco-GATT.

In our study, there was a significant decrease in AL and significant increases in ACD, ACA, ACV, and CCT after combined surgery. The Kane formula produced a higher predictability of IOL power calculation compared to SRK/T and Barrett-Universal II. The refractive outcomes in Hill-RBF were comparable with those in the Kane formula. The AS parameters and refractive outcomes did not differ between 360-degree GATT and 180- to 270-degree GATT.

In the published literature discussing the PE results in MIGS combined with cataract surgery, traditional IOL formulas have been used in all studies.[11,12,13,14,25] Luebke et al.[11] reported a mean PE of 0.53 D in patients who had combined cataract and trabectome surgery. In a study by Sieck et al.,[13] refractive error occurred in 20 (26.3%) of 76 eyes that underwent Kahook Dual Blade-goniotomy with phacoemulsification. Fifteen cases with refractive surprise in this group were between ±0.50 and ±1.00 D of the intended target. Scott et al.[14] reported 95% and 80% of 76 eyes were within ±1.0 D and ±0.50 D, respectively, in the combined trabecular micro-bypass stent and cataract surgery group. Ioannidis et al.[25] determined the MAE was 0.36±0.25 D, with 73.9% of 89 eyes within 0.50 D and with 98.9% within 1.00 D of the predicted refractive target after trabecular micro-bypass stent combined with cataract surgery. In the present

Table 2. Refractive outcomes after combined cataract surgery and GATT in four intraocular lens calculation formulas

Parameter mean ± SD	SRK/T	Barrett Universal II	Hill-RBF	Kane	p value
PE (D)	-0.076±0.45	0.011±0.43	0.010±0.41	0.001±0.39	0.004*
MAE (D)	0.38±0.32	0.36±0.30	0.32±0.28	0.30±0.28	<0.001*
PE > ±0.50 D (n, %) Myopic PE <-0.50 D Hyperopic PE >0.50 D	18 (34) 13 (24.5) 5 (9.4)	16 (30.2) 8 (15.1) 8 (15.1)	14 (26.4) 6 (11.3) 8 (15.1)	11 (20.8) 4 (7.5) 7 (13.2)	0.07 <0.001* 0.26
PE > ±0.75 (n, %)	5 (9.4)	4 (7.5)	3 (5.7)	3 (5.7)	0.46
PE > ±1.00 (n, %)	0	0	0	0	-
Pairwise comparisons		PE[a]	MAE[b]	Myopic PE <-0.50 D[c]	
SRK/T vs. Barrett Universal II		0.01*	0.58	0.06	
SRK/T vs. Hill-RBF		0.03*	0.01	0.01	
SRK/T vs. Kane		0.08	<0.001*	0.004*	
Barrett Universal II vs. Hill-RBF		>0.99	0.004*	0.50	
Barrett Universal II vs. Kane		>0.99	<0.001*	0.12	
Hill-RBF vs. Kane		>0.99	0.02	0.50	

*p<0.05, [a]Post-hoc analysis with Bonferroni correction (*p<0.05), [b]Post-hoc analysis with Wilcoxon signed rank test (*p<0.008), [c]Post-hoc analysis with McNemar test (*p<0.008). GATT: Gonioscopy-assisted transluminal trabeculotomy, SD: Standard deviation, SRK/T: Sanders-Retzlaff-Kraff/theoretical, Hill-RBF: Hill-radial basis function, MAE: Mean absolute error, PE: Prediction error, D: Diopters

Table 3. Comparisons between 360-degree GATT and 180- to 270-degree GATT

Parameter mean ± SD	360-degree GATT (n=33)	180- to 270-degree GATT (n=20)	p value
CDVA (logMAR)			
Preoperative	0.69±0.57	0.99±0.68	0.01*
Postoperative	0.10±0.13	0.08±0.10	0.80
Mean change	-0.58±0.54	-0.91±0.65	0.008*
IOP (mmHg)			
Preoperative	21.15±6.22	21.00±5.17	0.93
Postoperative	14.06±3.40	14.35±3.42	0.76
Mean change	-7.09±6.57	-6.65±5.65	>0.99
Mean K (D)			
Preoperative	43.89±1.56	43.60±1.25	0.48
Postoperative	43.75±1.64	43.65±1.41	0.91
Mean change	-0.13±0.41	0.05±0.49	0.34
Corneal astigmatism (D)			
Preoperative	0.78±0.44	0.86±0.56	0.59
Postoperative	0.85±0.41	0.80±0.44	0.67
Mean change	0.06±0.33	-0.05±0.54	0.29
AL (mm)			
Preoperative	23.88±1.46	23.66±1.08	0.77
Postoperative	23.69±1.46	23.45±1.05	0.65
Mean change	-0.19±0.12	-0.20±0.12	0.89
ACD (mm)			
Preoperative	2.74±0.40	2.64±0.32	0.25
Postoperative	3.54±0.37	3.53±0.37	0.93
Mean change	0.80±0.39	0.89±0.39	0.46
ACV (mm^3)			
Preoperative	138.54±29.17	132.15±21.21	0.39
Postoperative	177.78±23.87	174.35±22.91	0.60
Mean change	39.24±21.25	42.20±16.32	0.59
ACA (°)			
Preoperative	40.93±6.76	40.55±7.31	0.84
Postoperative	53.33±5.39	53.85±5.66	0.74
Mean change	12.39±6.20	13.30±4.82	0.39
CCT (µm)			
Preoperative	533.21±32.13	528.40±37.92	0.62
Postoperative	537.12±34.93	529.70±40.13	0.48
Mean change	3.90±10.38	1.30±8.64	0.60
LT (mm)	1.49±0.50	1.47±0.39	0.85

*p<0.05. GATT: Gonioscopy-assisted transluminal trabeculotomy, SD: Standard deviation, logMAR: Logarithm of the minimum angle of resolution, CDVA: Corrected distance visual acuity, IOP: Intraocular pressure, K: Keratometry, D: Diopters, AL: Axial length, ACD: Anterior chamber depth, ACV: Anterior chamber volume, ACA: Anterior chamber angle, CCT: Central corneal thickness, LT: Lens thickness

study, PE greater than ±0.50 D was demonstrated in 34%, 30%, 26%, and 21% of cases, respectively, in the SRK/T, Barrett II, Hill-RBF, and Kane formulas. There was no refractive error greater than ±1.0 D in any of the investigated IOL formulas.

A few studies have evaluated refractive results with different IOL formulas in combined cataract and glaucoma surgery.[26,27,28] Iijima et al.[26] compared the accuracy of IOL power calculation using the SRK/T and Barrett-Universal II formulas in 56 eyes

Table 4. Refractive outcomes of 360-degree GATT and 180- to 270-degree GATT

Parameter (mean ± SD)	360-degree GATT (n=33)	180- to 270-degree GATT (n=20)	p value
PE (D)			
SRK/T	-0.06±0.45	-0.09±0.47	0.84
Barrett Universal II	0.03±0.43	-0.02±0.45	0.67
Hill-RBF	0.01±0.38	0.00±0.47	0.96
Kane	0.01±0.34	-0.01±0.48	0.83
MAE (D)			
SRK/T	0.38±0.24	0.38±0.42	0.23
Barrett Universal II	0.35±0.23	0.36±0.39	0.39
Hill-RBF	0.31±0.20	0.34±0.39	0.37
Kane	0.27±0.19	0.34±0.38	0.70

GATT: Gonioscopy-assisted transluminal trabeculotomy, SD: Standard deviation, PE: Prediction error, D: Diopters, SRK/T: Sanders-Retzlaff-Kraft/theoretical, Hill-RBF: Hill-radial basis function, MAE: Mean absolute error

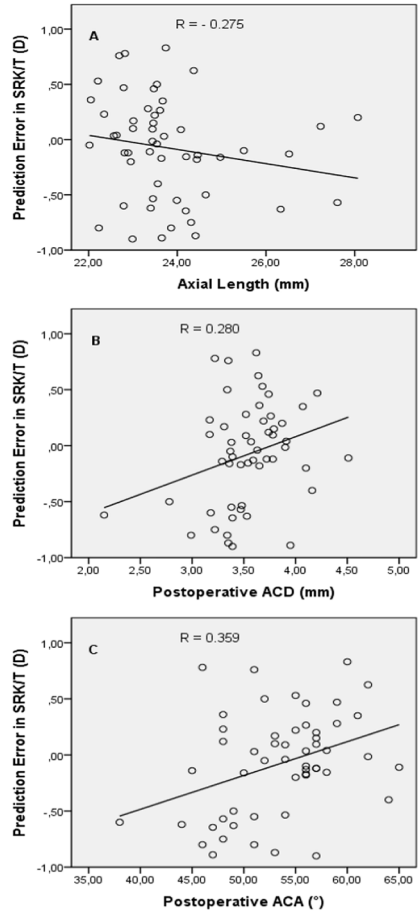

Figure 1. Scatterplot of mean prediction error in the SRK/T formula versus preoperative axial length (A), postoperative anterior chamber depth (ACD) (B), and postoperative anterior chamber angle (ACA) (C)
SRK/T: Sanders-Retzlaff-Kraft/theoretical, D: Diopters

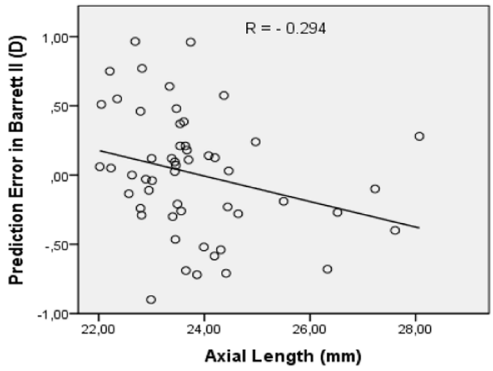

Figure 2. Scatterplot of mean prediction error in the Barrett Universal II formula versus preoperative axial length
D: Diopters

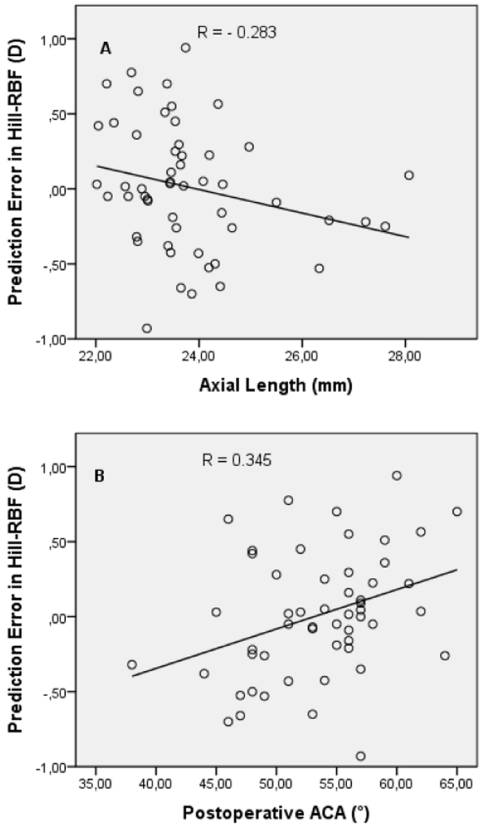

Figure 3. Scatterplot of mean prediction error in the Hill-RBF formula versus preoperative axial length (A) and postoperative anterior chamber angle (ACA) (B)
Hill-RBF: Hill-radial basis function, D: Diopters

after combined trabeculectomy and cataract extraction and found that the Barrett provided a smaller absolute error. Marta et al.[27] analyzed refractive errors in the Haigis, SRK/T, Holladay 1, Hoffer Q, Barrett-Universal II, Hill-RBF, and Kane formulas in combined cataract surgery and Ahmed glaucoma valve implantation. They reported that in the eyes with anterior chamber implant, the formula with the best PE was Barrett

