

**A COMPARISON OF CORNEAL ENDOTHELIAL CELL DENSITY
BETWEEN A GROUP OF BLACK SOUTH AFRICANS WITH GLAUCOMA
AND A GROUP OF BLACK SOUTH AFRICAN HEALTHY CONTROLS**

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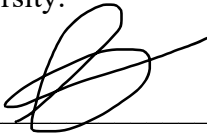
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DECLARATION

I, Catherine Jean Dold, declare that this Research Report is my own, supervised work. It is being submitted for the Degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



Signed

14th day of July 2025 in Johannesburg

CANDIDATE CONTRIBUTION TO THE PAPER

I, Professor Ismail Mayet, supervisor and co-author of this submitted research report, confirm that the research project was carried out by Catherine Dold. This project was carried out with the intention of submission for the Masters in Medicine degree during her time as a registered student. She conceptualised the research, drafted the protocol, collected the data, and wrote up the research report. She received assistance with analysis of the data from Brian Brummer. She received corrections and input from myself.



Signed

19th day of March 2025 in Johannesburg

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I am also grateful to Brian Brummer who assisted me with statistical analysis of my collected data. Your insight into biostatistics has been both a help and an inspiration.

I could also not have undertaken this journey without the help of my husband, Graham Wridgway. Your support for me during this project has kept my motivation high.

Abstract

Background: The endothelial layer of the cornea is crucial to corneal clarity and function and the cell density of this layer gradually decreases with age and due to disease processes.

Primary open angle glaucoma, a subtype of glaucoma, has been shown to be associated with reduced corneal endothelial cell density in various populations.

Methods: This cross-sectional, descriptive, observational study assessed the corneal endothelial density of a group of black South Africans with primary open angle glaucoma on topical medical management only and compared it to a control group from the same population.

Results: 112 participants were included in the study with an equal number in each group. The baseline characteristics of the two groups were similar. Corneal endothelial cell density in cells per square millimetre was found to be significantly lower in the group of participants with primary open angle glaucoma compared to the group of control participants with healthy corneas – mean endothelial cell density overall was 2323 (SD 331, 95% CI 2261-2385) with the glaucoma group having a mean of 2213 (SD 365, 95% CI 2115-2311) and the control group having a mean of 2433 (SD 251, 95% CI 2365-2500).

Conclusion: The corneal endothelial cell density of a group of black South Africans with primary open angle glaucoma only being managed with topical medication was found to be significantly lower than a control group from the same population. The clinical significance of this difference is not certain.

Introduction

Corneal endothelial cells form a single layer dividing the stroma of the cornea from the aqueous humour filled anterior chamber of the eye (1). The cells maintain the relative dehydration of the corneal stroma which is one of the contributing factors to corneal clarity (2). Corneal endothelial cells have a poor ability to divide and the density of cells reduces gradually throughout life (2). Endothelial cells in a healthy state are hexagonally shaped; as cells are lost, the remaining cells cover over the gaps and become irregularly shaped (2).

Corneal endothelial cell density (CECD) is the number of endothelial cells per mm^2 (3) . It is measured using a specular microscope which can assess “cellular size, shape, density and distribution”(1). Normal adult CECD is between 2000-3000 mm^2 and decreases throughout adult life as part of the ageing process: 0.6% density is lost per year in healthy individuals (3–5). As density is lost, the size and shape of corneal endothelial cells becomes less uniform; these phenomena are respectively termed polymegathism and pleomorphism (3).

A specular microscope is a high powered compound microscope which can obtain an image of the light reflected off the junction of the corneal endothelium and Descemet membrane (due to the differing refractive indices of the two layers) (6). The image obtained is analysed by the machine in order to measure cell size and density and calculate values of the coefficient of variance of cell size (1). Figure 1 illustrates the images and calculated values which can be obtained using a commercially available specular microscope set up to assess corneal endothelial density – the images are from the machine used in this study (TOMEY EM-4000)

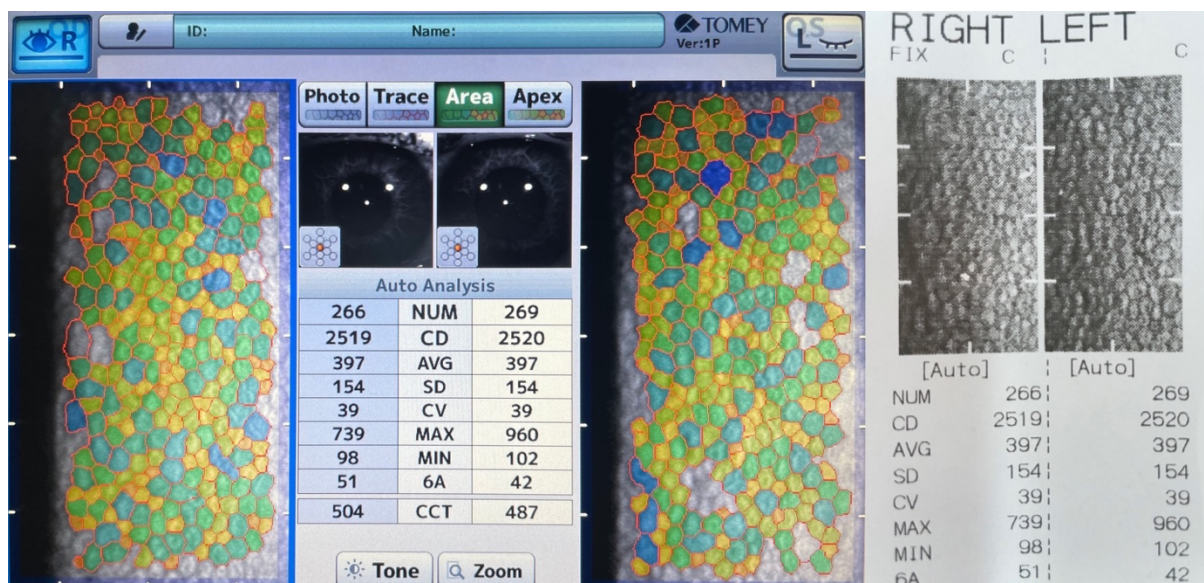


Figure 1: Example of automated corneal endothelial cell density measurement

There are disease processes which can cause significant decline in cell density which can cause corneal oedema and result in haze and reduced vision (2) . Intra-ocular surgery can also result in a dramatic loss of corneal endothelial cells; the most common surgery to do this is cataract surgery (7) although glaucoma surgeries which penetrate into the anterior chamber can also cause significant and ongoing endothelial loss (5,8–10). The use of mitomycin C as a

surgical adjunct in order to reduce fibrosis and increase chance of surgical success and longevity can also cause loss of endothelial cells (8). Patients with POAG who undergo phacoemulsification cataract surgery have been shown to lose an average of 9% of CECD by two years following surgery (7). This is similar to the 7% CECD loss at six months to one year following phacoemulsification surgery in the non-glaucomatous eyes (4,11). This loss appears to be due to the single insult of the intraocular surgery and the rate of loss after surgery slows to what would be expected with normal aging (8).

