RESEARCH ARTICLE

Corneal confocal microscopy identifies greater corneal nerve damage in patients with a recurrent compared to first ischemic stroke

Adnan Khan¹, Naveed Akhtar², Saadat Kamran², Hamad Almuhannadi¹, Georgios Ponirakis¹, Ioannis N. Petropoulos¹, Blessy Babu², Namitha R. Jose², Rumissa G. Ibrahim², Hoda Gad¹, Paula Bourke², Maher Saqqur²,³, Ashfaq Shuaib³, Rayaz A. Malik¹*¹

1 Department of Medicine, Weill Cornell Medicine-Qatar, Doha, Qatar, 2 Department of Neurology and Institute of Neurosciences, Hamad Medical Corporation, Doha, Qatar, 3 Department of Neurology, Stroke Program, University of Alberta, Alberta, Canada

* ram2045@qatar-med.cornell.edu

Abstract

Objectives

Corneal nerve damage may be a surrogate marker for the risk of ischemic stroke. This study was undertaken to determine if there is greater corneal nerve damage in patients with recurrent ischemic stroke.

Methods

Corneal confocal microscopy (CCM) was used to quantify corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL) and corneal nerve fiber tortuosity (CNFT) in 31 patients with recurrent ischemic stroke, 165 patients with a first acute ischemic stroke and 23 healthy control subjects.

Results

Triglycerides (P = 0.004, P = 0.017), systolic BP (P = 0.000, P = 0.000), diastolic BP (P = 0.000, P = 0.000) and HbA¹c (P = 0.000, P = 0.000) were significantly higher in patients with first and recurrent stroke compared to controls. There was no difference in age, BMI, HbA¹c, total cholesterol, triglycerides, LDL, HDL, systolic and diastolic BP between patients with a first and recurrent ischemic stroke. However, CNFD was significantly lower (24.98 ± 7.31 vs 29.07 ± 7.58 vs 37.91 ± 7.13, P < 0.05) and CNFT was significantly higher (0.085 ± 0.042 vs 0.064 ± 0.037 vs 0.039 ± 0.022, P < 0.05) in patients with recurrent stroke compared to first and healthy controls. CNBD (42.21 ± 24.65 vs 50.46 ± 27.68 vs 87.24 ± 45.85, P < 0.001) and CNFL (15.66 ± 5.70, P < 0.001 vs 17.38 ± 5.06, P = 0.003) were equally reduced in patients with first and recurrent stroke compared to controls (22.72 ± 5.14).
Conclusions

Corneal confocal microscopy identified greater corneal nerve fibre loss in patients with recurrent stroke compared to patients with first stroke, despite comparable risk factors. Longitudinal studies are required to determine the prognostic utility of corneal nerve fiber loss in identifying patients at risk of recurrent ischemic stroke.

Introduction

Recurrent stroke occurs in 11.1% of stroke patients within one year of the initial stroke [1], and is associated with greater disability and mortality [2]. A recent study from China has shown that the incidence of recurrent stroke has increased 3-fold between 1992 and 2012 [3]. Age [4], dyslipidemia [5], smoking [6], diabetes, hypertension, homocysteine levels, atrial fibrillation [1], metabolic syndrome [7] and other risk factors [8–10] are associated with recurrent stroke. Indeed, a recent study has shown that hypertension, prior symptomatic stroke and chronic infarcts on MRI were independently associated with recurrent stroke and this also doubled the all-cause mortality [11]. However, an artificial neural network model utilizing 19 independent variables generated only a moderate accuracy of 75% for predicting stroke recurrence at 1-year [12].

Brain imaging reveals that the presence of multiple white matter hyperintensities [13–15], silent lacunar infarcts and isolated cortical lesions are associated with recurrent stroke and the presence of white matter hyperintensities, micro-bleeds [16] and silent new ischemic lesions [17, 18] predict the risk of stroke [19]. Furthermore, the 5-year recurrent stroke risk in the presence of severe white matter changes is comparable to the presence of atrial fibrillation and hypertension [20].