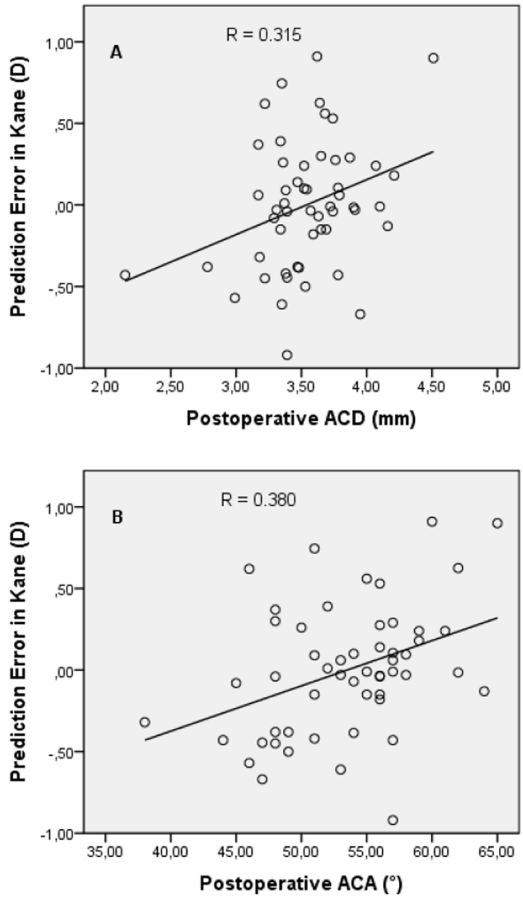

Figure 4. Scatterplot of mean prediction error in the Kane formula versus postoperative anterior chamber depth (ACD) (A) and postoperative anterior chamber angle (ACA) (B)
D: Diopters

II. Li et al.[28] evaluated the accuracy of SRK/T, Hoffer Q, Barrett II and Kane formulas in 111 eyes with primary angle-closure glaucoma (PACG) that underwent goniosynechialysis with phacoemulsification. The Kane (-0.06 D) and Barrett II (-0.07 D) formulas had a mean PE close to zero, while the Hoffer Q (-0.26 D) and SRK/T (-0.21 D) produced significantly myopic outcomes.[28] Although SRK/T showed significantly more myopic outcomes among the four formulas in our study, the mean PE (-0.08 D) was closer to zero than in the study by Li et al.[28] This difference may be due to the inclusion of eyes with PACG in the previous study.

In two previous studies that have reported the refractive outcomes of the latest formulas using the largest database in the literature, the Kane formula was found to be the most accurate compared to other traditional and newer formulas.[29,30] The Kane formula is a new formula that combines theoretical optics with artificial intelligence to calculate IOL power.[31] Similar to these studies, we obtained results closest to zero in mean PE and MAE with the Kane formula. The second-best outcomes were in the Hill-RBF formula, which uses artificial intelligence

and regression analysis of a large database of actual postsurgical refractive results for IOL power calculation.[32]

There are only three studies evaluating the AS changes in combined cataract surgery and MIGS.[22,23,24] In a study by Shao et al.,[22] ACA widened significantly after phaco-goniosynechialysis in 20 eyes with PACG. Moghimi et al.[23] indicated an improvement in gonioscopic measurements with AS-optical coherence tomography (AS-OCT) after phaco-viscogonioplasty in 45 eyes with PACG. Akil et al.[24] investigated AS parameters with AS-OCT following combined trabectome and cataract surgery in 20 OAG eyes and reported mean increases of 0.5±0.11 mm in ACD, 26.65±8.8 mm^3 in ACV, and 7.8±1.58° in trabecular iris angle. In our study, there was a mean increase of 0.83±0.39 mm, 40.35±19.43 mm^3, and 12.73±5.69° in ACD, ACV and ACA, respectively.

Postoperative ACD was shown to be a potential factor in postoperative refractive surprise.[33] IOP change, shallow ACD, worse preoperative visual acuity, and higher preoperative IOP were found to be risk factors for refractive error after combined cataract and glaucoma surgery.[8,10,13] In the present study, postoperative ACD and ACA correlated significantly with mean PE in the SRK/T and Kane formulas. In Hill-RBF, postoperative ACA was the only AS parameter significantly associated with PE. Preoperative AL correlated with the errors in all formulas except Kane. It is suggested that the Kane formula was not susceptible to AL, even in eyes undergoing phaco-GATT. This is consistent with previous studies reporting that the Kane formula was the most accurate IOL calculation formula for all ranges of ALs in cataract surgery alone when compared to the traditional and new-generation IOL formulas.[34,35]

Strengths of our study are the use of a single IOL model implanted by a single experienced surgeon and the exclusion of eyes with postoperative CDVA ≤20/400 and corneal astigmatism ≥2.0 D to ensure reliable refraction could be achieved. The results of both the traditional IOL calculation formula (SRK/T) and the newer IOL formulas (Barrett-Universal II, Hill-RBF, and Kane) were reported. Finally, this is a novel study reporting changes in AS parameters after phaco-GATT surgery and their effect on refractive outcomes.

Study Limitations

The study was performed retrospectively. We did not have a cataract surgery only control group, so the effect of GATT itself on refractive accuracy and AS configuration remains unclear. We could not analyze the effect of cataract density on refractive results, but LT was recorded and no significant relationship was found with the refractive results. Postoperative mean CCT was significantly greater than baseline. This may be due to the surgical parameters such as surgical time and cumulative dissipated energy. However, we could not record these parameters because of the retrospective nature of the study. As postoperative K values and corneal astigmatism did not differ from preoperative values, we think that the change in CCT did not affect our refractive outcomes. A Scheimpflug camera was used for the analysis of AS parameters in our study. Different associations may be found with different devices such as AS-OCT. Glaucoma subtype could have some effect on AS configuration and refraction, but our sample size was insufficient for subgroup analysis. A prospective study with a large number of patients would be helpful for determining the difference between POAG and PXG.

Conclusion

Our results support the view that the Kane formula may provide higher predictability of the IOL power calculation than the SRK/T and Barrett-Universal II in eyes undergoing phaco-GATT. The accuracy of Hill-RBF 3.0 was comparable to that of the Kane formula. The only PE that did not have a significant correlation with AL was in the Kane formula. Postoperative enlarged ACD and ACA were associated with more hyperopic PE. This information may be clinically helpful for choosing the most accurate IOL formula when planning combined cataract and GATT surgery, which may cause unexpected changes in AS and AL.

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Ethics

Ethics Committee Approval: Haydarpaşa Numune Training and Research Hospital Ethics Committee of the same hospital approved the protocol (decision no: HNEAH-KAK-KK-2022-210, date: 07.11.2022).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.İ., H.T., Concept: H.T., S.İ., Design: H.T., M.S.M., Data Collection or Processing: H.T., S.İ., M.S.M., Analysis or Interpretation: H.T., M.S.M., Literature Search: H.T., S.İ., Writing: H.T.

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Invited Review

Golden Indications and an Overview on the Use of Botulinum Toxin in Strabismus

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Abstract

Botulinum toxin A (BTXA) is considered a pharmacological agent that may provide an alternative treatment to surgery in strabismus. It can be used as both a diagnostic and therapeutic agent in various concomitant, paralytic, and restrictive disorders. The major advantage of BTXA treatment is that it is non-invasive and does not impact the patient's chance for future surgery in case of an unfavorable response. In some selected disorders, BTXA has become the primary choice of treatment, whereas surgery is found to be more effective in others. Accumulated knowledge and experience have demonstrated that BTXA is more than merely an alternative treatment and has additional specific indications such as in unstable deviations and as an adjunct to surgery. Patients with recurrent deviations despite multiple surgeries are also good candidates for BTXA treatment. Although the major expectation is to obtain a permanent result, BTXA can also be used as a maintenance treatment. This paper mainly focuses on the current indications for the use of BTXA in strabismology, with special emphasis on ideal first-choice applications referred to as "golden indications," within the scope of the author's own experience with the use of BTXA over 30 years.

Keywords: Botulinum toxin, strabismus, strabismus surgery, paralytic strabismus, pharmacological treatment of strabismus

In memory of John P. Lee and Alan B. Scott…

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Introduction

Botulinum toxin A (BTXA) has been used for the pharmacological treatment of strabismus for over three decades. In most of the strabismus literature it is mainly regarded as an alternative to surgery depending on the choice of the strabismologist. However, accumulated knowledge and experience have demonstrated that BTXA is not only an alternative therapy but has some additional indications where surgery is not a good option.[1,2] Despite a dearth of randomized controlled trials, BTXA seems to have comparable results with surgery in selected motility problems.[3,4,5] In this paper, the best indications for the use of BTXA, hereafter referred to as "golden indications," will be highlighted with literature results and the author's own experience with the use of BTXA in strabismus over 30 years.

Historical Perspective

The first person to conceive of injecting a pharmacological agent into the extraocular muscles (EOM) to weaken their function was Conrad Behrens, who had unsuccessful results with alcohol due to tissue necrosis and permanent paralysis.[6] Scott[6,7] found that BTXA could be used for the treatment of strabismus after testing various drugs in the EOM of monkeys. Human studies were started in 1977, and US Food and Drug Administration approval was obtained in 1989 for its use in adults and children over 12 years of age with strabismus.

Mechanisms of Effect

Eight antigenic types of botulinum toxin have been identified and type A is used in strabismus. BTXA blocks acetylcholine release, interferes with calcium metabolism, and creates a "chemodenervation" effect. After injection into an EOM, the maximum effect is reached in 5-7 days and the paralytic effect lasts for 2 months. The overall weakening effect of BTXA lasts for 6-9 months. During the effect of BTXA, a relative contracture of the antagonist is expected to occur.[8]

Although there are a number of new products either on the market or on the way, the main commercial BTXA products in the market with nearly worldwide approval are Botox (onabotulinumtoxin A, Allergan), Dysport (abobotulinumtoxin A, Ipsen), and Xeomin (incobotulinumtoxin A, Merz).

BTXA has a temporary effect on EOM but may result in a permanent decrease in deviation. Possible mechanisms of this permanent effect may be related to alterations in sarcomer number during the effect of BTXA, immunohistochemical changes, central adaptive mechanisms that manifest with improved binocularity, and as we suggested, a "traction suture-like" effect during the overcorrection period.[9,10,11,12]

Injection Techniques and Dosage

The gold standard method of BTXA injection is under topical anesthesia with electromyographic (EMG) guidance using a monopolar needle electrode to ensure the injection is administered to the target tissue, and it is mainly an outpatient treatment. The injection can also be performed using an insulin syringe by grasping the EOM with Mendonça forceps, although this inevitably means a more anterior injection and more discomfort for the patient. This method works well in medial rectus (MR) muscles without previous recession. In EOM with previous recession, grasping the EOM is challenging and there is less chance of reaching the target tissue. Inferior rectus (IR) muscle injections should better be performed with EMG guidance through the lower eyelid while the EMG signal is heard on depression. Without EMG guidance, it is not possible to determine whether the injection is made in the inferior oblique or IR muscle. Sub-Tenon injections are imprecise and have a higher risk of spread to neighboring EOM. Injection under direct visualization through a surgical opening is an invasive method which is antithetical to the non-invasive nature of BTXA treatment and should only be considered if the injection is done in combination with surgery.

In children, the injection should be done under ketamine anesthesia in order not to alter the EMG signals. We prefer injection without EMG control only in infants.

The usual dose for EOM is 2.5-10 units (U) and unlike the skeletal muscles, the same dosage may be used for both Botox and Dysport in the EOM. There is no real dose-response curve for BTXA injection. Our preferred standard dose is 5 U; we reduce the dose to 2.5 U in infants and increase the dose up to 10 U in thyroid orbitopathy. Larger doses result in more spread to adjacent tissues and increased complications rather than increased effect, and early overcorrection is a good indicator for a better outcome.[13,14] If the desired effect is not achieved, a repeat BTXA injection can be added in the 1-week follow-up visit.

The most commonly injected EOM are the MR, lateral rectus (LR), and IR. In general, oblique muscle injections are not as successful as rectus muscles.

Advantages of BTXA Treatment

The major advantages of BTXA treatment over surgery are the non-invasive nature of the treatment, absence of scar tissue formation, and ability to perform unlimited repeat injections without interfering with future chance of surgical treatment. BTXA injection may be used as a maintenance treatment in patients with multiple failed previous surgeries and those who refuse surgery for any reason. It is an outpatient treatment in adults and requires a very short period of ketamine anesthesia in children. In busy clinics, the cost of the drug does not incur much financial burden.