Glaucoma is an umbrella term for a variety of diseases which result in a common outcome of a typical pattern of progressive optic nerve atrophy caused by loss of retinal nerve fibre layer ganglion cells and consequential irreversible vision loss; primary open angle glaucoma (POAG) is the most common type of glaucoma (12,13). Glaucoma represents a significant burden of disease, especially in Africa where it is the second largest cause of blindness (14). Glaucoma disproportionately affects black people in Africa – it is responsible for approximately 15% of blindness (15,16) in Sub-Saharan Africa and an additional 4% of moderate to severe visual impairment (16). This represents a greater proportional burden of disease than in other parts of the globe – the average global rate of blindness due to glaucoma is 8,3% (16). In addition to higher rates of glaucoma, black people who have the disease tend to develop it earlier in life and have vision loss which progresses at a faster rate than other race groups (17,18).

Primary open angle glaucoma has been shown to be associated with lower corneal endothelial cell density in multiple populations (3–5,19,20). The cause of this reduced cell density has been postulated to potentially be due to multiple factors which include the disease process itself, raised intraocular pressure, topical medications used to treat the condition, and preservatives in these topical medications (3,8,19). In vitro studies have pointed to benzalkonium chloride (BAK), a commonly used preservative, being a cause of decreased CECD (4). Surgical and laser procedures form part of the management of primary open angle glaucoma. As discussed above, glaucoma and cataract surgery are associated with significant loss of corneal endothelial cells (5,7–10). Selective laser trabeculoplasty is a procedure used to reduce intraocular pressure by the application of laser spots to the trabecular meshwork situated in the iridocorneal angle – its effect on corneal endothelial cell density is not certain (21).

Normative databases of CECD have been described for various African populations but not for black South African people (22–24). These studies show contradictory findings. A Nigerian study of healthy eyes concluded that CECD in Nigerians is lower than “that reported in the Japanese, American and Chinese eyes, and is comparable to that seen in Indian and Malaysian eyes.” (22). Similar research done in the Democratic Republic of Congo and Egypt described normative values of CECD in healthy eyes (23,24). The normative values for the Congolese population are “greater than in most other populations” (23) while the Egyptian database showed similar CECD to the Nigerian database (24).

This study was undertaken in order to investigate whether the black South African population mirrors other groups. This study aimed to assess whether the corneal endothelial cell density of a group of people with primary open angle glaucoma is lower than a comparable group of healthy controls.

Methods

Study design and setting

This cross sectional, case-control study was conducted at St John Eye Hospital. St John Eye Hospital is part of the Chris Hani Baragwanath Academic Hospital complex of facilities in Diepkloof, Soweto, Johannesburg. It is staffed by specialist ophthalmologists, ophthalmology registrars in training and medical officers working exclusively in the discipline of ophthalmology. The hospital provides care across all of the sub-specialties of ophthalmology and serves the population of Soweto as well as being a referral centre for the South and West Rand areas of Johannesburg and North West Province. Primary through to tertiary level eye conditions are managed at this facility.

Included study participants were 18 years or older and self identified as black South African. The participants in the glaucoma group were confirmed to have a diagnosis of primary open angle glaucoma and were only on topical medication (one or more drugs). Participants in the control group were assessed to confirm the absence of pathology in the study eye (including but not limited to glaucoma and corneal disease) although cataract through which the optic nerve head was still visible was not an exclusion criterion.

Convenience sampling was used to recruit study participants who were either attending St John Eye Hospital for their own care (booked follow up for glaucoma or cataract pre-operative appointments) or to recruit healthy family and friends escorting people with visual complaints to the eye hospital into the control group.

Sample size was calculated by referencing previous studies done on the same topic in other populations. Sample size will be calculated using a Cohens D of 0.475, which is derived from a mean(+SD) CECD of 2692 (+358) and 2253(+291) in non-glaucomatous eyes and glaucomatous eyes respectively (10,11,15,25). These studies were adequately powered to achieve statistical significance. Using a power of 80% and alpha of 5%, the minimum sample size needed for a one-tailed t-test, with 1:1 sampling is 112. Required sample size was calculated as 112 participants with 1:1 sampling, resulting in 56 in each group. Only one eye of each participant was included in the study; if both eyes were eligible then a random number generator was used to decide which eye to include.

Non-contact specular microscopy was done using the TOMEY EM-4000 and the automated analysis of cell density and size generated by the machine was utilised. The machine does not run an automated analysis in the image obtained is of poor quality and thus quality of data collected was ensured.

This study followed the principles of the Declaration of Helsinki and ethical approval was obtained from the Wits Human Research Ethics Committee. The study was registered with an NHRD number: GP_202307_044. All participants gave written consent before inclusion.

Data collection

Participant data was collected on a data collection sheet and then transcribed onto a spreadsheet for use in data analysis. Data was collected between 23 November 2023 and 1 November 2024.

Study variables – demographics, clinical variables

Participant demographic data which was collected included age, sex, medical comorbidities, home language and province of birth in South Africa. Clinical data which was collected

included visual acuity, intraocular pressure, cup-disc ratio of the optic nerve head and keratometry. The TOMEY EM-4000 was used to do specular microscopy – the variables it provided are: corneal endothelial cell density, the coefficient of variance, average cell size, standard deviation, maximum and minimum cell sizes, central corneal thickness and 6A (a measure of the hexagonality of the cells).

Data analysis

Statistical analysis was performed using R version 4.1.2. For descriptive categorical analysis the Pearson Chi-square and Fisher exact test were used while for continuous variables the Wilcoxon rank sum test was used. An assessment of whether gender affected the outcome was performed as a sensitivity analysis.

Results

The baseline characteristics including age, sex, the eye examined and medical comorbidities of the 122 study participants are shown in Table 1. The glaucoma and control groups were similar in terms of participant age and the eye examined. Although there was a difference in the proportion of male to female participants in each group, this was later sub-analysed in order to assess whether any bias was introduced by this difference. The difference in hypertension between the two groups was not found to affect the outcome in a multivariate analysis.

The clinical indices which were similar when the two groups were compared were: visual acuity, intraocular pressure and keratometry. These clinical indices are also described in Table 1. The mean vertical cup-disc ratio of the control group was 0.3 (SD 0.08, 95% CI 0.28-0.33) while that of the glaucoma group was 0.85 (SD 0.17, 95% CI 0.8-0.9); with a $p < 0.001$ for this comparison.