Corneal confocal microscopy (CCM) is a noninvasive ophthalmic imaging technique for rapid, high-resolution imaging of corneal nerves. This technique has identified axonal loss in diabetes [21–23], impaired glucose tolerance [24], other peripheral neuropathies [25, 26], Parkinson’s disease [27], amyotrophic lateral sclerosis [28], multiple sclerosis [29] and dementia [30]. More recently we have shown a significant loss of corneal nerves in patients with TIA [31] and acute ischemic stroke [32–34], which was associated with elevated triglycerides and HbA1c. Vascular risk factors including dysglycemia and dyslipidemia [35] and hypertension [36] are associated with corneal nerve loss and an improvement in blood pressure, lipids, HbA1c [37] and glucose tolerance [38] are associated with an improvement in corneal nerve morphology.

Given that there are shared risk factors for stroke and corneal nerve loss, we hypothesized that patients with recurrent ischemic stroke will demonstrate greater corneal nerve abnormality compared to those with first ischemic stroke, reflecting the greater overall exposure to the risk factors for stroke.

Materials and methods

Thirty-one patients with a recurrent acute ischemic stroke, 165 patients with a 1st acute ischemic stroke and 23 age-matched healthy control participants were studied. The diagnosis of stroke was confirmed clinically andradiologically using AHA criteria [39]. Exclusion criteria included patients with intracerebral hemorrhage, a known history of ocular trauma or surgery, high refractive error, glaucoma, dry eye and corneal dystrophy [40]. Demographic (age,
gender, ethnicity) and clinical (blood pressure, HbA1c, lipid profile) data were obtained from patients’ health records. All patients underwent assessment of the National Institutes of Health Stroke Scale (NIHSS) at presentation. This study adhered to the tenets of the declaration of Helsinki and was approved by the Institutional Review Board of Weill Cornell Medicine (15–00021) and Hamad General Hospital (15304/15). Informed, written consent was obtained from all patients/guardians before participation in the study.

**Corneal confocal microscopy**

All patients underwent CCM (Heidelberg Retinal Tomograph III Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany). CCM uses a 670 nm wavelength helium neon diode laser, which is a class I laser and therefore does not pose any ocular safety hazard. A ×63 objective lens with a numeric aperture of 0.9 and a working distance, relative to the applanating cap (TomoCap; Heidelberg Engineering GmbH) of 0.0 to 3.0 mm, is used. The size of each 2-dimensional image produced is 384×384 pixels with a 15˚×15˚ field of view and 10 μm/pixel transverse optical resolutions. To perform the CCM examination, local anesthetic (0.4% benoxinate hydrochloride; Chauvin Pharmaceuticals, Chefaro, United Kingdom) was used to anesthetize both eyes, and Viscotears (Carbomer 980, 0.2%, Novartis, United Kingdom) was used as the coupling agent between the cornea and the cap. Patients were asked to fixate on an outer fixation light throughout the CCM scan and a CCD camera was used to correctly position the cap onto the cornea [41]. The examination took approximately 10 minutes for both eyes. The examiners captured images of the central sub-basal nerve plexus using the section mode. On the basis of depth, contrast, focus, and position, 6 images per patient were selected [42]. All CCM images were manually analyzed using validated, purpose-written software. Corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), corneal nerve fiber length (CNFL) and corneal nerve fiber tortuosity (CNFT) were analyzed using CCMetrics (M. A. Dabbah, ISBE, University of Manchester, Manchester, United Kingdom) [21].

**Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics software Version 25. Normality of the data was assessed using the Shapiro-Wilk test and by visual inspection of the histogram and a normal Q-Q plot. Data are expressed as mean ± standard deviation (SD). Each group was compared using ANOVA (for normally distributed variables) with Bonferroni as post hoc test and the non-parametric Kruskal-Wallis test (for non-normally distributed variables). To investigate the association between risk factors for corneal nerve parameters, Pearson and Spearman correlation were performed as appropriate. Multiple linear regression analysis was conducted to evaluate the independent association between corneal nerve loss and their covariates. The data used for statistical analysis in this study is available (https://figshare.com/s/ea4479b2063a26113cf0).

**Results**

**Clinical and metabolic characteristics**

The clinical and metabolic characteristics of the cohorts of participants studied are summarized in Table 1.

Thirty-one patients with a recurrent ischemic stroke were compared with 165 patients with a 1st ischemic stroke and 23 age-matched healthy controls. There was no significant difference in the percentage of patients with a 1st stroke compared to recurrent (2nd) stroke in relation to
the use of statins (95% vs 87%), ACE-inhibitors (52% vs 58%), Angiotensin II receptor blockers (6% vs 13%), Beta blockers (10% vs 29%), calcium channels blockers (16% vs 26%), aspirin (83% vs 77%) and clopidogrel (58% vs 45%) (Table 2).

Clinical and metabolic variables in patients with a 1st stroke, recurrent stroke and healthy controls

Systolic BP (P = 0.000), diastolic BP (P = 0.000, P = 0.000), HbA1c (P = 0.000, P = 0.000), total cholesterol (P = 0.035, P = 0.196) and triglycerides (P = 0.004, P = 0.017) were significantly higher in the patients with a 1st stroke and recurrent stroke (except cholesterol) compared to healthy controls (Table 1).

Table 2. Percentage of patients on different medications.

| Medications         | 1st Stroke | 2nd Stroke |
|---------------------|------------|------------|
| Statins (%)         | 153/165 (95%) | 27/31 (87%)|
| ACE Inhibitors (%)  | 85/165 (52%) | 18/31 (58%)|
| ARB’s (%)           | 09/165 (6%) | 4/31 (13%)|
| Beta blockers (%)   | 17/165 (10%) | 09/31 (29%)|
| Calcium channel blockers (%) | 26/165 (16%) | 5/31 (26%)|
| Aspirin (%)         | 137/165 (83%) | 24/31 (77%)|
| Clopidogrel (%)     | 96/165 (58%) | 14/31 (45%)|

https://doi.org/10.1371/journal.pone.0231987.t002
Clinical and metabolic variables in recurrent v 1st stroke

There was no significant difference in age, BMI, HbA1c, total cholesterol, triglycerides, LDL, HDL, systolic and diastolic BP between patients with a first and recurrent ischemic stroke (Table 1).

CCM in patients with a recurrent stroke, 1st stroke and healthy controls

CNFD (P < 0.001, P < 0.001), CNFL (P < 0.001, P < 0.001) and CNBD (P = 0.003, P < 0.001) were significantly lower, and CNFT (P = 0.028, P < 0.001) was significantly higher in patients with 1st and recurrent stroke compared to healthy controls (Table 1, Fig 1).

CCM in recurrent v 1st stroke

CNFD (P = 0.018) was significantly lower and CNFT (P = 0.013) was significantly higher in patients with recurrent stroke compared to 1st stroke. There was no significant difference in CNFL (P = 0.269) or CNBD (P = 0.269) between patients with recurrent compared to 1st stroke (Table 1, Fig 1).

Multiple linear regression

There were independent associations between corneal nerve and metabolic parameters in patients with stroke (Table 3). CNFD was significantly associated with age (β = –0.204,
P<0.001, BMI (β = -0.525, P = 0.001) and diastolic BP (β = 0.082, P = 0.014). CNFL was significantly associated with age (β = -0.105, P = 0.015) and BMI (β = -0.333, P = 0.000). CNFT was significantly associated with NIHSS (β = 0.001, P = 0.049). CNBD was skewed, therefore it was not included in the regression analysis.