General Indication Categories

Although the temporary effect of BTXA is regarded as a disadvantage, it is advantageous in certain cases where surgery is not an option, such as unstable deviations and those with the risk of postoperative diplopia. The use of BTXA during the acute phase of paralytic, restrictive, or concomitant deviations is an additional indication, as surgery is not an alternative during this unstable period.

The major indications of BTXA may be categorized as diagnostic use, therapeutic use, and adjunctive use to increase the success of surgery.

Diagnostic Use of BTXA

Botulinum toxin may be used to assess the potential function of the paretic muscle, which may be masked by antagonist contracture. If the function of the paretic EOM increases with release of the contracture of the antagonist muscle by BTXA injection, this alters the surgical plan.[15]

Assessment of postoperative diplopia risk is another major indication for diagnostic use. Patients who report diplopia in the postoperative diplopia test may not have diplopia when their eyes are surgically aligned, but some of those patients have permanent diplopia after surgery. Injection of BTXA provides a temporary orthophoric period and enables the identification of those who will have a permanent diplopia problem.[16,17]

Central fusion disruption is a challenging problem with an unfavorable outcome.[18,19,20] Intractable diplopia related to central fusion disruption is a golden indication for BTXA.[21,22,23] The main advantage of BTXA compared to prisms is to provide an orthophoric period under real-life conditions. Even conventional prisms have a negative effect on quality of vision, albeit to a lesser extent than Fresnel prisms, and this effect increases with prism power.[24,25] We previously reported a group of patients with intractable diplopia and strabismus related to intracranial problems or long-term uncorrected aphakia who underwent BTXA injection into the appropriate EOM.[23] In our study group, 64% of the patients regained fusion, some of whom achieved a permanent cure after BTXA injection. Patients who do not have the capacity for fusion should be identified before considering any surgery, and BTXA is an excellent choice that serves this purpose.

Therapeutic Use of BTXA

BTXA injection is used therapeutically in various types of concomitant, restrictive, and paralytic strabismus. The list of the indications for BTXA are summarized below, with special emphasis on golden indications.

BTXA in concomitant deviations

BTXA can be used as a therapeutic agent in various types of concomitant strabismus. In concomitant strabismus, BTXA is more effective for moderate angles. In some motility problems, repeat injections are required as maintenance therapy, where in others one or two injections provide a permanent decrease in the deviation. Binocular fusion is the determinant mechanism that locks the correction of the eyes for a permanent effect. However, even in patients who require regular injections, there is a tendency toward longer intervals and reduced deviation.[26,27]

The results of BTXA therapy were found to be similar in children and adults.[28,29,30,31] Our results in childhood strabismus revealed that better outcomes are achieved in those with small angles and binocularity. Additionally, patients without binocularity who have small angles and high risk of consecutive deviations are also good candidates for BTXA therapy.

The indications for BTXA in concomitant deviations can be summarized as below:

- Infantile esotropia
- Deviations associated with neurological impairment/ cerebral palsy
- Residual/consecutive deviations
- Multiple previous surgeries
- Intermittent deviations
- Convergence insufficiency
- Convergence spasm
- Small-angle deviations
- Sensory eso/exodeviations
- Acute comitant esotropia
- Cyclic deviations

In infantile esotropia, the results are comparable to surgery, with better outcome in deviations ≤30 prism diopter (PD) and early injections.[32,33,34,35,36,37] In larger angles, surgery was found to be more successful. Infantile esotropia with associated ocular abnormalities such as microphthalmos represents one of the golden indications of BTXA.

In childhood strabismus, another first-choice application for BTXA in concomitant deviations are cases associated with cerebral palsy or other neurological problems or developmental delay. In this group of patients there is a tendency to delay surgery because of the high risk of consecutive deviations and the potential risks of full general anesthesia in surgery. However, these patients may have significant gains in motor skills with the alignment of their eyes, and improved binocularity may yield permanent results. Previous reports suggested that these patients may benefit from BTXA injection.[38,39,40] In our recent series including 50 patients with neurological impairment, we found that the overall success rate was 60%, with better outcome in esodeviations and shorter duration of strabismus.[40] Our results demonstrated that instead of delayed surgery, these patients should receive prompt BTXA treatment for a better outcome. Therefore, such cases are considered a golden indication of BTXA in our clinical practice.

In patients who have undergone multiple previous surgeries but still have recurrent deviation, BTXA is a very good option to keep the eyes aligned and improve quality of life.[26,27] In this group who seem to have no other chance for surgery, BTXA is our first-line treatment as a golden indication, but repeat injections are usually required in these patients.

In intermittent exotropia, the results of BTXA injection are encouraging in children.[41,42,43] In adults, we prefer BTXA for those who had a recent decompensation of intermittent exotropia.

Sensory eso- or exodeviations represent another difficult group who may need to have multiple surgeries because of recurrent deviations related to poor visual acuity in one eye and lack of binocular fusion. These patients may present with very large deviations and in those cases our preference is to perform surgery first and then use BTXA in case of recurrence before the deviation increases.

Late-onset acute comitant esotropia has become a rising problem in recent years because of excessive screen use. BTXA provides an orthophoric period to allow the binocular system to recover and may provide a permanent cure. In comparative studies with surgery, similar success rates were observed in both adults and children.[44,45,46,47] One comparative study showed a lower success rate with BTXA, but this study included a wide range of age groups.[48] For late-onset acute comitant esotropia related to excessive screen use, the author's first-line treatment as a golden indication is BTXA injection, while surgery is reserved for those who do not achieve a permanent cure with BTXA.

Cyclic deviations represent a rare form of strabismus. Surgical treatment based upon the deviation on squinting days carries the risk for overcorrection. The results with BTXA injection were found to be encouraging in previous reports, including ours.[49,50,51] In our case study with long-term follow-up of 8 years, we found that BTXA may either provide a cure or break the cycle.[51] Thus, we consider cyclic deviations among the golden indications.

BTXA in paralytic strabismus

The use of BTXA during the acute stage of paralytic deviations represents an additional indication of BTXA use where surgery is not an alternative. In order to consider any surgery, a period of at least 6 months is required and this period may increase to up to 1 year, especially in third nerve palsy because of the possibility of late spontaneous recovery. BTXA injection during the acute stage of paralytic deviations aims to provide symptomatic relief of diplopia, decrease the deviation and abnormal head posture, reduce the antagonist contracture in large angles, and assess the fusion potential in those with central fusion disruption.[23,52,53,54,55]

The most common golden indication for BTXA injection is sixth nerve palsy, in which it provides rapid relief of symptoms. BTXA was found to have no effect on spontaneous recovery.[56,57] However, in total sixth nerve palsy, those who had BTXA treatment during the acute phase were found to have smaller final deviation compared to conservatively followed patients, which represents an advantage for further surgery.[58]

In chronic cases, BTXA injection into the MR muscle in combination with vertical rectus transposition reduces the risk of anterior segment ischemia.[59] BTXA injection may be performed either before or after surgery. Injection before surgery is advantageous to assess potential LR function and perform transposition surgery under the full effect of BTXA. The disadvantage is the overcorrection period, which can sometimes last more than 6 months. The benefit of postoperative BTXA injection is the ability to see the transposition effect first and then decide whether to inject or not. Our preferred method in cases who present in the chronic stage with a large angle of deviation is to administer the BTXA injection one week before surgery, while in those with moderate angles we prefer to perform transposition first.

In a primary case of total sixth nerve palsy, our approach is to inject BTXA during the acute period. In those with deviations under 35 PD 6 months after BTXA injection, the author's preference is modified Nishida transposition without further MR weakening, adding BTXA later if required. In a multicentric study on modified Nishida transposition in sixth nerve palsy, we found that the mean correction with modified Nishida transposition alone was 29.4 PD and increased to 62.6 PD when combined with MR recession or MR BTXA and 95 PD when combined with MR recession augmented by BTXA.[60]

In total third nerve palsy, LR contracture and orbital fibrosis are the major challenges.[52] BTXA may help to prevent contractures and thus increase the likelihood of success in future surgical treatment. In chronic cases it may also be used to function as traction sutures in combination with large recess-resect and superior oblique transposition surgery.[61]

BTXA is not in common use for fourth nerve palsy. Injections to the ipsilateral inferior oblique, contralateral IR for undercorrections, and ipsilateral IR for overcorrections after SR recession may be considered.[54,55,62,63,64,65] We do not use oblique muscle injections. In patients with long-standing fourth nerve palsy and SR contracture, SR recession may cause overcorrection, and ipsilateral IR injection in the acute phase may provide a permanent cure.[65] In a small case series, we found that BTXA injection into the SR in combination with inferior oblique disinsertion may be effective in the long term in patients with a large angle of deviation and SR contracture to prevent overcorrection.[66]

BTXA is effective in supranuclear palsies either in the acute or chronic phase.[67,68,69] These patients may have associated problems that limit their chance for surgery and BTXA may be very helpful both for symptomatic relief and a possible cure.

BTXA in restrictive strabismus

Of the congenital restrictions, BTXA injection may be beneficial in Duane syndrome.[70,71,72] It is known that the innervational pattern does not change in Duane syndrome. However, the balance between co-contracting EOM and secondary contracture may show alterations that can result in increased abnormal head posture and primary position deviation. BTXA may help in permanently reorganizing the paradoxical contractile forces. BTXA seems more effective when used at young ages, preferably during infancy.[70] It may also be helpful to control postoperative over- and undercorrections. In Duane syndrome, our indications for BTXA treatment are abnormal head posture during infancy and recently increased abnormal head posture at any age as well as residual and consecutive deviations.

In acquired restrictive problems, BTXA cannot release fibrotic changes but may be useful if fibrosis is not fully developed. Thyroid orbitopathy, orbital myositis, strabismus after retinal detachment surgery, postoperative restrictions, and the acute stage of adherence syndrome are indications for BTXA in selected cases.[12,73,74,75,76] Thyroid orbitopathy is a common restrictive problem and surgery must be performed during the inactive stage of the disease. However, the process of becoming fully inactive may be quite prolonged, and BTXA treatment may offer these patients symptom relief during this active inflammatory period before fibrotic changes develop.

Specific Indications of BTXA as an Adjunct to Surgery

BTXA may be used to increase the success of surgery in following categories:

1. In combination with recession to augment the effect of recession or recess-resect surgery,
2. Instead of recession to reduce the risk of anterior segment ischemia in transposition procedures,
3. As a replacement for traction sutures to overcome fibrosis or contracture problems,
4. To rescue surgical failures and complications.

BTXA to augment the effect of recession

BTXA may be used in combination with recession to augment the effect of recession or recess-resect surgery, which provides a greater effect without the disadvantage of permanently reduced EOM function in supramaximal recessions.[77,78] In our previous study we obtained satisfactory long-term results both in eso- and exodeviations with large angles with BTXA injection into the recessed muscle during surgery.[77] Lueder et al.[79] reported that BTXA-augmented bimedial recessions in infantile esotropia with large angles over 65 PD had a higher success rate with a lower rate of consecutive exodeviation in the long term compared to supramaximal recessions.[80] In another study of infantile esotropia with large angles, the authors calculated the numerical effect as 5.7 PD/mm and 4 PD/mm in BTXA-augmented and non-augmented recessions, respectively.[81]

We previously reported that in sensory eso- or exodeviations, BTXA effectively increased the effect of recession in recess-resect surgery in the long term.[82] The major advantage of combining BTXA with recession is to avoid the need for supramaximal surgeries or third and fourth rectus muscle surgeries in sensory deviations where surgery is not desired in the "good eye."

BTXA instead of recession to reduce anterior segment ischemia risk

In paralytic cases where full muscle transpositions are required in combination with third rectus muscle recession, BTXA can be used instead of recession. BTXA may also be used in some traumatic cases with EOM muscle and ciliary vessel damage to weaken the antagonist muscle with less risk of anterior segment ischemia.

BTXA as a replacement for traction sutures

Traction sutures are used in the treatment of complex strabismus problems to overcome severe contracture and orbital fibrosis. It is preferred they remain in place for 6 weeks, which is an unpleasant period for the patient. Augmentation of recession either in combination with transposition or with a large antagonist resection provides significant overcorrection during a similar period of time that serves as traction sutures, an effect referred to as "pharmacological traction" (Figure 1). In our clinical experience we found this method very effective and well-tolerated in third nerve palsy, long-standing sixth nerve palsy, and traumatic cases.[1,61] Using BTXA in combination with resection or transposition to obtain a traction suture effect has become one of our golden indications.