Table 1: Descriptive statistics of study participants – glaucoma patients and healthy controls

Characteristic	Overall N = 112	Control N = 56	95% CI	Glaucoma N = 56	95% CI	p-value
Age, Median (IQR)¹	64 (13)	65 (13)	62, 68	64 (12)	62, 67	0.70
Sex, n (%)²						0.005
Female	57 (51)	36 (64)	50%, 76%	21 (38)	25%, 51%	
Male	55 (49)	20 (36)	24%, 50%	35 (63)	49%, 75%	
Eye Examined, n (%)²						0.78
Left	49 (44)	25 (45)	32%, 59%	24 (43)	30%, 57%	
Right	62 (56)	30 (55)	41%, 68%	32 (57)	43%, 70%	
Unknown	1	1		0		
Self-Reported Comorbidities						
HIV, n (%) ³	8 (7.1)	2 (3.6)	0.62%, 13%	6 (11)	4.4%, 23%	0.27
Hypertension, n (%) ²	65 (58)	38 (68)	54%, 79%	27 (48)	35%, 62%	0.035
Asthma, n (%) ³	3 (2.7)	2 (3.6)	0.62%, 13%	1 (1.8)	0.09%, 11%	>0.99
Diabetes Mellitus, n (%) ²	27 (24)	17 (30)	19%, 44%	10 (18)	9.3%, 31%	0.12
Clinical Measures						
Visual Acuity (LogMAR), Mean (SD) ¹	0.38 (0.31)	0.37 (0.30)	0.28, 0.45	0.39 (0.32)	0.30, 0.49	0.77
Intraocular Pressure (mmHg), Median (IQR) ¹	13.00 (5.00)	13.00 (3.50)	13, 14	14.00 (5.00)	13, 15	0.90
Vertical Cup-Disc Ratio, Median (IQR) ¹	0.50 (0.60)	0.30 (0.10)	0.28, 0.33	0.90 (0.20)	0.80, 0.90	<0.001
Keratometry K1, Median (IQR) ¹	42.94 (1.95)	42.81 (1.96)	43, 43	43.24 (2.05)	43, 44	0.94
Keratometry K2, Median (IQR) ¹	43.63 (1.96)	43.58 (2.01)	43, 44	43.77 (1.91)	43, 44	0.78
Flat Axis, Median (IQR) ¹	95 (114)	94 (100)	77, 108	95 (123)	73, 105	0.91

¹Wilcoxon rank-sum test

²Chi-squared test

³Fisher's exact test

All participants in the glaucoma group were asked which topical medications they were using. The topical medications available for management of primary open angle glaucoma at St John Eye Hospital fall into one of the following categories: alpha agonists, beta blockers, prostaglandin analogues and carbonic anhydrase inhibitors. Table 2 details the medications which participants were using and shows that beta blockers and prostaglandin analogues were most commonly. Table 3 details the number and percentage of the total number of intra-ocular pressure lowering drugs used by participants in the glaucoma group.

Table 2: Table of medications among patients with glaucoma

Characteristic	N = 56
Beta Blocker, n (%)	52 (93)
Prostaglandin Analogue, n (%)	52 (93)
Alpha Blocker, n (%)	45 (80)
Carbonic Anhydrase Inhibitor, n (%)	19 (34)

Table 3: Table of number of agents among patients with glaucoma

Characteristic	N = 56
Number of Agents, n (%)	
1	2 (3.6)
2	13 (23)
3	24 (43)
4	17 (30)

The primary outcome of the study was corneal endothelial cell density – overall, the mean endothelial cell density was 2323 (SD 331, 95% CI 2261-2385). The control group had a mean endothelial cell density of 2433 (SD 251, 95% CI 2365-2500) while the mean of the glaucoma group was 2213 (SD 365, 95% CI 2115-2311) with $p < 0.001$. The other measurements and relevant statistics taken from the TOMEY EM-4000 relating to endothelial cell density are described in Table 4. Figure 2 is a box and whisker diagram which illustrates this primary outcome.

Table 4: Descriptive analysis of endothelial cell variables

	Overall N = 112	95% CI	Control N = 56	95% CI	Glaucoma N = 56	95% CI	p- value ¹
Corneal Endothelial Cell Density, Mean (SD)	2,323 (331)	2,261, 2,385	2,433 (251)	2,365, 2,500	2,213 (365)	2,115, 2,311	<0.001
Coefficient of Variation, Mean (SD)	40.7 (8.3)	39, 42	40.8 (5.9)	39, 42	40.6 (10.3)	38, 43	0.49
Average Cell Size, Mean (SD)	446 (88)	429, 462	422 (51)	408, 435	470 (108)	441, 499	0.003
Standard Deviation, Mean (SD)	181 (51)	171, 190	173 (41)	162, 184	188 (59)	173, 204	0.33
Maximum Cell Size, Mean (SD)	1,177 (413)	1,099, 1,254	1,120 (264)	1,049, 1,191	1,233 (518)	1,095, 1,372	0.53
Minimum Cell Size, Mean (SD)	112 (32)	105, 118	106 (22)	100, 111	117 (39)	107, 128	0.040
Central Corneal Thickness, Mean (SD)	481 (46)	473, 490	489 (58)	474, 505	473 (28)	466, 481	0.002
6a, Mean (SD)	44 (8)	42, 46	43 (9)	41, 46	45 (8)	43, 47	0.13

¹Wilcoxon rank sum test

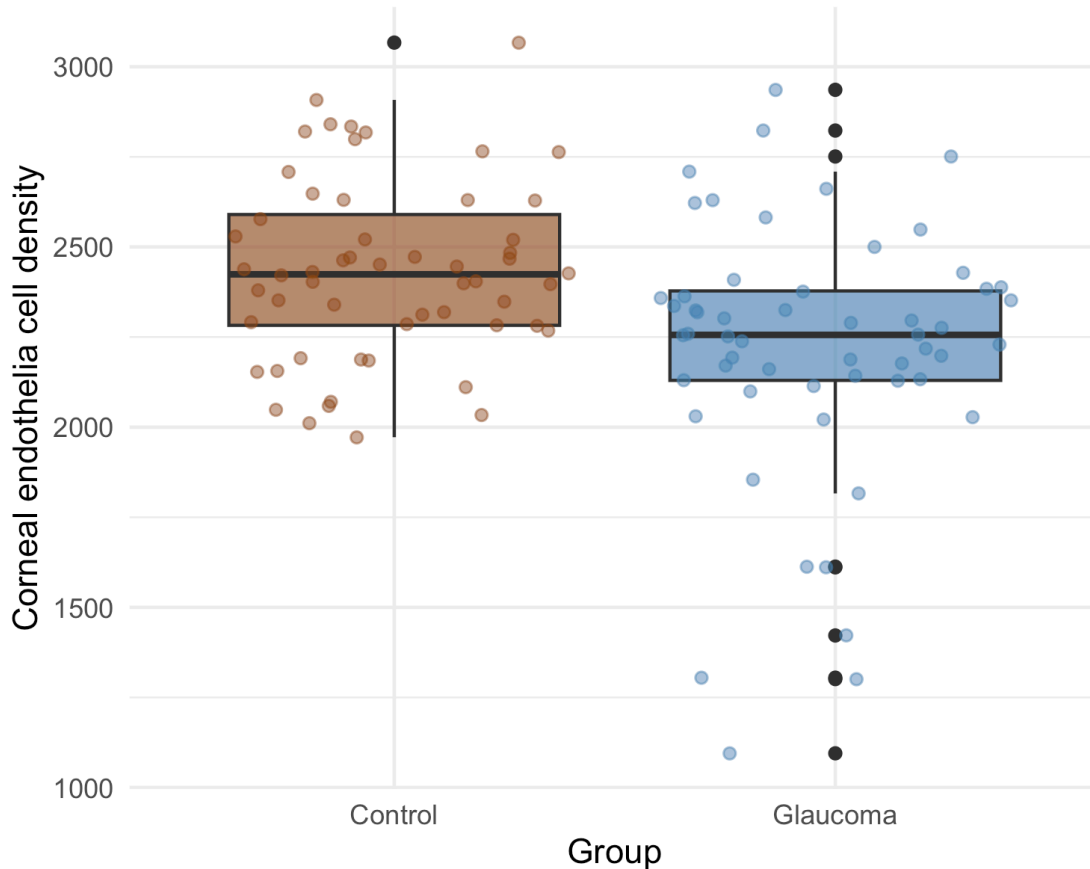


Figure 2: Boxplot of corneal endothelial cell density among patients with glaucoma and healthy controls

As a result of the differing proportion of baseline sex composition of the two groups, a sensitivity analysis was performed in order to assess the effect of sex on corneal endothelial cell density and other measured corneal variables. The analysis showed that sex had no effect on the outcomes with $p > 0.05$.