**Discussion**

There is a need to identify risk factors or surrogate markers for stroke recurrence, such that high risk individuals can be targeted for more aggressive risk factor reduction. This is the first study to show greater corneal nerve loss in patients with recurrent ischemic stroke compared to a 1st ischemic stroke. This extends our previous findings demonstrating corneal nerve loss in patients with TIA [31] and acute ischemic stroke [32, 33].

Individual vascular risk factors such as diabetes, hypertension, smoking, dyslipidemia and metabolic syndrome are associated with the risk of a first and recurrent ischemic stroke [5–7, 13, 43]. A recent study has shown that a greater increase in carotid intima media thickness (IMT) was associated with an increased incidence of major adverse cerebral and coronary events [44]. Similarly, in the J-STARS (Japan Statin Treatment Against Recurrent Stroke) study, patients with the greatest baseline IMT were at the highest risk of recurrent stroke, which was partially ameliorated by treatment with pravastatin [45]. Intervention with dual as opposed to single antiplatelet therapy reduces the risk of recurrent stroke, but it is also associated with an increased risk of adverse events [46]. It is therefore important to identify those patients who may benefit the most from more aggressive control of risk factors. Interestingly, a recent longitudinal study of patients with a myocardial infarction from Stockholm showed that whilst albuminuria was associated with an increased risk of recurrent myocardial infarction there was no association with ischemic stroke [47].

MRI studies have shown that structural alterations including white matter hyperintensities, lacunes and microbleeds are associated with an increased risk of recurrent ischemic stroke [9, 13, 15]. We have previously shown a loss of corneal nerves in subjects with a major ischemic stroke compared to controls and an association between corneal nerve loss with HbA1c and triglycerides [32, 33]. In the present study, despite age, BMI, HbA1c, lipids, BP and use of medications to treat blood pressure and lipids being comparable between those with recurrent stroke and 1st stroke, there was greater corneal nerve damage in patients with recurrent compared to 1st stroke. This suggests that corneal nerve loss may reflect the cumulative effect of known vascular risk factors and unknown risk factors for stroke and act as a surrogate marker for the risk of stroke and recurrent stroke.
This study is limited by the modest number of patients studied with recurrent ischemic stroke. We were also not able to include patients with severe stroke as CCM could not be performed in these individuals, due to their inability to cooperate during the CCM procedure. Whilst this may limit the utility of CCM across the spectrum of severity of stroke, it may also have biased the outcomes as the results may have been even more pronounced in those with more severe stroke. Nevertheless, we show greater corneal nerve abnormalities in patients with recurrent compared to a 1st acute ischemic stroke. Larger, longitudinal studies assessing corneal nerve fibre morphology in those at higher risk of stroke, perhaps with TIA and in relation to therapies to reduce risk factors for stroke are warranted to establish the clinical utility of corneal confocal microscopy in ischemic stroke.

Author Contributions

Conceptualization: Adnan Khan.

Data curation: Adnan Khan, Georgios Ponirakis, Blessy Babu, Namitha R. Jose, Rumissa G. Ibrahim, Paula Bourke.

Formal analysis: Adnan Khan.

Funding acquisition: Rayaz A. Malik.

Investigation: Adnan Khan, Naveed Akhtar, Saadat Kamran, Hamad Almuhannadi, Ioannis N. Petropoulos, Hoda Gad, Maher Saqqur.

Methodology: Adnan Khan, Ashfaq Shuaib, Rayaz A. Malik.

Project administration: Naveed Akhtar, Ashfaq Shuaib, Rayaz A. Malik.

Resources: Naveed Akhtar, Ashfaq Shuaib, Rayaz A. Malik.

Software: Adnan Khan.

Supervision: Ashfaq Shuaib, Rayaz A. Malik.

Validation: Adnan Khan, Ashfaq Shuaib, Rayaz A. Malik.

Visualization: Adnan Khan.

Writing – original draft: Adnan Khan.

Writing – review & editing: Adnan Khan, Ashfaq Shuaib, Rayaz A. Malik.