BTXA to rescue surgical complications

Over- and undercorrections are the most common problems after surgery, and BTXA may provide a "pharmacological adjustment" effect.[83,84] Both in acquired and infantile esotropia, BTXA was found to have an effect equal to surgery in the rescue of failures, and the BTXA group had better results when injections were given within the first 3 months postoperatively.[85,86] In postoperative injections, the mechanism of effect is mainly through alteration of mechanical contractile forces and soft tissue healing in the early phase and a central adaptive mechanism in the chronic phase.[1,2,84] In reoperations with stretched scars and or slipped muscles, the muscle becomes stiff and with advancement or resection of the stretched scar, overcorrections may occur despite adjustable sutures. BTXA is very useful to release the contracture in the early postoperative period and this is one of our golden indications.[76]

In cases with a lost muscle problem, it was shown that antagonist contracture develops in as little as 2 weeks if surgery is delayed for any reason.[87] Relaxation of the antagonist by BTXA injection may be useful for a successful outcome with the additional benefit of allowing more anterior attachment of the soft tissues surrounding the lost muscle to the globe (Figure 2).[76,88] In late interventions where any transposition surgery is being considered after failed attempts to find the lost muscle, BTXA injection is the appropriate choice to release the antagonist contracture to avoid anterior segment ischemia. Lost muscle with delayed surgery is considered among our golden indications of BTXA.

We have previously reported that BTXA may be highly effective in adherence syndrome, a very challenging complication, if injection is performed during the acute inflammatory period before the development of fibrosis.[12,76] If the eye can be kept in primary position during the inflammatory period, the attachments of fatty tissue develop more posteriorly, thus resulting in less limitation of ocular motility. Therefore, the acute period of adherence syndrome is among the golden indications of BTXA if used with appropriate timing (Figure 2).[1]

BTXA in Posttraumatic Strabismus

In posttraumatic EOM damage and/or adherence syndrome, BTXA injection to the appropriate EOM may be very useful either to reduce the risk of anterior segment ischemia, prevent antagonist contracture, or reduce adherence syndrome-related motility problems.[1,2] In the acute stage, BTXA injection may keep the eye in primary position, thereby reducing contracture of the antagonist EOM and allowing soft tissue healing with the eye in primary position, which may reduce the effect of orbital fibrosis on ocular motility (Figure 2). In the chronic stage, it may be used as maintenance therapy or to reduce the anterior segment ischemia risk in multiple rectus muscle surgery.

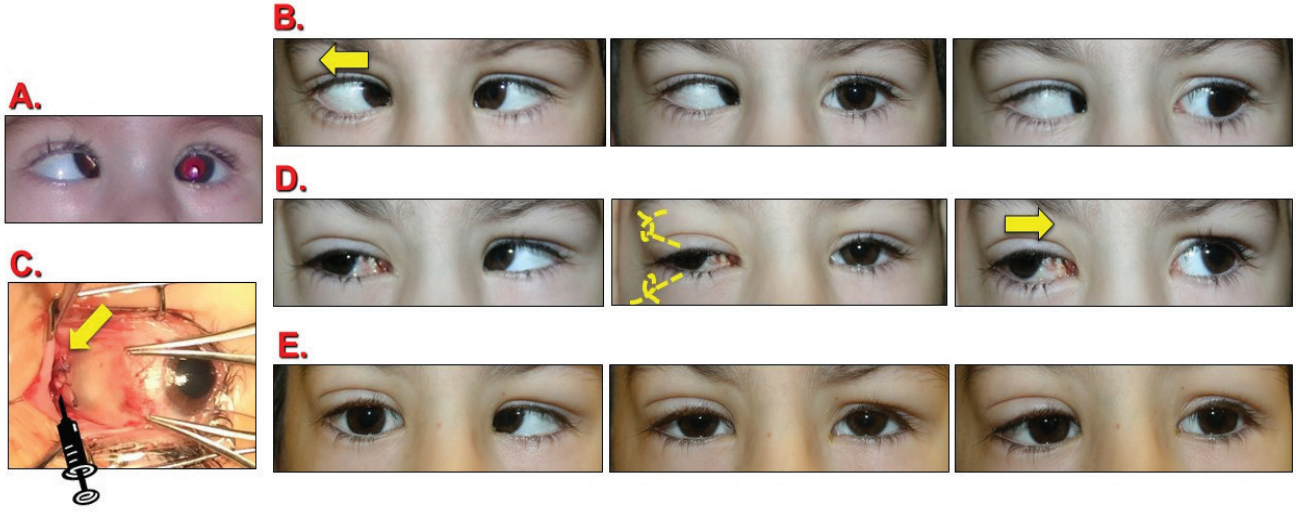

Figure 1. (A) A child with history of preterm birth had sixth nerve palsy in the right eye related to intracranial hemorrhage at 5 months of age. (B) The child was not treated and returned at 7 years of age with a very large right esotropia and severe limitation of abduction. There was severe antagonist contracture and orbital fibrosis. (C) The medial rectus muscle was recessed 14 mm from the insertion and botulinum toxin A was injected in combination with modified Nishida transposition in the same session. Forced duction test was still positive on abduction at the end of operation. (D) On postoperative day 13, there was severe overcorrection with limitation of adduction. The effect resembled that of traction sutures. (E) At postoperative 16 months, the result was stable with limited abduction and moderate limitation of adduction

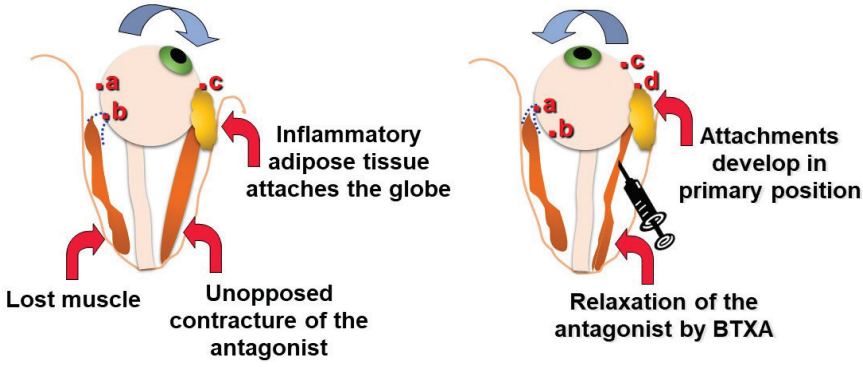

Figure 2. The mechanism of effect in lost muscle and adherence syndrome. When an extraocular muscle is lost, contracture of the antagonist develops and pulls the globe. Botulinum toxin A injection into the antagonist extraocular muscle in the acute phase prevents antagonist contracture and keeps the eye in primary position. Thus, any possible attachments around the extraocular muscle attach to the globe more anteriorly (at point "a" instead of "b"). Similarly, in adherence syndrome the inflammatory adipose tissue reaction pulls the globe and limits ocular motility. Botulinum toxin A injection during the acute phase keeps the eye in primary position and allows the attachments to develop at a more posterior point ("d" instead of "c"), thereby reducing limitation of ocular motility
BTXA: Botulinum toxin A

Complications of BTXA Treatment

The main problem with BTXA injection is related to its spread to neighboring tissues, with ptosis seen in 9%-42% of cases and neighboring EOM affected in 8.3%-18.5% of patients.[89] It was reported that ptosis occurs less when BTXA is injected with sodium hyaluronate.[90] Although diplopia in the acute phase due to overcorrection or limitation of ocular motility may be bothersome for some patients, this is actually not a complication but the natural effect of BTXA. Tonic pupil may occur in 0.16%-11% of patients and is likely related to needle injury rather than the effect of BTXA.[91] Accommodation deficiency, subconjunctival hemorrhage, and retrobulbar hemorrhage are other possible complications of BTXA treatment. The least common but most serious complication is scleral perforation, which was reported at a frequency of 0.28% in one series.[89] We have not observed scleral perforation in our clinical practice. Patients with excessive scar tissue and those with myopia-related large globes are at high risk for globe perforation. In an experimental study it was demonstrated that BTXA was non-toxic for the retinal tissue.[92]

Problems with BTXA treatment

Despite the many advantages of BTXA treatment there are some problems that limit its use in some instances. These problems can be summarized as follows:

- Difficulty reaching the target tissue: Despite EMG guidance this is still a problem.
- Uncertainty of the effect during the early post-BTXA period: Full paralytic effect is obtained in some cases, whereas only a decrease of the deviation without overcorrection or limitation of ocular movement may be obtained in others.
- Inefficiency in established fibrosis: The decision to perform BTXA injection must be made quickly in most cases to obtain a benefit before fibrosis has fully developed.
- Possibility of repeat injections: If the desired effect is not obtained, repeat injections are required during the early post-injection period, which may be a significant problem in children especially. In adults, an additional dose at the 1-week post-injection visit usually solves the problem in those with inadequate effect.
- Off-label use in children.
- Cost/insurance problems-varies by region.
- Lack of dose-response grading: In the author's view, the effect is more related to reaching the target tissue than the applied dose.

Golden indications for BTXA treatment in strabismus

Considering all the advantages and disadvantages of BTXA treatment and the author's experience using this agent in clinical practice, the golden indications of BTXA can be summarized as follows:

- Late-onset acute comitant esotropia,
- Unstable concomitant deviations with cerebral palsy,
- Infantile esotropia with associated abnormalities,
- Paralytic/restrictive strabismus-acute phase,
- Early over- and undercorrections,
- Early adherence syndrome,
- Lost muscle with late intervention,
- Intractable diplopia related to central fusion disruption,
- The need to weaken a rectus muscle in the presence of the risk of anterior segment ischemia,
- Recurrent deviations despite multiple previous surgeries,
- Cyclic deviations.

Conclusion

In summary, there are five indication categories of BTXA treatment in strabismus:

- Alternative to surgery and prisms in selected concomitant, restrictive, and paralytic deviations,
- A necessary additional agent in acute deviations where surgery is not an alternative,
- Good choice in surgical failures,
- Good choice to increase surgical success,
- Only choice if surgery is not an option for any reason.

In conclusion, BTXA treatment has become the primary option in certain strabismus problems. Strabismologists are recommended to be familiar with BTXA treatment, as it has gained its own non-surgical indications and has an adjunctive role in the management of challenging motility problems rather than being only an alternative treatment based on physician preference.

Ethics

Peer-review: Internally peer-reviewed.

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Case Report

Extraretinal Fibrovascular Proliferation in a Neonate Possibly Associated with an *ESAM* Gene Variant

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Abstract

A female infant born with a gestational age of 35 weeks and birth weight of 2500 g was referred for ophthalmic examination on the second postnatal day. Bilateral venous dilatation and arterial tortuosity, severe extraretinal fibrovascular proliferation, and peripheral ischemia were detected. Fluorescein angiography showed profoundly delayed arteriovenous transit and peripheral avascularity. Both eyes were treated with diode laser photocoagulation and bevacizumab injection. Cranial magnetic resonance imaging (MRI) revealed hydrocephalus, ventricular dilatation, and cerebral atrophy. Her family history revealed that the patient's brother presented to the ophthalmology outpatient clinic at postnatal 3 months with inoperable total retinal detachment and similar cranial MRI findings. No systemic or ocular findings were detected in the parents. A recent study showed that in 13 cases, including our patients, bi-allelic variants in the *ESAM* gene lead to a new neurodevelopmental disease whose main clinical features include impaired speech and language development, seizures, varying degrees of spasticity, ventriculomegaly, intracranial hemorrhage, and developmental delay/mental disability. Newborn siblings of children with serious pathological retinal findings should undergo a detailed ophthalmic examination as soon as possible after birth to prevent total retinal detachment, even without a diagnosis of specific inherited retinal vascular diseases. Further investigations performed in collaboration with an international network may reveal more candidate gene variants possibly related to retinopathy of prematurity-like ophthalmological findings such as extraretinal fibrovascular proliferation.

