SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

Araştırma Makalesi / Research Article

Selcuk Med J 2022;38(2): 82-86
DOI: 10.30733/std.2022.01552

NEÜ YAYINEVİ

Reliability of Corneal Subbasal Nerve Plexus Analyses Using Semi-Automated Software

Yari-Otomatik Bir Yazilim Kullanilarak Yapilan Korneal Subbazal Sinir Pleksusu Analizlerinin Güvenilirliği

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Geliş Tarihi/Received: *7 February 2022*
Kabul Tarihi/Accepted: *18 April 2022*

Öz

Amaç: Bu çalışmanın amacı yarı-otomatik bir yazılım kullanılarak yapılan kantitatif korneal subbazal sinir pleksusu (KSSP) analizlerinin gözlemciler-arası ve gözlemci-içi güvenilirliğinin değerlendirilmesidir.
Hastalar ve Yöntem: Çalışmaya Necmettin Erbakan Üniversitesi Meram Tıp Fakültesi Göz Hastalıkları Bölümüne 20 Aralık 2021 – 20 Ocak 2022 tarihleri arasında başvuran 40 gönüllü dahil edildi. Katılımcıların sağ gözlerinden Heidelberg Retina Tomografisi III ile entegre Rostock Kornea Modülü kullanılarak KSSP'nu gösteren görüntüler alındı. Her bir gözden en kaliteli üç görüntü seçildi. ImageJ yazılımı için NeuronJ eklentisi ile sinir lifleri işaretlendi ve sinir lifi uzunluğu (SLU), sinir lifi dansitesi (SLD) ve sinir dalı dansitesi (SDD) hesaplandı. Tüm bu ölçümler iki farklı gözlemci tarafından yapıldı ve bir gözlemci tarafından bir hafta ara ile ikinci kez tekrar edildi ve sınıf-içi korelasyon katsayısı (SKK) kullanılarak gözlemciler-arası ve gözlemci-içi güvenilirlik analiz edildi.
Bulgular: Çalışmaya dahil edilen 12'si kadın, 28'i erkek 40 katılımcının ortalama yaşı 34.70±5.86 yıldır. Gözlemciler-arası güvenilirlik analizinde SKK değerleri SLU için 0.967 (95% CI 0.939-0.982), SLD için 0.826 (95% CI 0.696-0.904) ve SDD için 0.949 (95% CI 0.906-0.973) tespit edilmiş olup iyi-mükemmel güvenilirliği göstermekteydi. Gözlemci-içi güvenilirlik analizinde SKK değerleri SLU için 0.964 (95% CI 0.932-0.981), SLD için 0.803 (95% CI 0.657-0.891) ve SDD için 0.890 (95% CI 0.802-0.941) tespit edilmiş olup iyi-mükemmel güvenilirliği göstermekteydi.
Sonuç: Yarı-otomatik yazılım kullanılarak yapılan kantitatif KSSP analizlerinin gözlemciler-arası ve gözlemci-içi güvenilirliği yüksektir ve bu sayede hem klinik pratikte, hem de klinik çalışmalarda, kornea sinirlerinin iyilik halinin ve hasarının tespitinde, takibinde ve tedaviye yanıtın değerlendirilmesinde kullanılabilir.

Anahtar Kelimeler: Korneal subbazal sinir pleksusu, NeuronJ, güvenilirlik analizi