References

1. Hankey GJ. Secondary stroke prevention. Lancet Neurol. 2014; 13(2):178–94. https://doi.org/10.1016/S1474-4422(13)70255-2 PMID: 24361114
2. Petty G, Brown R, Whisnant J, Sicks J, O’Fallon W, Wiebers D. Survival and recurrence after first cerebral infarction: a population-based study in Rochester, Minnesota, 1975 through 1989. Neurology. 1998; 50(1):208–16. https://doi.org/10.1212/wnl.50.1.208 PMID: 9443482
3. Zhao W, Wu J, Liu J, Wu Y, Ni J, Gu H, et al. Trends in the incidence of recurrent stroke at 5 years after the first-ever stroke in rural China: a population-based stroke surveillance from 1992 to 2017. Aging (Albany NY). 2019; 11(6):1686.
4. Pennlert J, Eriksson M, Carlberg B, Wiklund P-G. Long-term risk and predictors of recurrent stroke beyond the acute phase. Stroke. 2014; 45(6):1839–41. https://doi.org/10.1161/STROKEAHA.114.005060 PMID: 24788972
5. Park J-H, Lee J, Ovbiagele B. Nontraditional serum lipid variables and recurrent stroke risk. Stroke. 2014; 45(11):3269–74. https://doi.org/10.1161/STROKEAHA.114.006827 PMID: 25236873
6. Chen J, Li S, Zheng K, Wang H, Xie Y, Xu P, et al. Impact of smoking status on stroke recurrence. Am Heart J 2019; 8(8):e011696.
7. Chan W, Pan Y, Jing J, Zhao X, Liu L, Meng X, et al. Recurrent stroke in minor ischemic stroke or transient ischemic attack with metabolic syndrome and/or diabetes mellitus. Am Heart J. 2017; 6(6):e005446. https://doi.org/10.1016/甲状.116.005446 PMID: 28572281.

8. Kauw F, Takx RA, de Jong HW, Velthuis BK, Kappelle LJ, Dankbaar JW. Clinical and imaging predictors of recurrent ischemic stroke: A systematic review and meta-analysis. Cerebrovasc Dis. 2018; 45 (5–6):279–87. https://doi.org/10.1159/000490422 PMID: 29936515

9. Yu L, Yang L, Zhang X, Yuan J, Li Y, Yang S, et al. Age and recurrent stroke are related to the severity of white matter hyperintensities in lacunar infarction patients with diabetes. Clin Interv Aging. 2018; 13(7):2487–94. https://doi.org/10.2147/CI.184463 PMID: 30584289

10. Charidimou A, Kakar P, Fox Z, Werring DJ. Cerebral microbleeds and recurrent stroke risk: systematic review and meta-analysis of prospective ischemic stroke and transient ischemic attack cohorts. Stroke. 2013; 44(4):995–1001. https://doi.org/10.1161/STROKEAHA.111.000038 PMID: 23493732

11. Khanevski AN, Bjerkreim AT, Novotny V, Naess H, Thomassen L, Logallo N, et al. Recurrent ischemic stroke: incidence, predictors and impact on mortality. Acta Neurol Scand. 2019; 140(1):3–8. https://doi.org/10.1111/anec.13093 PMID: 30929256

12. Chan KL, Leng X, Zhang W, Dong W, Qiu Q, Yang J, et al. Early identification of high-Risk TIA or minor stroke using artificial neural network. Front Neurol. 2019; 10:1–7. https://doi.org/10.3389/fneur.2019.00001

13. Park JH, Heo S, Lee M, Kwon H, Kwon S, Lee J, et al. White matter hyperintensities and recurrent stroke risk in patients with stroke with small-vessel disease. Eur J Neurol. 2019.