Discussion

This study highlights the severe and frequently advanced nature of the glaucomatous disease seen in this population. The mean vertical cup:disc ratio of 0.85 (SD 0.17, 95% CI 0.8-0.9) illustrates this. The fact that the mean intraocular pressure in the glaucoma group was 13.71 (SD 3.02, 95% CI 13-15) demonstrates that this group of participants had well controlled intraocular pressure at the time of inclusion in the study. This good control of intraocular pressure could have been a contributing factor to their being available to be included in the

study as they had not needed additional glaucoma management in the form of surgery or laser treatment.

In this small study, it was shown that the corneal endothelial cell density of black South Africans with primary open angle glaucoma is lower than age matched healthy controls. This mirrors studies in other populations which showed a difference between endothelial cell density between eyes with glaucoma on medical management and healthy eyes (4,19). This study is the first of its kind done in a black South African population. A 2021 review of all glaucoma therapies on endothelial cell density concluded that high intraocular pressure in glaucoma was associated with increased loss of endothelial cells; in addition, other glaucoma subtypes such as angle closure glaucoma can result in very high pressure and significant reduction in corneal endothelial cell density over a short period of raised pressure (4). As this study was cross sectional, it is not possible to infer the rate of loss of endothelial cells in participants nor is it possible to determine when the loss occurred – prior to or following diagnosis of glaucoma.

The medications (in drop formulation) used to lower intra-ocular pressure as well as the compounds used to preserve them have not conclusively been found to be contributory to this loss of endothelial cells(4). The drops available for lowering intraocular pressure at St John Eye Hospital are all preserved, almost all with BAK – BAK has been associated with corneal decompensation due to significant endothelial cell loss but case reports are centred around incidences of frequent drop administration(26). The effects of individual drug classes on endothelial cell density have been studied and, in a review by Realini et al, no one glaucoma medication has been shown to significantly reduce endothelial cell density (4). Janson notes that the studies of individual medications on corneal endothelial cells are generally undertaken for a period of weeks or months and that years or decades of use of these medications may be having effects that are not fully understood (8). A 2019 study found significantly lower endothelial cell density in participants with primary open angle glaucoma compared to healthy controls; this study also included glaucoma participants not yet on topical medication and found that glaucoma medication use was associated with lower corneal endothelial cell density – the authors concluded that this difference might be attributable to glaucoma medication toxicity(19).

With the increase of the use of selective laser trabeculoplasty for management of primary open angle glaucoma (as driven by the results of the LiGHT trial(27)), there are likely to be less patients with primary open angle glaucoma who will only be on topical medical treatment for the disease.

Rho kinase (ROCK) inhibitors are a relatively new class of drug which have been developed to lower intraocular pressure and manage glaucoma (28) – they have been found to “promote corneal endothelial cell proliferation, increase intercellular adhesion, and suppress apoptosis” as well as affecting cellular migration and are thus have applications in treatment of endothelial pathology as well as in glaucoma management. They are not yet licenced for use in South Africa and so no study participants were using them. Once they are commercially available, their effect on corneal endothelium in this population is a potential area of study.

There are several limitations to this study. As it was cross sectional in nature, it was unable to assess the rate of loss of endothelial cell density. Only participants on topical medical management were included in the glaucoma arm of the study and thus a large proportion of the population with primary open angle glaucoma was ineligible to participate as they have either undergone intraocular surgery or have had a laser procedure for the management of their glaucoma.

This study compared the corneal endothelial cell density and other parameters between the two groups of participants and found a statistically significant difference. As there is no established normative database of corneal endothelial cell density in this population, it is not possible to comment on whether the group of healthy participants are representative of the population as a whole.

Despite the statistical significance of the difference between the two groups assessed in this study, it is not possible to infer whether this translates to any clinical significance which could result in loss of corneal clarity or bullous keratopathy.

Conclusion

The comparison of the endothelial cell density of these groups of black South African participants showed a significantly lower density in the glaucoma group. This is in line with

what previous authors have described. The awareness of this parameter is important for the ophthalmologist when managing patients with primary open angle glaucoma – surgical decision making and long term post-operative corneal clarity will be affected by endothelial cell density. A larger study establishing a normative database of corneal endothelial cell density in this population would also potentially add to how glaucoma in a black South African population is understood.

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Appendix A: Plagiarism declaration



PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I, Catherine Jean Dold (Student number: 0700827 V) am a student registered for the degree of Master of Medicine in the academic year 5th.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: 

Date: 17 March 2025

Appendix B: Ethics clearance

 UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
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Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr C Dold
School of Clinical Medicine
Department of Neurosciences
Division of Ophthalmology
St John's Eye Hospital
Chris Hani Baragwanath Academic Hospital

E-mail: cjdold@gmail.com

CC: Supervisor: Professor I Mayet
eyemay.im@gmail.com
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 21 November 2023

REF: R14/49

PROTOCOL NO: **M230708** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *A comparison of corneal endothelial cell density between a group of Black South Africans with glaucoma and a group of Black South African healthy controls*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps



R49 Dr C Dold

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230708**

NAME: Dr C Dold **DEGREE:** MMed
(Principal and Co-Investigators)

DEPARTMENT: School of Clinical Medicine
Department of Neurosciences
Division of Ophthalmology
St John's Eye Hospital
Chris Hani Baragwanath Academic Hospital

PROJECT TITLE: *A comparison of corneal endothelial cell density between
a group of Black South Africans with glaucoma and a group
of Black South African healthy controls*

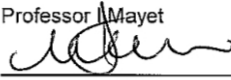
DATE CONSIDERED: 11 August 2023

DECISION: Approved unconditionally

CONDITIONS:

NOTE: If contact information regarding student study participants is required,
please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Professor M Mayet

APPROVED BY: 
Ms M van Niekerk, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 21 November 2023 **EXPIRY DATE:** 20 November 2028

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report in the format available at <https://wits.ac.za/research/researcher-support/research-ethics/ethics-committees/>. This report is due annually, for the duration of the Clearance Certificate, beginning on the first anniversary of Date of Approval above. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).