Abstract

Aim: The aim of this study was to evaluate the interobserver and intraobserver reliability of quantitative corneal subbasal nerve plexus (CSNP) analyses using semi-automated software.
Patients and Methods: Forty volunteers who applied to the Ophthalmology Department of the Necmettin Erbakan University Meram Faculty of Medicine between 20 December 2021 and 20 January 2022 were enrolled in the study. Images showing CSNP were obtained from the right eyes of the participants by using Heidelberg Retina Tomograph III with Rostock Cornea Module. Three best quality images were selected from each case. NeuronJ plugin for ImageJ software was used to trace nerve fibers and calculate nerve fiber length (NFL), nerve fiber density (NFD), and nerve branch density (NBD). All these measurements were performed by two different observers, and repeated for the second time by one of the observers with an interval of one week, and interobserver and intraobserver reliability were determined using the intraclass correlation coefficient (ICC).
Results: The mean age of 40 participants (12 female and 28 male) was 34.70±5.86 years. The ICCs for interobserver reproducibility were 0.967 (95% CI 0.939-0.982) for NFL, 0.826 (95% CI 0.696-0.904) for NFD, and 0.949 (95% CI 0.906-0.973) for NBD indicating good to excellent reliability. The ICCs for intraobserver repeatibility were 0.964 (95% CI 0.932-0.981) for NFL, 0.803 (95% CI 0.657-0.891) for NFD, and 0.890 (95% CI 0.802-0.941) for NBD indicating good to excellent reliability.
Conclusion: Quantitative CSNP analyzes using semi-automated software have high interobserver and intraobserver reliability and can therefore be used in both clinical practice and clinical studies for detection and follow-up of corneal nerves' well-being, damage, and evaluation of response to treatment.

Key words: Corneal subbasal nerve plexus, NeuronJ, reliability analysis

Cite this article as: Belviranli S, Gundogan AO, Mirza E, Adam M, Oltulu R. Reliability of Corneal Subbasal Nerve Plexus Analyses Using Semi-Automated Software. Selcuk Med J 2022;38(2): 82-86

Disclosure: *None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. All authors have agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

INTRODUCTION

The cornea is the foremost transparent layer of the eye and is one of the tissues with the most intense innervation in the human body. Corneal nerves are mainly sensory and most of them originate from the ophthalmic branch of the trigeminal nerve. In addition to sensory functions, corneal nerves also play an important role in the blink reflex, tear production, corneal wound healing, and maintaining a healthy ocular surface (1,2). Corneal nerves enter the cornea radially from the stroma layer at the periphery and branch out parallel to the corneal surface. Then they turn anteriorly and penetrate the Bowman's layer and form the corneal subbasal nerve plexus (CSNP), which runs parallel to the corneal surface, under the basal epithelial layer. Branches originating from here also terminate as free nerve endings in the corneal epithelium (1).

It is not possible to evaluate the corneal nerves in detail with standard examination methods. Ex vivo light microscopy and electron microscopy studies provide information about corneal nerves (3). In vivo confocal microscopy (IVCM) is a relatively new, non-invasive technique that provides high-resolution and cellular imaging of corneal layers. Nowadays, IVCM is widely used in the diagnosis of various corneal pathologies and in monitoring the response to treatment (4). IVCM also enables visualization of the corneal nerves and CSNP in detail (5). Various parameters in CSNP images can be quantitatively evaluated using manual, semi-automated or automated software (6).

In this study, we aimed to evaluate the interobserver reproducibility and intraobserver repeatability of nerve fiber length (NFL), nerve fiber density (NFD) and nerve branch density (NBD) parameters that were analyzed using a semi-automated software on CSNP images obtained with IVCM.

PATIENTS AND METHODS

Forty volunteers who applied to the Ophthalmology Department of the Necmettin Erbakan University Meram Faculty of Medicine between 20 December 2021 and 20 January 2022 and met the study criteria were included in the study. The study was carried out with the approval of the Ethics Committee of the Necmettin Erbakan University, approval number 2021/3565. The study was conducted in accordance with the terms of the Declaration of Helsinki. After the participants were given detailed information about the study, informed consent was obtained from each participant. All participants underwent a complete ophthalmologic examination, including measurement of refraction, measurement of intraocular pressure, best corrected visual acuity, and slit-lamp examination of the anterior segment and posterior segment. Patients with any systemic disease that may affect corneal innervation such as diabetes mellitus, connective tissue diseases, autoimmune diseases; patients with any ocular pathology such as uveitis, glaucoma, blepharitis, conjunctivitis, keratitis, dry eye, corneal dystrophy, eyelid disorders; patients with a history of chemical or thermal ocular injury; patients with a scar or nephelia on the cornea; those using contact lenses; those with a history of intraocular or periocular surgery including refractive surgery; patients using any topical ocular medication including artificial tears, patients with a refractive error greater than ± 3 diopters of spherical equivalent; patients who had a history of COVID-19 and who were pregnant or breastfeeding were excluded.