14. Moerch-Rasmussen A, Nacu A, Waje-Andresen U, Thomassen L, Naess H. Recurrent ischemic stroke is associated with the burden of risk factors. Acta Neurol Scand. 2016; 133(4):289–94. https://doi.org/10.1111/anec.12457 PMID: 26177064

15. Kim G-M, Park K-Y, Avery R, Helenius J, Rost N, Rosand J, et al. Extensive leukoaraiosis is associated with high early risk of recurrence after ischemic stroke. Stroke. 2014; 45(2):479–85. https://doi.org/10.1161/STROKEAHA.113.003004 PMID: 24370756

16. Thijss V, Lemmens R, Schoofs C, Görner A, Van Damme P, Schrooten M, et al. Microbleeds and the risk of recurrent stroke. Stroke. 2010; 41(9):2005–9. https://doi.org/10.1161/STROKEAHA.110.588020 PMID: 20651265

17. Kang D-W, Han M-K, Kim H-J, Sohn H, Kim BJ, Kwon SU, et al. Silent new ischemic lesions after index stroke and the risk of future clinical recurrent stroke. Neurology. 2016; 86(3):277–85. https://doi.org/10.1212/WNL.0000000000002289 PMID: 26683639.

18. Kang D-W, Lattimore SU, Latour LL, Warach S. Silent ischemic lesion recurrence on magnetic resonance imaging predicts subsequent clinical vascular events. Arch Neurol. 2006; 63(12):1730–3. https://doi.org/10.1001/archneur.63.12.1730 PMID: 17172612

19. de Groot M, Verhaeren BF, de Boer R, Klein S, Hofman A, van der Lugt A, et al. Changes in normal-appearing white matter precede development of white matter lesions. Stroke. 2013; 44(4):1037–42. Epub 2013/02/23. https://doi.org/10.1161/STROKEAHA.112.680223 PMID: 23429507.

20. Melkas S, Sibott G, Oksala N, Putaala J, Pohjasvaara T, Kaste M, et al. Extensive white matter changes predict stroke recurrence up to 5 years after a first-ever ischemic stroke. Cerebrovasc Dis. 2012; 34(3):191–8. https://doi.org/10.1159/000341404 PMID: 23006549

21. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, Dabbah MA, et al. Rapid automated diagnosis of diabetic peripheral neuropathy with in vivo corneal confocal microscopy. Invest Ophthalmol Vis Sci. 2014; 55(4):2071–8. Epub 2014/02/27. https://doi.org/10.1167/iov.13-13787 PMID: 24569580; PubMed Central PMCID: PMC3979234.

22. Fadavi H, Tavakoli M, Foden P, Ferdousi M, Petropoulos I, Zejzorska M, et al. Explanations for less small fibre neuropathy in South Asian versus European people with type 2 diabetes mellitus in the UK. Diabetes Metab Res Rev. 2018; 34(7):a3044. Epub 2018/07/05. https://doi.org/10.1002/dmrr.3044 PMID: 29972725.

23. Chen X, Graham J, Petropoulos IN, Ponirakis G, Asghar O, Alam U, et al. Corneal nerve fractal dimension: A novel corneal nerve metric for the diagnosis of diabetic sensorimotor polyneuropathy. Invest Ophthalmol Vis Sci. 2018; 59(2):1113–8. Epub 2018/03/01. https://doi.org/10.1167/iovs.17-23342 PMID: 29483948; PubMed Central PMCID: PMC5830988.

24. Asghar O, Petropoulos IN, Alam U, Jones W, Zejzorska M, Marshall A, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. Diabetologia. 2014; 57(9):2643–6. Epub 2014/06/28. https://doi.org/10.2337/dc14-0279 PMID: 24965851.

25. Ferdousi M, Azmi S, Petropoulos IN, Fadavi H, Ponirakis G, Marshall A, et al. Corneal confocal microscopy detects small fibre neuropathy in patients with upper gastrointestinal cancer and nerve regeneration in chemotherapy induced peripheral neuropathy. PLoS One. 2015; 10(10):e0139394. Epub 2015/
Corneal nerves in recurrent stroke

10/03. https://doi.org/10.1371/journal.pone.0139394 PMID: 26430773; PubMed Central PMCID: PMC4592260.