Signature of Principal Investigator

22 November 2023
Date

Appendix C: Change of wording of research report name



APPLICATION FOR CHANGE OF TITLE OF APPROVED RESEARCH REPORT, DISSERTATION OR THESIS

Student Surname and Initials: Dold, CJ

Student Number: 0700827V

Degree: Master of Medicine (MMed)

Department: Ophthalmology (Division Neurosciences)

Telephone: 084 580 9077

E-mail: cjdold@gmail.com or 0700827v@students.wits.ac.za

Current Title: Comparison of corneal endothelial cell density between black South Africans with glaucoma and healthy controls

New Title: A comparison of corneal endothelial cell density between a group of black South Africans with glaucoma and a group of black South African healthy controls

Motivation / Reason for title change: HREC chair found original title ambiguous and requested a clarification in order to specify exactly who both groups of participants were in the study. Ethics approval was granted after this change.

Approvals / signatures:

Student Signature: _____

Date: 17 March 2025

=====

Supervisor – Name & Surname: Professor Ismail Mayet

Department: Ophthalmology, Division of Neurosciences

Supervisor Telephone: 084 410 5769

Supervisor E-mail: eyemay.im@gmail.com

Supervisor Signature: _____

Date: 18/03/2025

=====

***HEAD OF DEPARTMENT / HEAD OF SCHOOL:** **(Where the HOD is Supervisor, the HOS must sign)*

Professor Aubrey Makgotloe
(Name and Surname)

(Signature)

20 March 2025
(Date)

DECISION OF CHAIR OF THE PG AFFAIRS:

Comments:

Signature: _____

Date: _____

Application for Change of Approved Title ➤ Inbox x

J

Junia Mthombeni

<Junia.Mthombeni@wits.ac.za>

to me

▼

Mon, Apr 29, 2024, 12:05 PM

Dear Catherine

Please the form, get all the necessary signatories. Kindly attach it to your final research submission to the postgrad office.

Regards

Junia Mthombeni

Senior Secretary: Department of Neurosciences | School of Clinical Medicine

E: Junia.Mthombeni@wits.ac.za

T: +27 11 717 2774

W: www.wits.ac.za/clinicalmed/

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG





Wits Medical School, 4th Floor, 7 York Road,
Parktown, Johannesburg, South Africa



WJCM Author Guidelines

The Wits Journal of Clinical Medicine aims to provide peer reviewed publication of original articles, reviews, opinion papers, case and meeting reports dealing with all facets of Clinical Medicine practice and research. All articles will be independently peer-reviewed. Material accepted for publication is done on the undertaking that it has not been submitted for publication anywhere else.

Submissions

All submissions should follow the style of the Journal and be clear and concise. The word count, including references, should not be exceeded. Articles must be submitted via the website:

https://www.scienceopen.com/collection/WITS_WJCM or emailed to the Journal Manager, **Pam Singh** pam@wjcm.co.za

Article Structure

All articles should have a title page, specify the type of article being submitted and include the following: a short descriptive title, authors surname and first name, affiliations, full postal address, telephone numbers and email addresses for the author, as well as co-authors. The corresponding author should be identified with an Asterix. All authors will require an ORCID number which should also be submitted as an alphanumeric string. Please register at www.orcid.org if you do not already have one. The Editors may later request validation authorisation for this publication.

The Text

Please submit all text in Times New Roman, 12 points with 1.5 spacing in English (UK or South African). Text must be submitted as a Word document. Tables and figures placement must be indicated in the text as part of the sentence example, see Table 1, or in parentheses (Table 1). All tables and figures must include a descriptive heading with appropriate attribution and permissions indicated. Figures and tables must be submitted within the Word document. All tables and graphs (containing original data or which are not archival in nature) may be redrawn to suit the house style. Please ensure that all elements are included especially legends, scale indicators and all axes. Images must be of High quality.

References

References should be numbered in the order of appearance in the text (in parentheses after punctuation) and be presented according to Vancouver styling.

For articles

- Author A, Author B, Author XYZ. Title of article. Journal title abbreviated Year; volume: xx-zz.
- Leurs R, Church MK, Taglialatela M. H1 -antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects. Clin Exp Allergy. 2002; 32(4):489-498.
- List first 5 authors. If more than 5 authors list the first three and add et al.

For chapters in edited books

- Author A, Author BC. Title of the book chapter. In: Editor E, Editor FG, ed. Title of the book, X edn. Location: Publisher, year: xxx-zzz.

- Glennon RA, Dukat M. Serotonin receptors and drugs affecting serotonergic neurotransmission. In: Williams DA, Lemke TL, editors. Foye's principles of medicinal chemistry. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2002.

For authored books

- Author A, Author B, Author C. Title. Edition. Location: Publisher; Year.
- Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

For all other reference queries eg datasets, websites and social media please consult the Vancouver style guide. When submitting articles to the Journal please try to adhere to the conventions outlined above as closely as possible.

Structure

The abstract (250 words) should outline the main purpose of the study, the key methods, the main results, and conclusion. The Introduction provides a background to the study and should be sufficient to permit non-specialists to understand the framework of the work. Include a well-defined statement regarding the purpose of the study. The methods: provide a description of the study design, all procedures, techniques, and statistical analyses. The results: stipulate the study findings and ensure to cross-reference figures and tables. The discussion should elicit an interpretation of the study set against the background of current knowledge of the field and provide definitive conclusions. Reviews will comprise a title page as above; an abstract (250 words), providing the setting of the review, any key messages and conclusions drawn. The body should be presented with headings and subheadings and lead to summary with conclusions to end. A few (2-5) key statements could be itemised in separate box. Acknowledgements, references, tables, and figures must be as detailed above.

All images should be of a suitably high resolution because we want to ensure the highest possible reproducibility in both print and digital. We require these images to have a resolution of 600 dpi.

Research Article

Total word count should not exceed 4500 words.

1. The format should include an abstract (max 250 words), the text (introduction, methods, results, discussion, and conclusion), a maximum of 5-6 tables, figures, or images - images must be of High quality.
2. The reference count should not exceed 30.
3. The Ethics Clearance/Approval Certificate from the relevant institution should accompany the submission.

Research Letter

1. The word count should not exceed 1600 words.
2. The abstract should have a maximum of 250 words.
3. The reference count should not exceed 15.
4. The Research letter should have a maximum of 5 tables, figures, or images - images must be of High quality.
5. The Ethics Clearance/Approval Certificate from the relevant institution should accompany the submission.

Opinion Piece or Perspective

1. The word count should be between 2000 – 2300 words
2. The abstract should not exceed 250 words
3. The article should have a maximum of 2 figures or images - images must be of High quality.
4. A maximum of 15 references are permitted

Case Reports

1. The word count should not exceed 1600 words
2. The article should have a maximum of 3 figures, tables, or images - images must be of High quality.
3. A maximum of 6 references are permitted
4. The Ethics Approval/Clearance Certificate from the relevant institution should accompany the submission

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Appendix E: Turnitin report



Digital Receipt

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The first page of your submissions is displayed below.