CSNP imaging was performed using Heidelberg Retina Tomograph III (HRT III) in combination with the Rostock Cornea Module (RCM) (Heidelberg Engineering, Heidelberg, Germany). Topical anesthetic drops (0.5% proparacaine HCl, Alcaine®, S.A. Alcon-Couvreur N.V., Puurs, Belgium) were applied before the procedure. Only the right eyes of all participants were examined in the study. Serial images of 400*400 microns showing the CSNP were taken from the central cornea. Corneal confocal microscopy imaging for all participants was performed under the same standard conditions by the same experienced investigator. Then, for each eye included in the study, three images with the best quality display of the CSNP were selected. An open-source semi-automatic plugin (NeuronJ by Erik Meijering; ver. 1.4.3) for ImageJ software (Wayne Rasband and contributors, National Institutes of Health, USA; ver. 1.53f) was used to trace nerve fibers in selected images. Figure-1 shows an example for tracing of the nerve fibers with NeuronJ. After the nerve fibers were traced, the total nerve length calculated by the software on 400*400 micron images was converted to mm/mm^2 and recorded as nerve fiber length (NFL). The nerve fiber density (NFD; the number of major nerves per mm^2) and the nerve branch density (NBD; the number of branches emanating from major nerve trunks, per mm^2) in each selected image were recorded in units of fibers/mm^2 and branches/mm^2, respectively, as described by Malik et al. (7). The results from 3 different CSNP images from each eye examined in the study were averaged. All these measurements were

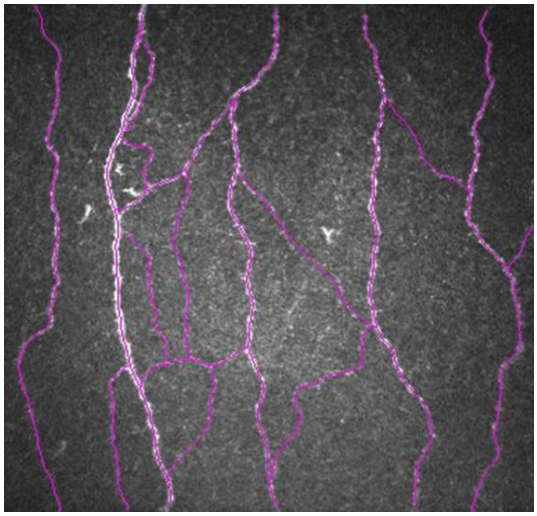

Figure 1. An example for tracing of the nerve fibers with NeuronJ plugin. (400x400 micron image)

performed by two different observers (Observer-1 and Observer-2), and repeated for the second time by one of the observers (Observer-1) with an interval of one week. Interobserver reproducibility was evaluated by analyzing the consistency of the results obtained by Observer-1 (SB) and Observer-2 (AOG). Intraobserver repeatability was evaluated by analyzing the consistency of the results obtained by Observer-1 (SB) at two different times.

IBM SPSS statistics software version 20.0 was used for statistical analysis (IBM Corp, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables as number (n) and percentage (%). Conformity of continuous variables to normal distribution was evaluated by Shapiro-Wilk test. Intraclass correlation coefficient (ICC) was used for reliability analyzes. For interobserver reproducibility analysis, ICC two-way random-effects model, absolute agreement type and single measures were preferred. For intraobserver repeatability analysis, ICC two-way mixed-effects model, absolute agreement type and single measures were preferred. When evaluating ICC results, an ICC value of <0.5 was considered poor reliability, a value of 0.5-0.75 was considered moderate reliability, a value of 0.75-0.9 was considered good reliability, and a value of >0.90 was considered excellent reliability per recommendation published by Koo et al. (8). In all statistical analyses, two-sided p<0.05 was considered statistically significant.