26. Khan A, Petropoulos IN, Ponirakis G, Menzies RA, Chidiac O, Pasquier J, et al. Corneal confocal microscopy detects severe small fiber neuropathy in diabetic patients with Charcot neuroarthropathy. J Diabetes Investig. 2018; 9(5):1167–72. Epub 2018/01/31. https://doi.org/10.1111/jdi.12806 PMID: 29380548.

27. Kass-Illyia L, Javed S, Gosdal S, Kobylecki C, Marshall A, Petropoulos IN, et al. Small fiber neuropathy in Parkinson's disease: A clinical, pathological and corneal confocal microscopy study. Parkinsonism Relat Disord. 2015; 21(12):1454–60. Epub 2015/11/19. https://doi.org/10.1016/j.parkreldis.2015.10.019 PMID: 26578039; PubMed Central PMCID: PMC4671992.

28. Ferrari G, Grisan E, Scarpa F, Fazio R, Comola M, Quattrini A, et al. Corneal confocal microscopy reveals trigeminal small sensory fiber neuropathy in amyotrophic lateral sclerosis. Front Aging Neurosci. 2014; 6:278. Epub 2014/11/02. https://doi.org/10.3389/fnagi.2014.00278 PMID: 25360111; PubMed Central PMCID: PMC4199282.

29. Mikolajczak J, Zimmermann H, Kheirkhah A, Kadars EM, Oberwahrenbrock T, Muller R, et al. Patients with multiple sclerosis demonstrate reduced subbasal corneal nerve fibre density. Mult Scler. 2017; 23 (14):1847–53. https://doi.org/10.1177/1352458516677590 PMID: 27811337.

30. Ponirakis G, Al Hamad H, Sankaranarayanan A, Khan A, Chandran M, Ramadan M, et al. Association of corneal nerve fiber measures with cognitive function in dementia. Ann Clin Transl Neurol. 2019; 6 (4):686–97. https://doi.org/10.1002/acn3.746 PMID: 31019993.

31. Gad H, Khan A, Akhtar N, Kamran S, El-Sotouhy A, Dargham SR, et al. Corneal nerve and endothelial cell damage in patients with transient ischemic attack and minor ischemic stroke. PLoS One. 2019; 14 (3):e0213319. https://doi.org/10.1371/journal.pone.0213319 PMID: 30875374.

32. Khan A, Akhtar N, Kamran S, Ponirakis G, Petropoulos IN, Tunio NA, et al. Corneal confocal microscopy detects corneal nerve damage in patients admitted with acute ischemic stroke. Stroke. 2017; 48 (11):3012–8. Epub 2017/10/12. https://doi.org/10.1161/STROKEAHA.117.018289 PMID: 29018135.

33. Khan A, Kamran S, Akhtar N, Ponirakis G, Al-Mu Hannadi H, Petropoulos IN, et al. Corneal confocal microscopy detects a reduction in corneal endothelial cells and nerve fibres in patients with acute stroke. Sci Rep. 2018; 8(1):17333. https://doi.org/10.1038/s41598-018-35298-3 PMID: 30478334.

34. Kamran S, Khan A, Salam A, Akhtar N, Petropoulos I, Ponirakis G, et al. Cornea: A window to white matter changes in stroke; corneal confocal microscopy a surrogate marker for the presence and severity of white matter hyperintensities in ischemic stroke. J Stroke Cerebrovasc Dis. 2020; 1–8.

35. Asghar O, Petropoulos IN, Alam U, Jones W, Jezierska M, Marshall A, et al. Corneal confocal microscopy detects neuropathy in subjects with impaired glucose tolerance. Diabetes Care. 2014; 37 (9):2643–6. https://doi.org/10.2337/dc14-0279 PMID: 24969581.