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Introduction

Retinopathy of prematurity (ROP) occurs in infants with low gestational age (GA) and low birth weight as a result of retinal hypoxia and hyperoxia. Extraretinal fibrovascular proliferation and retinal detachment may develop in infants with ROP.[1] Usually, ROP disease occurs 4-10 weeks after birth, depending on the GA of the infant and oxygen therapy method. Rare diseases such as familial exudative vitreoretinopathy (FEVR), Norrie, and incontinentia pigmenti (IP) may cause proliferation in the retina, leading to findings that can be difficult to differentiate from ROP. This report presents a case that was not compatible with ROP, FEVR, Norrie, or IP in terms of genetics and systemic findings, in which progression to retinal detachment was prevented with laser and anti-vascular endothelial growth factor treatment on the third postnatal day.

Case Report

A female infant born with a GA of 35 weeks and birth weight of 2500 g was referred for ophthalmic examination on the second postnatal day due to a family history of bilateral retinal detachment. Anterior segment examination revealed bilateral neovascularization of the iris and pupillary rigidity. Fundus examination revealed bilateral venous dilatation and arterial tortuosity. Both eyes showed severe extraretinal fibrovascular proliferation in 6-8 clock hours, peripheral ischemia in all quadrants, and vitreous hemorrhage in the left eye, which prevented visualization of the posterior pole and temporal periphery

(Figures 1, 2). Fluorescein angiography showed profoundly delayed arteriovenous transit (>90 s), incomplete venous filling, and peripheral avascularity. Both eyes were treated with diode laser photocoagulation to the peripheral ischemic retina and intravitreal injection of 0.3125 mg bevacizumab. Additional laser photocoagulation was performed on the left eye after the vitreous hemorrhage regressed at 45 weeks' postmenstrual age (Figure 2f). The disease fully regressed after treatment; no reactivation was observed during 4 years of ophthalmological follow-up. Although the retina was anatomically attached and other optical structures were normal, the patient had nystagmus and severe visual impairment.

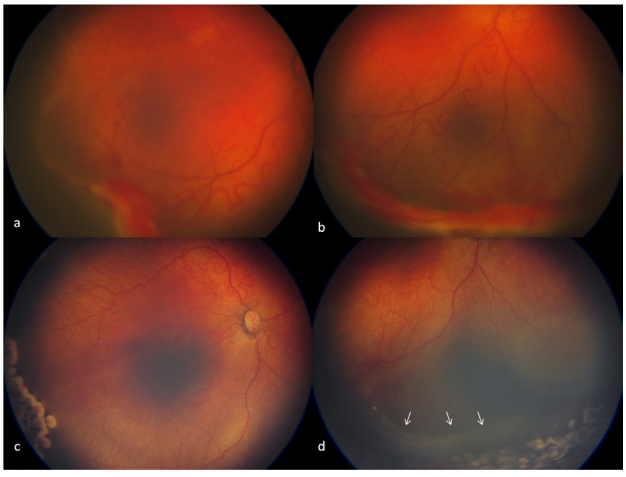

Figure 1. (a, b) Wide-field fundus photos of the right eye showing venous and arterial dilation, arterial tortuosity, and severe extraretinal fibrovascular proliferation on postnatal day 2. The eye was treated with combined laser and intravitreal 0.3125 mg bevacizumab. (c, d) At two months after treatment, the vascular dilation had regressed

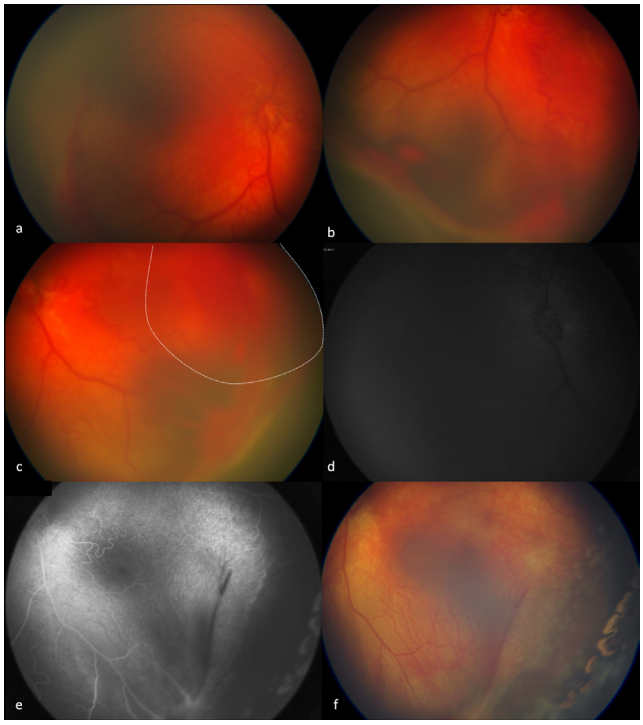

Figure 2. (a, b) Wide-field fundus photos of the left eye on postnatal day 2. (c) Vitreous hemorrhage prevents visualization of the temporal quadrant (circled area). (d) Fluorescein angiogram demonstrated profoundly delayed arteriovenous transit on postnatal day 3. The eye was treated with combined laser and intravitreal 0.3125 mg bevacizumab. (e) Fluorescein angiography performed after the vitreous hemorrhage regressed at the age of 2 months showed avascular retina between the laser scars and the normal retina. (f) Laser was applied to the residual avascular retinal area. Significant regression of the vascular dilation and fibrovascular proliferation are observed in wide-field fundus photos obtained at 2 months

On physical examination, triangular face, smooth philtrum, and prominent chin were present. Cranial magnetic resonance imaging (MRI) revealed a thin corpus callosum, diffuse calcification in the periventricular white matter, hydrocephalus, ventricular dilation, and cerebral atrophy (Figure 3). The patient had severe neuromotor retardation (severe developmental delay and intellectual disability, absence of speech and language development, hypotonia, and severe epileptic seizures). Hypertrophic cardiomyopathy was detected during follow-up.

The patient's family history revealed that her brother presented with bilateral iris coloboma and total retinal detachment at postnatal 3 months. Dysmorphic features, MRI and neurologic findings, and neuromotor development were similar between the siblings. No systemic or ocular findings were detected in the parents. Whole exome sequencing revealed the homozygous c.115del (p.Arg39Glyfs*33) frameshift variant in the *ESAM* gene in both siblings. The parents were heterozygous for the variant.

Discussion

Although our case had findings similar to severe stage 3 ROP and plus disease, we considered that the presented case may be related to inherited disease with retinal vaso-occlusive findings. This conclusion was based on the lack of low birth weight and GA, the lack of a history of oxygen therapy, and the very early presentation for the development of ROP, as well as the presence of family history and cranial findings.

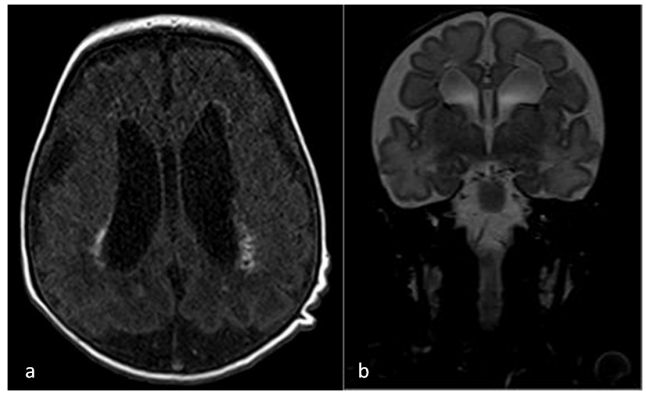

Figure 3. (a, b) Cranial MRI revealed diffuse calcification in the periventricular white matter, hydrocephalus, dilation of the ventricles, and cerebral atrophy
MRI: Magnetic resonance imaging

The Wnt signaling pathway is essential in ocular angiogenesis and the pathogenesis of inherited ocular vascular diseases such as Norrie disease, FEVR, and osteoporosis-pseudoglioma syndrome. *NDP, LRP5, FZD4,* and *TSPAN12* gene variants have been found to be related to the disruption of the Wnt signaling pathway.[2] Dysplastic retina with pseudoglioma appearance is the main characteristic ocular finding of Norrie disease.[3] The absence of a dysplastic retina in the present case may distinguish it from Norrie disease according to the ocular findings.

While subretinal exudation and radial retinal folds are remarkable findings of FEVR, the disease has a wide range of retinal and angiographic findings.[4,5] According to the clinical staging system, stage 1 can present with only avascular peripheral retina without extraretinal vascularization and exudation.[4] On the other hand, in later stages, patients can present with total retinal detachment. Although our case meets all three diagnostic criteria for FEVR that were previously defined by Kashani et al.[4] and Ranchod et al.,[5] we consider our case to have differential features from FEVR, such as the presence of intracranial pathologies and the lack of specific gene variants for FEVR, as well as the lack of remarkable findings such as retinal folds or subretinal exudation.

In addition to Wnt-related retinal vasculopathy, variants of the *IKBKG* gene (inhibitor of the kappa light polypeptide gene enhancer in B-cells, kinase gamma) play an essential role in the pathogenesis of IP and infantile retinopathy. Although IP is often considered a primarily dermatological disease, ophthalmic and intracranial pathologies may accompany skin lesions.[6] The vaso-occlusive nature of the disease may cause retinal avascularity, neovascularization, and exudative and tractional detachments.[6,7,8,9] Cerebral atrophy, dilated ventricles, hydrocephalus, and corpus callosum lesions have been reported in IP patients.[10] Therefore, the cranial imaging findings in the present case were comparable with previous reports of IP. Nevertheless, due to the lack of dermatological and dental lesions and *IKBKG* gene variant, the diagnosis of IP was ruled out.

In a recently published study conducted with an international collaborative network, bi-allelic variants in the *ESAM* gene were identified in these siblings as well as another 11 individuals with similar neurological findings.[11] However, severe extraretinal fibrovascular proliferation was noted during the neonatal period only in the present siblings. In the aforementioned study, retinal ischemia and retinal hemorrhage were reported in only two other individuals.[11] In one of them, vascular tortuosity, retinal ischemia, and new vessels were reported at the age of 10, although not as severe as the cases we presented, which seem to be in the same spectrum as our cases. This data suggest that *ESAM* gene variants may present different expressivity or other unidentified gene variants may contribute to these severe findings.

Laser photocoagulation of the ischemic avascular retina was the gold standard treatment for ROP and FEVR for the last three decades.[4,12] Nevertheless, combination therapy (laser and anti-vascular endothelial growth factor) for ROP and FEVR has been reported in the literature.[13,14,15] We preferred combination therapy because vitreous hemorrhage in the left eye prevented the completion of laser treatment and severe extraretinal fibrovascular proliferation was present in both eyes.

In conclusion, our case did not have gene variants that were previously described for the Wnt signaling pathway and *IKBKG* gene. Bi-allelic *ESAM* gene variants may cause extraretinal retinal vascularization during the neonatal period. Further investigations performed in collaboration with an international network may reveal more candidate gene variants that may be related to ROP-like ophthalmological findings such as extraretinal fibrovascular proliferation. While both eyes of the presented patient were treated successfully on postnatal day 3, her brother could not be treated for advanced disease. Therefore, newborn siblings of children with serious pathological retinal findings should undergo a detailed ophthalmic examination as soon as possible after birth to prevent total retinal detachment, even without a diagnosis of specific inherited retinal vascular diseases.

Ethics

Informed Consent: Obtained.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.E.B., N.S., M.E., S.D., A.G. M.Ç., Concept: S.E.B., A.G., Design: S.E.B., A.G., Data Collection or Processing: S.E.B., Analysis or Interpretation: S.E.B., N.S., M.E., S.D., A.G. M.Ç., Literature Search: S.E.B., Writing: S.E.B., A.G.

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Neurofibromatosis Type 1 Vasculopathy Presenting as Branch Retinal Vein Occlusion: Case Report and Review of the Literature

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Abstract

Systemic vascular occlusive disease associated with neurofibromatosis type 1 (NF1) has been reported in the aortic, cerebral, renal, celiac, and mesenteric vessels and is referred to as NF1 vasculopathy. Although retinal vascular involvement in patients with NF1 usually manifests as retinal capillary hemangiomatosis, a few cases of NF1 with retinal vascular occlusive disease have also been described. Here, we report a 2-year-old girl with NF1 who presented with branch retinal vein occlusion and peripheral retinal ischemia secondary to NF1. This case demonstrates that NF1-related retinal occlusive vasculopathy may occur in very young patients and that detailed fundus examination with fluorescein angiography is necessary in all patients with NF1.