RESULTS

The ages of the 40 participants included in the study were in the range of 25–44 years, with a mean of 34.70±5.86 years. Twelve (30%) of the participants were female and 28 (70%) were male. The mean NFL found in the first measurement of Observer-1 was 19.06±3.75 mm/mm^2, mean NFD was 24.79±5.08 fibers/mm^2 and mean NBD was 54.43±17.97 branches/mm^2. The mean NFL found in the second measurement of Observer-1 was 18.99±4.01mm/mm^2, mean NFD was 24.89±4.42 fibers/mm^2, and mean NBD was 54.69±20.26 branches/mm^2. The mean NFL found by Observer-2 was 19.04±3.52 mm/mm^2, mean NFD was 24.48±4.80 fibers/mm^2, and mean NBD was 54.84±16.78 branches/mm^2.

The ICCs for interobserver reproducibility were 0.967 (95% CI 0.939-0.982) for NFL, 0.826 (95% CI 0.696-0.904) for NFD, and 0.949 (95% CI 0.906-0.973) for NBD indicating good to excellent reliability. (Table 1). The ICCs for intraobserver repeatability were 0.964 (95% CI 0.932-0.981) for NFL, 0.803 (95% CI 0.657-0.891) for NFD, and 0.890 (95% CI 0.802-0.941) for NBD indicating good to excellent reliability. (Table 2)

DISCUSSION

In addition to their sensory functions, corneal nerves play an important role in maintaining a healthy ocular surface via their trophic effects (2). Deterioration of corneal innervation can lead to a degenerative process called neurotrophic keratopathy. Quantitative evaluation of corneal innervation is of great importance in the evaluation of the well being of the cornea, in the diagnosis and follow-up of neurotrophic processes, and in the evaluation of the response to treatment.

Table 1. Interobserver Reproducibility analyses for NFL, NFD and NBD

	Observer-1 (mean±SD)	Observer-2 (mean±SD)	Interobserver Reproducibility (ICC [95% CI])
Nerve Fiber Length (mm/mm^2)	19.06±3.75	19.04±3.52	0.967 [0.939-0.982]
Nerve Fiber Density (fiber/mm^2)	24.79±5.08	24.48±4.80	0.826 [0.696-0.904]
Nerve Branch Density (branch/mm^2)	54.43±17.97	54.84±16.78	0.949 [0.906-0.973]

SD standard deviation, ICC intraclass correlation coefficient, CI confidence interval

Table 2. Intraobserver reliability analyses for NFL, NFD and NBD

	Observer-1 Measurement-1 (mean±SD)	Observer-1 Measurement-2 (mean±SD)	Intraobserver Repeatability (ICC [95% CI])
Nerve Fiber Length (mm/mm^2)	19.06±3.75	18.99±4.01	0.964 [0.932-0.981]
Nerve Fiber Density (fiber/mm^2)	24.79±5.08	24.89±4.42	0.803 [0.657-0.891]
Nerve Branch Density (branch/mm^2)	54.43±17.97	54.69±20.26	0.890 [0.802-0.941]

SD standard deviation, ICC intraclass correlation coefficient, CI confidence interval

CSNP can be viewed in detail with IVCM. In addition, various features such as length, density, branching, and torticity of the nerves that make up the CSNP can be evaluated from these images. NFL, NFD, and NBD, which were defined by Malik et al. (7) in their study published in 2003, constitute the most commonly used parameters in the quantitative analysis of CSNP. These parameters determine potential indicators of corneal nerve fiber damage and repair. In the literature, corneal nerve fiber damage has been detected in various ocular and systemic diseases by using these parameters (9–14). In addition, the effects of various surgical procedures such as cataract surgery, refractive surgery and pterygium excision on corneal nerve fibers have also been shown with these parameters (15–17).