Submission author: Catherine Dold
Assignment title: Draft submission (all, regardless of course/programme)
Submission title: corneal endothelial cell density article for submssion.docx
File name: corneal_endothelial_cell_density_article_for_submssion.docx
File size: 58.75K
Page count: 13
Word count: 3,843
Character count: 21,000
Submission date: 12-Mar-2025 04:35PM (UTC+0200)
Submission ID: 2612642283

Title page

Title: A comparison of corneal endothelial cell density between a group of black South Africans with glaucoma and a group of black South African healthy controls

Authors:

1) Dold, Catherine *
cjdold@gmail.com
ORCID 0000-0001-8498-2707

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89 5th Avenue, Melville, 2002
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ismeyrjz@gmail.com
ORCID 0000-0003-1720-941X

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corneal endothelial cell density article for submssion.docx

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Publication

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Publication

5 Ismail, Sarah. "Retinal Nerve Fibre Layer Thickness in a Normal Black South African **1%**

Population", University of the Witwatersrand,
Johannesburg (South Africa), 2025

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Solmaz Abdolrahimzadeh, Mariachiara Di
Pippo, Edoardo Sordi, Mattia Cusato, Andrew
John Lotery. "Subretinal drusenoid deposits
as a biomarker of age-related macular
degeneration progression via reduction of the
choroidal vascularity index", Eye, 2022

Publication

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Exclude quotes

On

Exclude matches

< 20 words

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On

Appendix F: Copy of approved protocol



CANDIDATE'S SURNAME: Dold [Please print]		FIRST NAME/S: Catherine Jean	STUDENT NUMBER: 0700827V
CURRENT QUALIFICATIONS: MBChB (UCT), DipOphth			
TEL:	CELL: 0845809077	E-MAIL: cjdold@gmail.com	FAX:
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: MMed (in ophthalmology)			
PART-TIME OR FULL-TIME: Full time			
FIRST REGISTERED FOR THIS DEGREE:	TERM: Second semester	YEAR: 2021	
DEPARTMENT: Neurosciences			
TITLE OF PROPOSED RESEARCH: Comparison of corneal endothelial cell density between South Africans with glaucoma and healthy controls			
CANDIDATE'S SIGNATURE: 		DATE: 19/04/2023	
SUPERVISOR 1 (NAME & SURNAME): Prof I Mayet		% Supervision 100%	
SUPERVISOR'S QUALIFICATIONS: MBBCh, FCS, PhD			
SUPERVISOR'S DEPARTMENT: Neurosciences, ophthalmology			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: St John Eye Hospital, CHBAH / 0844105769 / eyemay.im@gmail.com			
SUPERVISOR 2 (NAME & SURNAME):		% Supervision	
SUPERVISOR'S QUALIFICATIONS			
SUPERVISOR'S ADDRESS / TEL / E-MAIL:			
SUPERVISOR 3 (NAME & SURNAME):		% Supervision	
SUPERVISOR'S QUALIFICATIONS			
SUPERVISOR'S ADDRESS / TEL / E-MAIL:			

SYNOPSIS OF RESEARCH: (Brief summary of proposed research project; between 200-300 words only; with sub-headings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s)
[Use reverse side of this page if more space is required]

Introduction and justification for the study

The cornea of the eye is lined by a layer of endothelium. This layer is unable to regenerate and the density of corneal endothelial cells slowly declines as part of the ageing process. A very low corneal endothelial cell density can lead to clouding of the cornea and severely reduced vision necessitating corneal transplantation procedure. The cell density of this layer has been shown to be lower in populations with primary open angle glaucoma than in healthy controls. This phenomenon has never been investigated in people of South African ancestry. Primary open angle glaucoma tends to occur earlier and be more aggressive in this population. It is thus worth assessing whether corneal endothelial cell density is lower people with primary open angle glaucoma in this population than in age matched controls.

Study aims

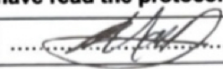
The aim of this study is to establish if primary open angle glaucoma is associated with a lower corneal endothelial cell density than a healthy cohort of African patients.

Proposed methodology

This will be a prospective case-control study. Cases will be people with primary open angle glaucoma; controls will be age matched healthy individuals. The study will be done at St John Eye Hospital, Soweto. Patients at the hospital will be recruited to participate. 112 participants will be included with 56 in each arm.

Expected outcomes

The corneal endothelial cell density of all participants will be measured and the groups will be compared to assess whether there is a difference in corneal endothelial cell density between those with primary open angle glaucoma and healthy controls.

WITS ETHICS NOT REQUIRED: Yes No WITS ETHICS PENDING: Yes No WITS ETHICS APPROVED: Yes No (circle appropriate symbol)*		IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
*Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research		
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.		
SIGNATURE OF SUPERVISOR/S:		
SIGNATURE PG OFFICE STAFF	REGISTERED YES..... NO.....	STAMP

Statement of principles for postgraduate supervision

In a context of academic freedom and within a framework of individual autonomy and the pursuit of knowledge, this statement is written in the belief that there is a reciprocal relationship and mutual accountability between supervisor and student

THE SUPERVISOR AND THE STUDENT:	THE STUDENT:
1. Will establish agreed roles and clear processes to be maintained by both parties. 2. Will meet regularly and as frequently as is reasonable to ensure steady progress towards the completion of the proposal, research report, dissertation or thesis. This time varies but the normal minimum requirement for face-to-face contact spread across each year of registration is: 10 contact hours for an Honours project, 15 contact hours for a Masters by coursework and research report and 24 contact hours for a Masters by dissertation and a PhD. 3. Will keep appointments, be punctual and respond timely to messages. 4. Will keep one another informed of any planned vacations or absences as well as changes in his or her personal circumstances that might impact on the work schedule. Unplanned absences or delays should be discussed as soon as possible and arrangements should be made, to catch up lost time. 5. Will ensure that research on animal or human subjects is conducted with prior approval and according to the procedures and the requirements of the relevant Ethics committee. 6. Will both complete Progress Reports on the research project as required/requested by the relevant Faculty Graduate Studies Committee.	1. Takes full responsibility for the research and its successful completion, including managing the process under the guidance of supervisor(s). 2. Will attend such courses and lectures that are compulsory for the degree, and undertakes to catch up fully on any work, lectures and/or assignments, that are missed. 3. Undertakes to work independently under the guidance of the supervisor(s). This includes reading widely and critically to ensure that the seminal and current literature pertinent to his/her chosen topic has been identified, consulted and critiqued. 4. Undertakes to work in accordance with the academic standards expected by the University for the commensurate NQF level of qualification. 5. Is obliged to make appointments to consult the supervisor(s) and arrange meeting times convenient to both parties well in advance. 6. Should submit written work for discussion with the supervisor(s) well in advance of a scheduled meeting. The kind and frequency of written work should be agreed with the supervisor(s) at the outset of the research. 7. Written work that is submitted to the supervisor, including final submissions to examiners, should be relatively free from basic spelling mistakes, incorrect punctuation and grammatical errors. Responsibility for the accuracy of language, the overall structure and coherence of the final research report, dissertation or thesis rests with the student. 8. Cannot expect the supervisor to be proof-reader and editor of his/her work or to approve work with any of the weaknesses spell out in 7 above. 9. Undertakes to heed the advice given by the supervisor(s) and to engage in discussion around suggestions made. Ultimately the student has to take responsibility for the quality, integrity and presentation of the work. 10. Should strive to maintain a focus on his/her research area and to work diligently within the agreed time schedule. 11. Agrees to honour agreements about ownership of the research and in accordance with the University's guidelines and rules in relation to co-authorship, copyright and intellectual property. 12. Will ensure that the work contains no instances of plagiarism, violation of intellectual property and research integrity standards, that all citations are properly referenced, and that the list of references is accurate, complete and consistent. 13. Agrees to work in accordance with the criteria of acceptability as supplied by the supervisor(s). 14. Undertakes not to place the supervisor(s) under undue pressure to submit work for examination until the supervisor is satisfied that it has reached an acceptable, <u>examinable</u> level of quality.
We confirm that we have read and understood this statement and agree to be guided by its principles for as long as we continue to work together. Name of student: <u>C.J. Dold</u> Student Number: <u>0700827V</u> Student's signature: <u>[Signature]</u> Name of Supervisor: <u>Prof. I. Mayet</u> Supervisor's signature: <u>[Signature]</u> Name of Co-Supervisor: _____ Co-Supervisor's signature: _____ The broad area of study is: <u>Cardiac endocrinology</u> Provisional submission date is: <u>2024</u> Degree: <u>M Med</u> School: <u>Clinical Medicine</u> Faculty: <u>Health Sciences</u> Date: <u>19/04/2023</u> Specific agreements pertaining to: ownership, joint publication, funding, confidentiality and disclosures pertinent to the Certificate of Clearance and ETD Form which the student and/or supervisor are required to sign, must be attached to this agreement as and when appropriate and kept in the Faculty Office. In the event of disagreements between the supervisor(s) and student, the parties should act in accordance with the University Grievance Policy. *Note: Consent by supervisor(s) to submit work for examination does NOT guarantee that the work will pass. The appointed examiners assess and determine whether the work is of a passable standard.	