Keywords: Neurofibromatosis type 1, NF1 vasculopathy, occlusive vascular disease, branch retinal vein occlusion

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Introduction

Neurofibromatosis type 1 (NF1) is an inherited multisystem disease that gives rise to cutaneous findings such as café au lait spots, intertriginous freckling, skin and nervous system tumors, osseous lesions, and vascular pathologies. The eye is frequently involved in patients with NF1. Iris Lisch nodules, optic pathway gliomas, neurofibromas of the orbit and eyelid, and choroidal nodules are among the most common ocular findings and serve as diagnostic criteria.[1] Recently, retinal vascular abnormalities have been shown to occur more frequently in this group of patients than previously thought.[2,3,4] Although these abnormalities mostly included structural changes and different microvascular arrangements, a limited number of reports have also documented different presentations of retinal vascular occlusion.[5,6,7,8,9]

Here, we report a unique case of branch retinal vein occlusion in a patient with NF1 and bilateral optic glioma.

Case Report

A 2-year-old girl with a known history of NF1 was referred for retinal detachment (RD) in the right eye (RE). She had a history of falling from the sofa 8 months ago, and was previously evaluated for retinoblastoma and persistent fetal vasculature as possible causes of the RD. NF1 had previously been diagnosed on the basis of multiple café au lait spots and bilateral optic nerve glioma (Figure 1A). The patient was born full term without complications. There was no history of consanguinity or ocular disease in the family.

During ocular examination, the patient showed intense objection to occlusion of the left eye (LE), indicating very poor vision in the RE. The LE could fixate on and follow small objects. Pupillary dilation was poor due to posterior synechiae in the lower quadrant of the RE (Figure 1B). The retina was behind the lens with overlying hemorrhagic fibrous membranes, no retinal break

was detected, and ultrasonography showed a closed-funnel RD in the RE (Figure 1C). The anterior segment was normal in the LE, while fundus examination revealed subtle vascular abnormalities and mild fibrous proliferation along the distal portion of the vein in the inferotemporal arcade (Figure 2A). An examination under general anesthesia was planned. Fluorescein angiography (FA) of the LE showed delayed filling of the distal inferotemporal vein, significant surrounding capillary non-perfusion, and highly tortuous corkscrew-shaped vessels bordering the ischemic areas (SW8000 Widefield Fundus Camera, Suoer, Tianjin, China) (Figure 2C, D). The entire temporal periphery was also avascular, with vessels abruptly terminating by forming arteriovenous anastomoses and bordering the perfused and non-perfused retina (Figure 2E). FA of the RE demonstrated diffuse capillary loss in the detached retina. The inferior half was totally avascular, along with some neovascularization (Figure 1D). Tractional RD could not be ruled out in the RE because of the retinal vascular findings.

The patient underwent vitreoretinal surgery in the RE and sectoral panretinal photocoagulation of the ischemic areas and pathological vessels in the LE (Figure 2F). During surgery in the RE, following limbal lensectomy, hemorrhagic coagula and membranes were removed with forceps and scissors and the funnel could be opened from the center to reach the optic nerve head. This revealed a large macular tear within the funnel, along with avascular peripheral retina and intraretinal, subretinal, and preretinal proliferative vitreoretinopathy membranes (Figure 1E, F). After seeing the possibly traumatic macular tear-related RD, the surgery was continued mainly for anatomical preservation of the globe. Extensive membrane peeling with retinotomy and peripheral ischemic retinectomy resulted in flattening of the retina, which was tamponaded with 5000 centistoke silicone oil. The patient has been followed up without any silicone-oil related complications in the RE during 1 year of follow-up.

Discussion

Systemic vascular occlusive disease affecting the aortic, cerebral, renal, celiac, and mesenteric vessels has been previously reported in NF1.[10,11] In fact, the term "NF1 vasculopathy" has been used in the literature to describe aneurysms, stenoses, and arteriovenous malformations that occur in NF1 patients. The pathogenesis of these NF1-related vascular abnormalities is largely unknown. Previous hypotheses suggested that it may result from cellular proliferation within the vessel walls

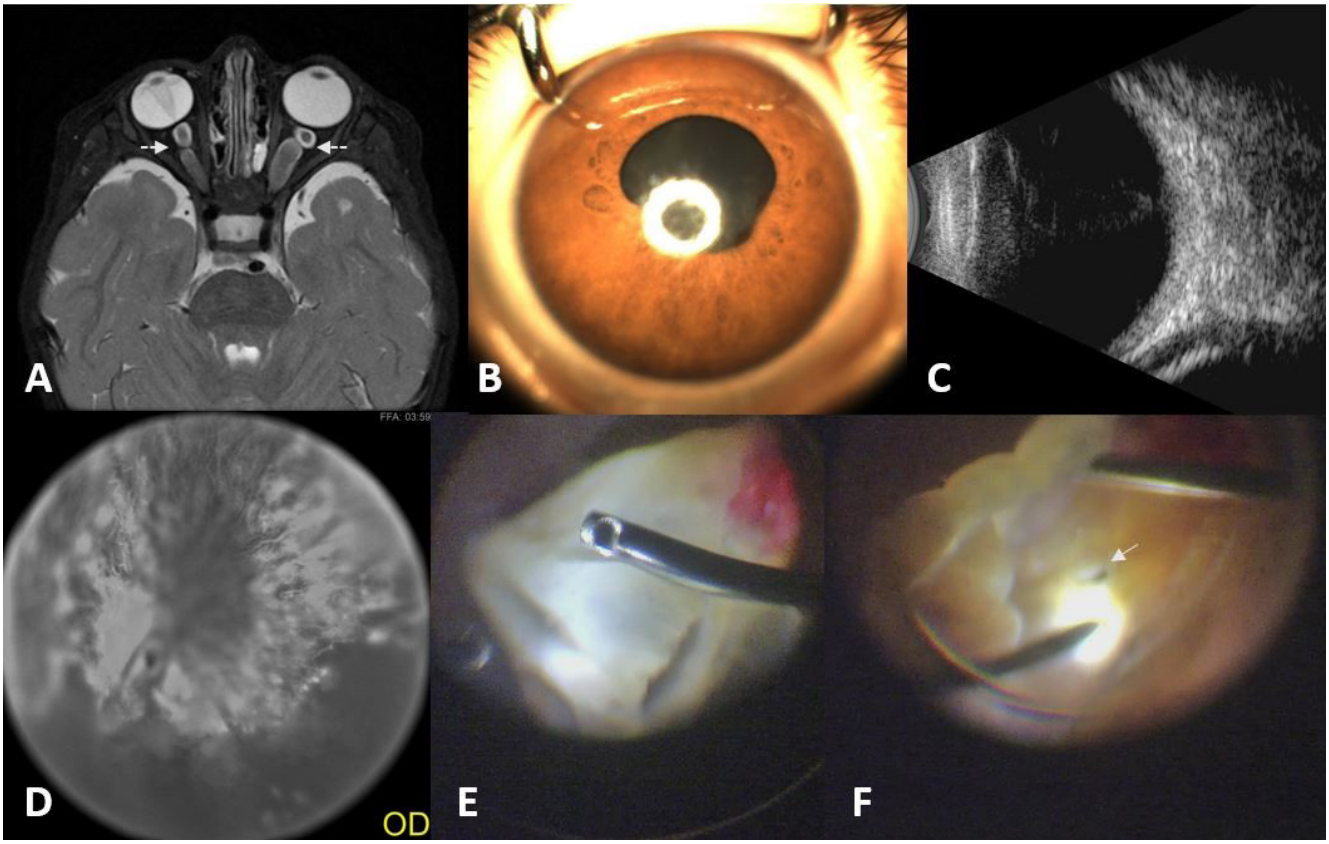

Figure 1. Cranial MRI showing bilateral optic glioma (arrows) (A). Pupillary dilation was poor due to posterior synechiae in the right eye and leukocoria was noted (B). B-scan ultrasonography demonstrated closed funnel-shaped retinal detachment (C). Fluorescein angiography showed diffuse leakage and capillary loss, which was more apparent in the inferior periphery of the right eye (D). Following lensectomy and removal of the retrolental fibrotic membranes, a macular tear was seen within the funnel (arrow) (E, F). Fundus images and fluorescein angiography images were taken with the SW8000 widefield fundus camera by Suoer (Tianjin, China)
MRI: Magnetic resonance imaging

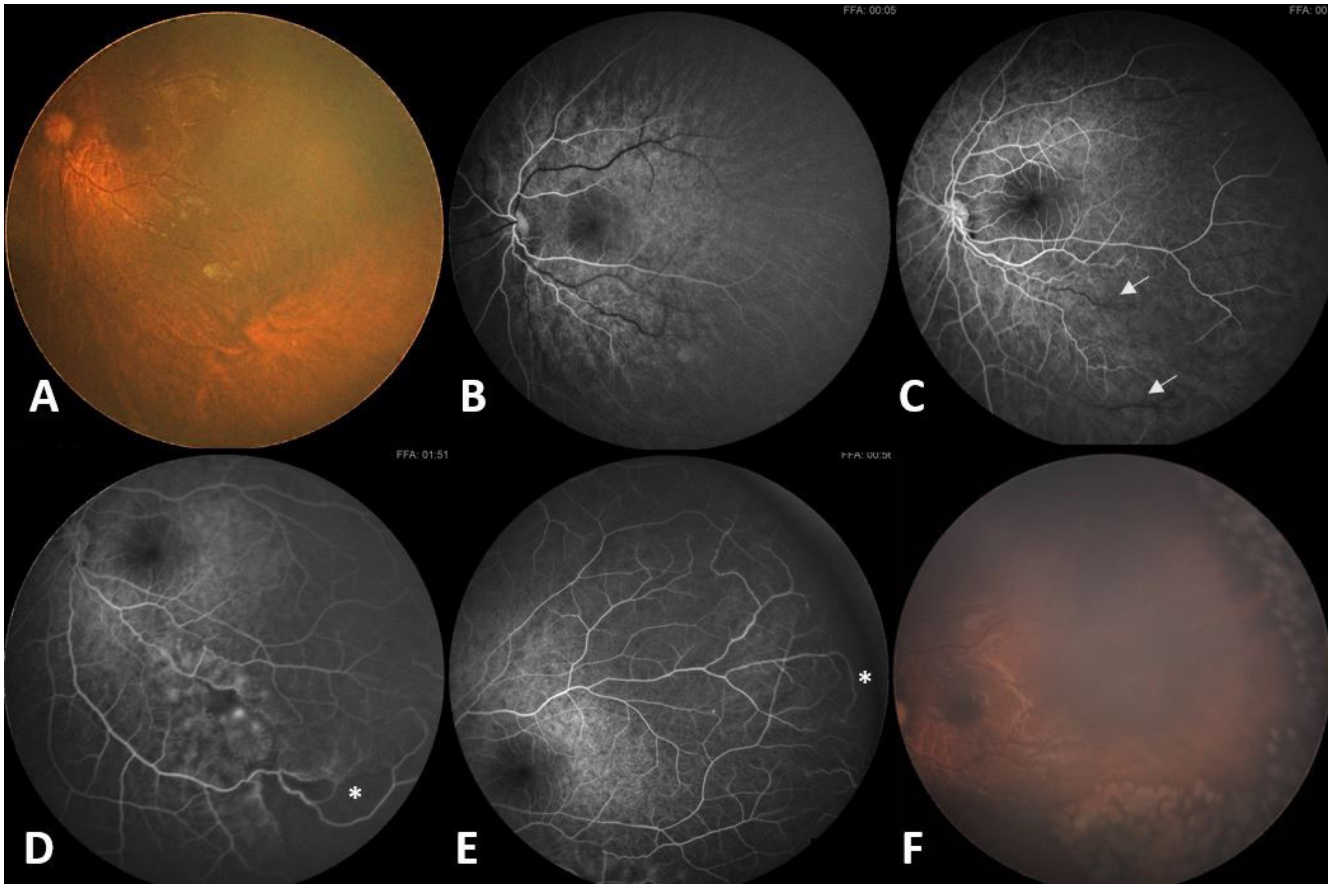

Figure 2. Fundus image of the left eye demonstrates subtle fibroglial proliferation along the distal portion of the inferotemporal vein (A). Fluorescein angiography showed normal arterial filling at 5 seconds after dye injection (B) and delayed filling of distal branches of the inferotemporal vein (arrows) at 20 seconds after dye injection (C). Areas of capillary non-perfusion and tortuous collaterals became evident in the inferotemporal quadrant in later frames (asterisk) (D). The temporal and inferior periphery were avascular, with arteriovenous communications bordering the perfused and non-perfused retina (asterisk) (E). Note that there were capillary non-perfusion areas in the tract of the inferotemporal retinal vein with a few leaking neovascular tufts at the vascular-avascular border. Fundus image of the left eye after laser photocoagulation treatment (F). Fundus images and fluorescein angiography images were taken with the SW8000 widefield fundus camera by Suoer (Tianjin, China)

or from direct compression or invasion by neural tumors.[10] However, the latter hypothesis does not seem to correlate well with clinical findings. More frequently, histologic findings indicate fibromuscular dysplasia with a predominance of intimal thickening in such cases.[11]

According to recent reports, retinal microvascular abnormalities have now been recognized in up to one-third of NF1 cases.[2,3,4] Several authors have demonstrated a spectrum of vascular abnormalities that range from simple tortuosities to the more complex corkscrew and moyamoya-like configurations.[2,3,4] While earlier studies described these lesions as congenital and stable,[2] recent ones have mentioned dynamic changes over the years.[3] Nevertheless, the clinical significance of these microvascular lesions remains unknown other than being a possible marker of NF1 disease.