Another very important point in the quantitative analysis of CSNP is the evaluation of these parameters objectively, accurately and consistently. These conditions must be met in order to use these parameters in clinical practice and to compare the results obtained from different studies. With the understanding of the importance of quantitative analysis of CSNP, studies on this subject have also increased, and various manual, semi-automated and automated software have been prepared for this purpose. The CCMetrics software (University of Manchester, Manchester, UK) calculates parameters such as NFL, NFD and NBD after manual tracing of all the nerve fibers that can be seen in the CSNP images. In the ACCMetrics software (University of Manchester, Manchester, UK) developed later by the same group, nerve fibers are automatically detected and parameters are calculated. In the study of Petropoulos et al. (18), in which they used the manual CCMetrics software, it was shown that interobserver reproducibility (ICC 0.70 and 0.74, respectively) and intraobserver repeatability (ICC 0.66 and 0.82, respectively) were good for NFL and NFD, but the reliability was low in NBD measurements. Chin et al.'s study (19), which included 20 patients who underwent refractive surgery, compared manual measurements performed with the CCMetrics software and automatic measurements performed with the ACCMetrics software. In their study, the ICCs for interobserver reproducibility in NFL, NFD and NBD measurements performed with CCMetrics software were 0.740, 0.728 and 0.591, respectively, while the ICCs for intraobserver repeatability was 0.799, 0.757 and 0.653, respectively. In the same study, all NFL, NFD and NBD measurements performed with the ACCMetrics software were lower than the measurements performed with the CCMetrics software. It has been stated that this may be due to the inability of the ACCMetrics software to detect thin or pale nerve fibers and nerve fibers in low contrast areas.

NeuronJ (by Erik Meijering), a plugin for ImageJ software (Wayne Rasband and contributors, National Institutes of Health, USA), can trace nerve fibers in a semi-automatic way. In this plugin, when the mouse pointer is brought closer to the starting point of the nerve fiber in a CSNP image, the most likely line for it is drawn by the software, and the nerve fiber can be traced by following it to the point where it ends. NFL can be calculated in mm/mm^2 by proportioning the total nerve fiber length calculated by the software to the total area of the image. NFD and NBD can be calculated in terms of fibers/mm^2 and branches/mm^2, respectively, by proportioning the number of main nerve trunks and branchs to the total area of the image. In the study by Dehghani et al. (20), NFL analyses using automated, semi-automated and manual software were shown to give consistent results in both healthy controls and diabetics. In the study by Cottrell et al. (21) in which corneal nerve damage was evaluated in eyes of patients with herpes simplex keratitis, it was reported that NFL measurement with NeuronJ, and NFD and NBD calculations showed good interobserver reproducibility with the ICCs of 0.96, 0.90 and 0.97, respectively. In the study by Parissi et al. (22), which included 106 people between the ages of 15-88 years, the mean NFL detected with the NeuronJ software was 19 mm/mm^2 and was

negatively correlated with age.

Analyzes performed using NeuronJ software in our study showed that NFL, NFD and NBD measurements had good to excellent interobserver (ICC 0.967, 0.826 and 0.949, respectively) and intraobserver (ICC 0.964, 0.803 and 0.890, respectively) reliability. Both interobserver and intraobserver reliability were highest in NFL, and lowest in NFD. In Chin et al.'s study (19), similar to our study, the parameter with the highest interobserver and intraobserver reliability was NFL, but the parameter with the lowest reliability was NBD. The most important limitation of this study is that the analyses were performed using a single software. Consistency between different softwares can also be evaluated by analyzing the same images with different softwares. In addition, the relatively low number of participants and observers is another limitation. The reliability of this method can be confirmed by the results obtained from studies in which images from more participants are analyzed by three or more observers.

In conclusion, quantitative CSNP analyzes with semi-automatic NeuronJ software have good to excellent interobserver and intraobserver reliability. This technique can be used both in clinical practice and in clinical studies for detection and follow-up of corneal nerves' well-being and damage, and in evaluating the response to treatment.

Conflict of interest: *Authors declare that there is no conflict of interest between the authors of the article.*

Financial conflict of interest: *Authors declare that they did not receive any financial support in this study.*

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