(2019/09/30)



University of the Witwatersrand Student Ethics Declaration Form

(To be completed during the protocol assessor meeting)

Background

All Research conducted by a University of the Witwatersrand student, with human subjects or animals, requires approval by the Wits Human Research Ethics Committee or Animal Research Ethics Committee, respectively.

If research has been undertaken without the necessary ethics approvals, this is considered an ethics violation. This will be reported to the relevant structures, the data will have to be discarded, and in the case of students, they cannot use the data towards their degree.

To prevent any ethics violations, the ethics requirements for the proposed project will be discussed with you at the protocol assessment.

Declaration

Based on the current protocol assessment (and any proposed changes suggested by the assessor committee), we, the undersigned, understand that the proposed research requires:

1. Human Research Ethics clearance certificate

a. Covered under existing supervisor ethics

b. Requires a new HREC application

2. Animal Research Ethics clearance certificate

3. No Human or Animal Ethics Clearance

4. Unclear, will seek appropriate guidance from the HREC/AREC committees (whichever relevant)

☒ Yes	☐ No
---	-----------------------------

☐ Yes	☒ No
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☒ Yes	☐ No
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☐ Yes	☒ No
------------------------------	--

☐ Yes	☒ No
------------------------------	--

☐ Yes	☒ No
------------------------------	--

Signatures

Supervisor/s:

Student:

Date:

19/04/2023

Comparison of corneal endothelial cell density between black South Africans with glaucoma and healthy controls

Dr C Dold

Registrar

Student number 0700827V

Department of Neurosciences

Division of Ophthalmology

University of the Witwatersrand

May 2023

Supervisor: Prof I Mayet

Contents

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2. Literature review
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4. Research aim
5. Research objectives
6. Research methods
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 - 6.9 Time schedule (including Gantt chart)
 - 6.10 Ethical considerations
 - 6.11 Costs and required funding
7. Reporting plans
8. Role players and investigators
9. References
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Appendix A – Participant information sheet

Appendix B – Participant consent form

Appendix C – Data collection sheet

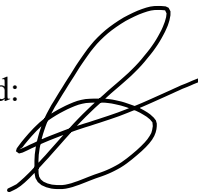
Appendix D – Turnitin report

Appendix E – Plagiarism declaration

Table of protocol corrections

<u>Location</u>	<u>Revision made</u>
All pages	Page numbers included
Cover page, page 1	Title amended to “black South Africans” from “South Africans”
Introduction, page 5	Clarification of term “progressive optic atrophy” to “progressive optic nerve atrophy”
Research question, page 9	Research question clarified to read: : “Is the corneal endothelial cell density of black South Africans with glaucoma lower than that of black South African controls with healthy eyes?”
Research question, page 10	Rationale for study expanded on underneath the clarified research question
Research objectives, page 10	Objective 2 removed
Sampling, page 12	Additional explanation of how sample size was calculated included
Time frame, page 15	Expanded description of the expected time frame for collection of data included above Gantt chart. Gantt chart adjusted to fit current expected date of permission to begin collection of data
Data collection, page 13 and Data collection sheet, page 27	Superfluous data collection points removed from data collection list and sheet

Signed:



Catherine Dold

Date 16 July 2023

1. Introduction

The human cornea is comprised of five layers, the innermost of which is called the endothelium – the endothelium is a single layer of hexagonal shaped cells.¹ The function of this cell layer is to be a barrier to water and to pump ions out of the relatively dehydrated cornea in order to maintain corneal clarity.² The cells of the corneal endothelium do not regenerate.³ As cells are lost through aging or disease process, the remaining endothelial cells cover over the gaps and the cells go from all having a hexagonal shape to being irregularly shaped.¹

Glaucoma is an umbrella term for a variety of diseases which result in a common outcome of a typical pattern of progressive optic nerve atrophy caused by loss of retinal nerve fibre layer ganglion cells and consequential irreversible vision loss; primary open angle glaucoma (POAG) is the most common type of glaucoma.^{4,5} Glaucoma represents a significant burden of disease, especially in Africa where it is the second largest cause of blindness.⁶ Glaucoma disproportionately affects black people in Africa – it is responsible for approximately 15% of blindness^{7,8} in Sub-Saharan Africa and an additional 4% of moderate to severe visual impairment.⁸ This represents a greater proportional burden of disease than in other parts of the globe – the average global rate of blindness due to glaucoma is 8,3%.⁸ In addition to higher rates of glaucoma, black people who have the disease tend to develop it earlier in life and have vision loss which progresses at a faster rate than non-black people.^{9,10}

There are multiple risk factors for the development and progression of primary open angle glaucoma; intraocular pressure (IOP) is seen as the most important modifiable risk factor to prevent progression; genetic factors also have a significant contributing role in glaucoma.^{4,5,11,12}

2. Literature review

Corneal endothelial cell density (CECD) is the number of endothelial cells per mm². It is measured using a specular microscope which can assess “cellular size, shape, density and distribution”.¹ Normal adult CECD is between 2000-3000/mm² and decreases throughout adult life as part of the ageing process: 0.6% density is lost

per year in healthy individuals.^{2,3,13} As density is lost, the size and shape of corneal endothelial cells becomes less uniform; these phenomena are termed polymegathism and pleomorphism respectively.²