On the other hand, retinal vascular occlusive diseases can also be seen in these patients, albeit rarely, and can lead to clinical consequences. The literature review yields several case reports of different types of vascular involvement in NF1 patients (Table 1). Three of these cases presented with diffuse involvement with both major and peripheral vessel occlusion and were diagnosed in the later sequelae stage.[5,6,7] They all had diffuse sheathing of retinal vessels, arteriovenous communications, avascular peripheral retina, and secondary fibroglial proliferation. One case presented with isolated macular artery involvement while the periphery was spared.[9] Two patients with peripheral retinal ischemia complicated with neovascular glaucoma have been presented as well.[12,13] Other examples included central retinal artery and ophthalmic artery occlusions during the course of NF1.[14,15]

While most of these previous reports on NF1 vasculopathy documented arterial system occlusions as the primary pathology, to our knowledge, one case of NF1 with branch retinal vein occlusion has been previously reported.8 This was a 64-year-old woman with no systemic pathology other than NF1 who presented with superotemporal vein occlusion and areas of capillary loss at the posterior pole. Differently than this case, we observed an occlusion in a distal branch vein, and the temporal periphery was totally avascular. Moreover, previous reports emphasized

Table 1. Literature review of NF1-related retinal vascular occlusion cases

Author[ref]	Year	Age (years)	Presentation	Laterality	Vascular occlusion type
Moadel et al.[5]	1994	4	Exodeviation	Unilateral	Peripheral retinal ischemia
Thölen et al.[6]	1998	20	Routine exam	Unilateral	Peripheral retinal ischemia and BRAO
Saatci et al.[14]	1998	15	Sudden painless vision loss	Unilateral	Ophthalmic artery occlusion
Mori et al.[8]	2001	64	Gradual vision loss	Unilateral	BRVO
Kadoi et al.[7]	2003	23	Poor vision since childhood	Unilateral	Peripheral retinal ischemia
Lecleire-Collet et al.[9]	2006	26	Sudden painless vision loss	Unilateral	Macular arteriolar occlusion
Elgi et al.[12]	2010	12	Pain and poor vision	Unilateral	Peripheral retinal ischemia, NVG
Pichi et al.[13]	2013	13	Pain and poor vision	Unilateral	Peripheral retinal ischemia, NVG
Umunakwe et al.[15]	2019	36	Episodes of sudden painless vision loss	Unilateral	CRAO

BRAO: Branch retinal artery occlusion; BRVO: Branch retinal vein occlusion; NVG: Neovascular glaucoma; CRAO: Central retinal artery occlusion, NF1: Neurofibromatosis type 1

the unilateral appearance of NF1 vasculopathy.5,6,7,8,9,12,14 However, the angiographic and surgical findings in the fellow eye suggested possible bilateral involvement in the presented case. Vascular anomalies and ischemia observed in both eyes on FA suggested that the source of RD in the RE might not be just a standard traumatic rhegmatogenous one but might be a tractional RD. As a result, surgical intervention was considered as a potential treatment option. The presence of the avascular ischemic peripheral retina in addition to the macular tear found during the operation suggested that a rhegmatogenous RD may have developed in an already ischemic retina after trauma. Although these findings might be confusing in a patient with closed funnel RD and advanced PVR, they may indicate that unilaterality is not a rule in NF1 vasculopathy and that both eyes should be meticulously investigated. The detection of such a marked vasculopathy in a patient with a near-normal retina is also striking, emphasizing the importance of routine FA in NF1 patients.

Another issue is that our patient was much younger and had bilateral optic glioma. One might argue that direct compression of the tumor may have caused the retinal vascular disturbances if it was a central retinal vascular occlusion. However, given the occlusion of the peripheral retinal vessels, this seems unlikely. We believe the underlying pathogenesis was consistent with previous cases and likely involves vascular smooth muscle cell proliferation due to abnormal signaling between smooth muscle and endothelial cells expressing the NF1 gene product neurofibromin, a negative regulator of mitogenic signaling.

In conclusion, NF1 may cause retinal vascular occlusions that can manifest in different ways affecting both the arterial and venous systems. Findings can be subtle, confined to small venules or located in the periphery, and can easily go unnoticed, especially in a young child, as in our case. Therefore, we recommend a detailed fundus examination and FA in all patients with NF1. Also, NF1 vasculopathy should be recognized as an etiology of retinal vascular occlusive disease in young patients.

Ethics

Informed Consent: Obtained.

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Authorship Contributions

Surgical and Medical Practices: Ş.Ö., E.Ö.Z., Concept: E.Ö.Z., Ş.Ö., Design: E.Ö.Z., Ş.Ö., Data Collection or Processing: E.Ö.Z., Analysis or Interpretation: E.Ö.Z., Ş.Ö., Literature Search: E.Ö.Z., Ş.Ö., Writing: E.Ö.Z., Ş.Ö.

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Case Report

Surgical Treatment of Bullous Exudative Retinal Detachment Secondary to Atypical Bilateral Central Serous Chorioretinopathy

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Abstract

This study aimed to report the diagnostic process, treatment, and follow-up of a patient with bullous exudative retinal detachment (RD) associated with an atypical variant of bilateral central serous chorioretinopathy (CSCR). A 28-year-old woman was referred to our clinic for total bullous RD in the right eye with a vision level of light perception only. She had been previously diagnosed with idiopathic uveal effusion syndrome and treated with systemic corticosteroid therapy with no response, and was referred to us for scleral window surgery. Four-quadrant scleral window surgery with external drainage of the subretinal fluid was performed, resulting in a transient partial attachment of the retina. RD started to progress again within 3 weeks, which prompted comprehensive imaging together with more advanced systemic workup for systemic lupus erythematosus and other rheumatological and immunological diseases. Systemic corticosteroid therapy was initiated during this period but did not stop the progression and was discontinued after a short time. Fluorescein angiography and indocyanine green angiography revealed multifocal choroidal leakage foci and large choroidal vessels without any intraocular inflammation findings and led to the diagnosis of atypical CSCR. Pars plana vitrectomy (PPV), internal drainage of the subretinal fluid, endolaser to the focal leakage areas, and intravitreal aflibercept injection were performed. Visual acuity increased to 0.8 within 8 months after the surgery with no recurrence. Bullous exudative RD is a very rare and atypical form of CSCR, and a favorable outcome can be obtained with PPV and surgical drainage of subretinal fluid followed by laser photocoagulation.

Keywords: Atypical central serous chorioretinopathy, bullous exudative retinal detachment, bilateral involvement, corticosteroid therapy, drainage of subretinal fluid, laser photocoagulation, pars plana vitrectomy

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Introduction

Central serous chorioretinopathy (CSCR) is a disorder characterized by serous macular detachment and/or focal changes in the retinal pigment epithelium (RPE), frequently limited to the macula and associated with fluid leakage in the subretinal space.[1] The main pathogenesis of CSCR has not been clearly defined but some theories focus on the role of the choroid and RPE. Choroidal hyperpermeability and RPE dysfunction caused by stasis, ischemia, or inflammation lead to vascular dilatation and leakage into the interstitial or stromal space.[1]

An atypical CSCR variant with exudative bullous retinal detachment (RD) is observed very rarely in comparison with acute and chronic CSCR. The differential diagnosis of bullous exudative RD includes Vogt-Koyanagi-Harada (VKH) disease, posterior scleritis, choroidal tumors (malignant melanoma, hemangioma, metastasis), uveal effusion syndrome, nanophthalmos, retinal vasculitis, lupus choroidopathy, multifocal choroiditis, Coats' disease, retinal hemangioblastoma, vasoproliferative tumors, malignant hypertension, and even rhegmatogenous RD.[2] Corticosteroid therapy administered as a result of misdiagnosis causes worsening of CSCR findings and delay of appropriate treatment.[3]

In the present case, we report the diagnostic steps, treatment, and follow-up of a patient with atypical CSCR manifesting with massive bullous exudative RD.

Case Report

A 28-year-old woman with no known systemic disease presented 1 year earlier to another center with sudden vision loss in her left eye (LE). She was diagnosed with RD and underwent pars plana vitrectomy (PPV). Visual acuity (VA) in the LE was no light perception postoperatively. She presented again to the same clinic with sudden vision loss in the right eye (RE) that started 2 weeks earlier. She was diagnosed with idiopathic uveal effusion syndrome following a limited systemic workup and received intravenous pulse steroid therapy for 3 days, followed by oral steroids. When no response was observed, she was referred to our center for possible scleral window surgery.

On admission to our clinic, VA was light perception only in the RE and no light perception in the LE. Intraocular pressures were 16 mmHg (RE) and 18 mmHg (LE). Anterior segment examination of the RE revealed leukocoria due to the bullous detachment of the retina coming to the back of the clear lens (Figure 1A). There was no flare or cells in the anterior chamber. The LE was aphakic with total fibrotic RD associated with subretinal fibrosis (Figure 1B).

Ultrasonography was done to rule out a tumor or posterior scleritis. Systemic steroid therapy was rapidly tapered and a systemic workup was started for rheumatologic and inflammatory diseases. However, laboratory tests were inconclusive. A 4-quadrant scleral window surgery with external drainage of subretinal fluid was performed under general anesthesia and resulted in almost complete reattachment of the retina at the end of surgery. At postoperative 2 weeks, BCVA had increased to counting fingers at 10 cm with shallow inferior detachment and subretinal yellow-white fibrin deposits. There was no vitritis or vitreous haze in the RE. Optical coherence tomography (OCT) images showed shallow foveal detachment with subretinal hyperreflective material suggestive of fibrin. The patient was followed up without additional treatment.

At the next visit 6 weeks after surgery, VA in the RE had decreased to hand motions and subretinal fluid had increased to become bullous again in the inferior hemisphere (Figure 2A, B, C). Ocular ultrasound revealed RD and a thick choroid (Figure 2B). Choroidal thickness was measured as 397 µm with enhanced depth image-OCT. The patient was hospitalized to conduct further laboratory testing for systemic lupus erythematosus and other causes of systemic vasculitis. Intravenous 1000 mg pulse methylprednisolone (Prednol, Mustafa Nevzat İlaç Sanayi, Türkiye) was given for 3 days and azathioprine (Imuran, ASPEN Europe GmbH, South Africa) 25 mg twice daily was initiated while preparing for fluorescein angiography (FA) and indocyanine green angiography (ICGA). ICG dye is not readily available in our country and must be imported from abroad. However, RD continued to progress during the 3-day period after steroid treatment. FA demonstrated multifocal hyperfluorescence in the early phase and multifocal staining (multiple hot spots) in the late phase in all quadrants (Figure 2D). There was no leakage from the retinal vessels or staining in the optic nerve head on FA. ICGA revealed widespread diffuse dilated choroidal vessels with no evidence of choroiditis (Figure 2E). Disease progression after corticosteroid treatment, absence of intraocular inflammation, thick choroid, and the FA and ICGA findings led us to the diagnosis of atypical CSCR with bullous exudative RD. Corticosteroid and azathioprine were stopped. A psychiatry consultation was arranged to start antidepressants. A second surgery was planned for the bullous RD, which again reached the back of the lens during this period.