36. Ponirakis G, Petropoulos IN, Alam U, Ferdousi M, Asghar O, Marshall A, et al. Hypertension contributes to neuropathy in patients with type 1 diabetes. Hypertension. 2019; 32(7):963–6. https://doi.org/10.1093/ajh/hpz058 PMID: 31013342.

37. Tavakoli M, Kalinitikos P, Iqbal A, Herbert A, Fadavi H, Efron N, et al. Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. Diabetic Medicine. 2011; 28(10):1261–7. https://doi.org/10.1111/j.1464-5491.2011.03372.x PMID: 21699561.

38. Azmi S, Ferdousi M, Petropoulos IN, Ponirakis G, Alam U, Fadavi H, et al. Corneal confocal microscopy identifies small-fiber neuropathy in subjects with impaired glucose tolerance who develop type 2 diabetes. Diabetes Care. 2015; 38(8):1502–8. https://doi.org/10.2337/dc14-2733 PMID: 25877914.

39. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. 2018 Guidelines for the early management of patients with acute ischemic stroke: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2018; 49(3):e46–e110. Epub 2018/01/26. https://doi.org/10.1161/STROKEAHA.117.018289 PMID: 29367334.

40. Shukla AN, Cruzat A, Hamrah P. Confocal microscopy of corneal dystrophies. Semin Ophthalmol. 2012; 27(0):107–16. https://doi.org/10.3109/08820538.2012.707276 PMC3970842. PMID: 23163262.

41. Tavakoli M, Malik RA. Corneal confocal microscopy: a novel non-invasive technique to quantify small fibre pathology in peripheral neuropathies. J Vis Exp. 2011;(47):1–7. Epub 2011/01/21. https://doi.org/10.3791/2194 PMID: 21248693; PubMed Central PMCID: PMC3182840.

42. Vagenas D, Pritchard N, Edwards K, Shahidi AM, Sampson GP, Russell AW, et al. Optimal image sample size for corneal nerve morphometry. Optom Vis Sci. 2012; 89(9):812–7. Epub 2012/03/13. https://doi.org/10.1097/OPX.0b013e31824e8c9 PMID: 22407254.

43. Hankey GJ, Jamrozik K, Broadhurst RJ, Forbes S, Burvill PW, Anderson CS, et al. Long-term risk of first recurrent stroke in the Perth Community Stroke Study. Stroke. 1998; 29(12):2491–500. https://doi.org/10.1161/01.str.29.12.2491 PMID: 9836757.
44. Gacoń J, Przewlocki T, Podolec J, Badacz R, Pieniazek P, Ryniewicz W, et al. The role of serial carotid intima-media thickness assessment as a surrogate marker of atherosclerosis control in patients with recent myocardial infarction. Advances in Interventional Cardiology. 2019; 15(1). https://doi.org/10.5114/aic.2019.81705 PMID: 31043988

45. Wada S, Koga M, Minematsu K, Toyoda K, Suzuki R, Kagimura T, et al. Baseline carotid intima-media thickness and stroke recurrence during secondary prevention with pravastatin. Stroke. 2018; 50 (6):1586–9. https://doi.org/10.1161/STROKEAHA.119.024968 PMID: 31035902

46. Xie X, Wang X, Laskowitz DT, Zhao X, Miao Z, Liu L, et al. Effect of dual versus mono antiplatelet therapy on recurrent stroke modulated by activated partial thromboplastin time. Eur J Neurol. 2019; 26 (9):1168–e78. https://doi.org/10.1111/ene.13961 PMID: 30972875

47. Mok Y, Ballew SH, Sang Y, Grams ME, Coresh J, Evans M, et al. Albuminuria as a predictor of cardiovascular outcomes in patients with acute myocardial infarction. Am Heart J. 2019; 8(8):e010546. https://doi.org/10.1161/JAHA.118.010546 PMID: 30947615