PPV with internal drainage through a superior small retinotomy was performed and endolaser was applied to the staining foci by looking at the FA images during the surgery. Sulfur hexafluoride 20% was used as a tamponade and intravitreal aflibercept 2 mg/0.05 mL was injected at the end of the surgery.

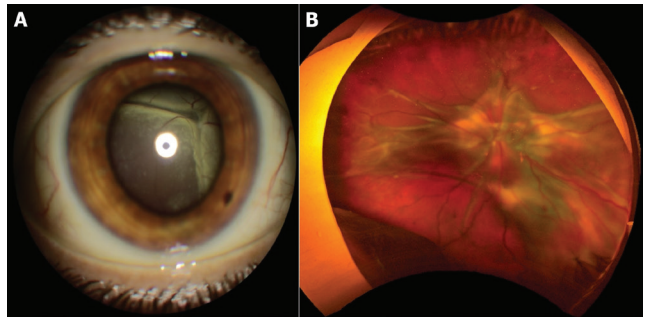

Figure 1. Anterior segment image of the right eye and fundus photograph of the left eye at first admission. (A) Total bullous retinal detachment was seen behind the clear lens in the right eye. (B) Wide-angle fundus photograph of the left eye showed subretinal fibrotic bands and detached retina at the posterior pole

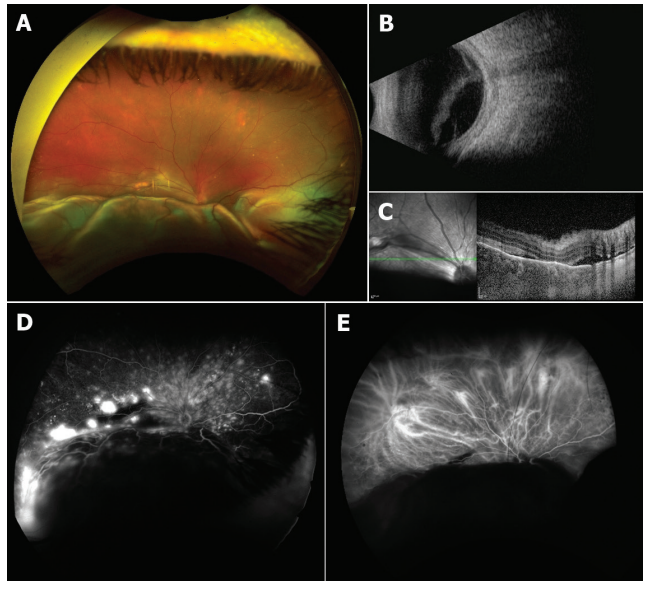

Figure 2. Multimodal imaging of the right eye at 6 weeks after the first surgery. (A) Inferior bullous retinal detachment. Ultrasonography (B) and enhanced depth imaging optical coherence tomography (C) revealed a thick choroid. (D) Fluorescein angiography demonstrated widespread leakage from multiple choroidal foci in the late phase at 4:15 min. Note the absence of retinal vascular leakage or staining of the optic nerve head. (E) Diffuse dilated choroidal vessels were observed on indocyanine green angiography

The retina was totally attached but VA remained at the level of counting fingers with resorption of the gas at the end of the first postoperative month. OCT revealed severe damage to the outer retinal layers. Follow-up FA revealed almost total regression of the leaking foci. The patient was followed up without further intervention. At the final examination 8 months after surgery, the retina remained attached and VA had increased to 20/25. OCT demonstrated restoration of the outer retinal layers starting from the fovea (Figure 3).

Discussion

An atypical form of CSCR, severe exudative bullous RD is a very rare clinical entity characterized by bilateral occurrence, multiple leakage foci, and severe vision loss.[4,5,6] This clinical presentation may be misdiagnosed as inflammatory diseases such as VKH disease, posterior scleritis, multifocal choroiditis, idiopathic posterior uveitis, lupus choroidopathy, or uveal effusion syndrome.[2] Multimodal imaging is crucial for the differential diagnosis, which is key in the management of these cases.

Several risk factors have been described, including male sex, type A personality, conditions that cause high corticosteroid levels (i.e., Cushing syndrome, pregnancy), systemic or local corticosteroid use, and psychological stress. The exact pathogenesis is still unclear. Some authors have suggested that the effects of steroids on the blood-retina barrier, choriocapillaris, and RPE lead to hyperpermeability and subretinal fluid accumulation.[3] This atypical severe CSCR associated with bullous exudative RD may be an exacerbated form of CSCR with possible risk factors such as corticosteroids. Sharma et al.[7] reported that 23 of 29 CSCR patients with exudative RD were using systemic corticosteroids. Gass and Little[6] reported a case of bilateral bullous RD that occurred after the administration of systemic and sub-Tenon corticosteroid in a patient with CSCR who was misdiagnosed as having choroiditis. Similarly, Cebeci et al.[5] reported bilateral bullous RD in a patient with CSCR who was given systemic and sub-Tenon corticosteroids with the misdiagnosis of VKH disease.

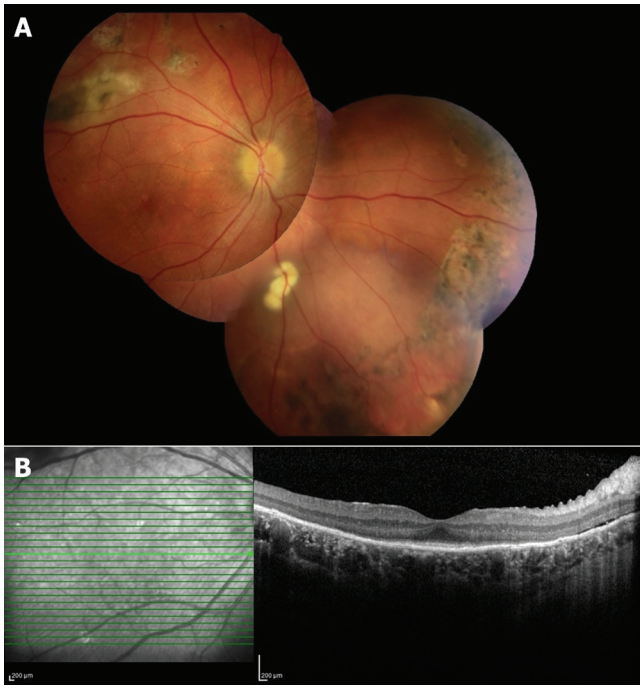

Figure 3. Imaging of the right eye at postoperative 8 months. (A) Composite fundus photograph showed the retina remained attached, with laser scars and some subretinal fibrin deposits. There was only a small area of shallow detachment in the inferonasal retina. (B) Optical coherence tomography showed macular attachment and marked improvement of the outer retinal layers, with reformation of the ellipsoid zone

Our patient was referred to our clinic for scleral window surgery with a possible diagnosis of uveal effusion syndrome. Although she received a course of steroid treatment with no response, it was not the steroid that initially triggered the formation of bullous exudative RD. There was also no history of steroid therapy during the loss of vision in the previously affected LE. In addition, recurrence of bullous RD after the first surgery was not related to steroid use in the present case. However, continued progression of exudative RD despite intravenous steroid therapy, the presence of pachychoroid, and the findings of FA (multifocal choroidal leakage without leakage from the retinal vessels or staining of the optic nerve head) and ICGA (dilated choroidal vessels) led us to the diagnosis of atypical CSCR.

Subretinal fibrosis and scar formation seem to be associated with severe CSCR when treated with corticosteroids. Hooymans[8] reported a patient with CSCR who developed subretinal fibrotic scar formation during systemic corticosteroid therapy. Sharma et al.[7] reported 29 multifocal CSCR patients with subretinal fibrosis and exudative RD, most of whom were given systemic steroids. The formation of subretinal bands and scarring at the posterior pole causes severe vision loss.[4,5] Our patient had subretinal fibrosis leading to tractional RD and no light perception in the fellow eye. Although she had a history of PPV in this eye a year earlier in a different clinic, we do not know the details of that surgery.

Differential diagnosis is essential for the treatment of this variant of CSCR. Cessation of corticosteroid therapy (if started) should be the first step of treatment. If there is no regression of the RD after steroid cessation, treatment should be considered. Non-surgical treatments like photodynamic therapy (PDT) or focal argon laser photocoagulation may be an option in cases with limited exudative RD.[3,5] However, cases with extensive bullous RD should be treated with surgical techniques such as external drainage or PPV with internal drainage to prepare for the application of any laser treatment. In the present case, scleral window surgery with external drainage of the subretinal fluid resulted in partial reattachment of the retina in the early period but could not prevent RD recurrence in a very short time. The second surgery included PPV with internal drainage of subretinal fluid, as well as endolaser to the leakage foci observed on FA, which we believe was the "sine qua non" of the surgery to

obtain the successful outcome. Anti-vascular endothelial growth factors (VEGFs) are thought to have beneficial effects in chronic CSCR and cases with subretinal fibrin exudates.[9] Yannuzzi[9] suggested that subretinal fibrin in CSCR is a result of leakage from abnormal choroidal vessels and recommended intravitreal anti-VEGF injections in cases with fibrin exudates, as we did in the present case.

The systemic mineralocorticoids eplerenone and spironolactone have been used with limited success for the treatment of this variant of CSCR.[1] Cebeci et al.[5] used eplerenone in combination with laser photocoagulation and PDT on an eye with bullous RD in a patient with atypical CSCR and asymmetric bilateral exudative RD, also with limited success. Kang et al.[4] performed a surgical technique similar to ours in their bilateral case but reported increased VA in only one eye because of the development of subretinal fibrosis in the fellow eye, which exhibited more severe involvement. Ng et al.[10] administered a half dose of verteporfin PDT to a patient with inferior exudative RD, which resulted in complete resolution of the RD within 3 months. Both laser photocoagulation and PDT seem to be effective for the treatment of CSCR with limited exudative RD.

In retrospect, we criticize ourselves for starting a second course of steroids for the recurrent RD following the first surgery, which resulted in more rapid progression. During that time, the RD was already progressing without steroids and we had to wait 3 more days to get ICG because it is not readily available in our country. We should not have challenged with steroids during this short time period just to be sure that it did not respond to steroids.

The present case may be unique in demonstrating a quick response to PPV and internal drainage, which enabled evacuation of the subretinal fibrin deposits and thereby prevented late subretinal fibrosis. Intravitreal anti-VEGF injection at the end of the surgery may have provided an additional benefit to prevent subretinal fibrosis. Intraoperative FA-guided endolaser application to the choroidal leakage foci was another important feature of the surgery. The restoration of the outer retinal layers and very satisfactory increase in VA within months after the surgery were also unique to this case.

In conclusion, the differential diagnosis of exudative RD should be done with multimodal imaging and careful systemic investigation to exclude malignancies and inflammatory and vascular diseases. An atypical variant of CSCR should be considered, especially when there is progression in response to steroid therapy. Steroid cessation, mineralocorticoid therapy, PDT, and laser photocoagulation therapy can be tried in moderate cases. However, surgical treatment with PPV, internal drainage, and endolaser photocoagulation to the leaking choroidal foci may be useful in the severe form of the disease, offering rapid recovery and good visual improvement. Anti-VEGFs injected at the end of surgery may be beneficial in reducing fibrin accumulation.

Ethics

Informed Consent: Obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Ö., H.B.Ö., M.H., Concept: Ş.Ö., H.B.Ö., G.G., İ.T.T., Design: Ş.Ö., H.B.Ö., M.Y., Data Collection or Processing: H.B.Ö., M.Y., Ş.Ö., A.M.S., Analysis or Interpretation: Ş.Ö., G.G., A.M.S., M.H., İ.T.T., Literature Search: M.Y., H.B.Ö., Writing: H.B.Ö., M.Y., Ş.Ö.

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