A specular microscope is a high powered compound microscope which can obtain an image of the light reflected off the junction of the corneal endothelium and Descemet membrane (due to the differing refractive indices of the two layers).¹⁴ The image obtained is analysed by the machine in order to measure cell size and density and calculate values of the coefficient of variance of cell size.¹

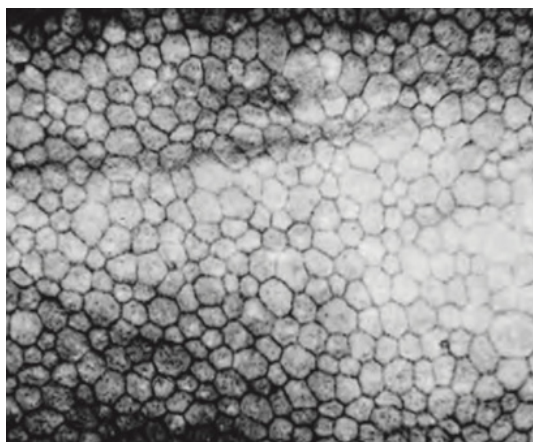


*Figure 1 – an example of an image of normal corneal endothelium generated by a specular microscope*¹ Kanski's Clinical Ophthalmology 9th Edition. Salmon, J. 2020. p 208

It is well described in multiple populations that people with glaucoma have a lower CECD than the healthy population.^{2,3,13,15,16} The reason for this lower CECD is not fully understood and may be multifactorial; it may be due to a combination of factors including raised intraocular pressure, topical medications, laser procedures and intraocular surgeries which are performed in order to control intraocular pressure.^{2,15,17} Intraocular pressure and CECD have been shown to have an inverse relationship in patients with glaucoma.^{17,18}

Measurement of CECD by automated devices is not perfectly accurate at low cell densities.¹⁹ Minami et al looked at automated cell counts in patients with glaucoma and found that the automated counts were falsely high in corneas with very low endothelial density.¹⁹ They concluded that accepting the automated CECD reading could endanger patients as those at risk of corneal decompensation would not be

picked up as being at risk. Patients with glaucoma may be at risk of having very low cell density because of their disease process as well as use of topical medications preserved with endothelial-toxic preservatives, glaucoma surgery where anti-metabolites are used, and laser procedures.¹⁷

Cataract extraction by means of phacoemulsification can cause trauma to the endothelium and reduced CECD.²⁰ Patients with POAG who undergo phacoemulsification cataract surgery have been shown to lose an average of 9% of CECD by two years post surgery.²⁰ This is similar to the 7% CECD loss at six months to one year following phacoemulsification surgery in the non-glaucomatous eyes.^{3,21} This loss appears to be due to the single insult of the intraocular surgery and the rate of loss after surgery slows to what would be expected with normal aging.¹⁷

Surgical procedures to manage glaucoma also cause a reduction in endothelial density.^{13,17,22} These surgeries include procedures where drainage tubes are left in the eye in relatively close relation to but not in contact with the cornea (eg. Ahmed glaucoma valve and Baerveldt implant) as well as trabeculectomy where no drainage device remains but rather an alternative route for aqueous humour drainage is created.^{17,23} Procedures where tubes remain in an eye cause a higher rate of endothelial loss over time than those that do not involve drainage via an implanted tube.^{17,24} Glaucoma surgeries which involve the implantation of very small devices into the anterior chamber drainage angle (such as EX-PRESS) appear to cause even less loss of endothelial density.^{13,17} Mitomycin C is an anti-metabolite used during glaucoma surgery to prevent scarring and long term procedure failure – this medication is toxic to corneal endothelium.¹⁷ In contrast to endothelial cell loss following cataract surgery, loss of endothelial density following the above glaucoma surgeries happens at a higher rate than expected for many years following the procedure.¹⁷

Selective laser trabeculoplasty (SLT) is commonly performed as part of the management of primary open angle glaucoma and its short term effects have been studied: a transient increase in dark spots on specular microscopy imaging has been documented.^{25,26} The long term effect of SLT on CECD is less certain.²⁵

Multiple classes of topical medication are used to reduce intraocular pressure (IOP) as part of the management of glaucoma and, although the effects of individual medications are difficult to study in a clinical setting, evidence points away from these medications affecting CECD.^{3,17} In vitro studies have pointed to benzalkonium chloride (BAK), a commonly used preservative, being a cause of decreased CECD.³ A large study of data from more than 19 000 donor corneas from an eye bank showed no difference in CECD between corneas from people on topical glaucoma medication and those not on medication.²⁷

Normative databases of CECD have been described for various African populations but not for black South African people.²⁸⁻³⁰ In 2018, a cross sectional study, the South African Eye Study (SAES), was carried out – IOP and central corneal thickness (CCT) of people in Cape Town, South Africa were measured. The participants self-reported as either African, mixed ethnicity or Caucasian.⁷ This study reported significant differences between the CCT of these 3 groups with African participants having the lowest CCT and Caucasian patients having the highest. The measured IOP in all 3 groups was similar when measured by the investigators but was significantly different when the Dresden formula was used to adjust the IOP for CCT. It is unclear if this statistically significant difference is clinically significant. The study did not assess CECD. A study of healthy Nigerian eyes concluded that CECD in Nigerians is lower than “that reported in the Japanese, American and Chinese eyes, and is comparable to that seen in Indian and Malaysian eyes.”²⁸ Similar research done in the Democratic Republic of Congo and Egypt described normative values of CECD in healthy eyes.^{29,30} The normative values for the Congolese population are “greater than in most other populations”²⁹ while the Egyptian database showed similar CECD to the Nigerian database.³⁰

Low CCT is known to be a risk factor for development and progression of glaucoma³¹; especially for development of primary open angle glaucoma in patients with ocular hypertension. The Goldmann applanation tonometer works on the Imbert-Fick principle and is prone to underestimating the IOP in an eye with a thinner cornea.³² There are calculations which can be used to compensate for this

phenomenon and glaucoma specialists take this into account when measuring IOP in patients known to have thin corneas.^{4,31}

It is thus apparent that there is a need for more research into glaucoma in African populations. The high burden of irreversible blindness due to glaucoma as well as the fast rate at which glaucoma progresses in this population point to the fact that there is much to be done to prevent blindness caused by glaucoma. An investigation into whether African people with glaucoma have lower CECD than the general population will serve to broaden the knowledge base on glaucoma in this population. It will also serve to assist those managing glaucoma to know what range of CECD to expect to measure in the patients they are treating when making decisions about potential glaucoma interventions and other procedures such as cataract surgery. Decisions on the use of anti-metabolites as well as the approach to cataract surgery could be affected by CECD and this investigation may highlight the need for more frequent measurement of CECD to find those at risk of endothelial decompensation. As the population of South Africa ages,³³ an increasing number of cataract surgeries will be performed. Some of these surgeries will be done on patients with glaucoma. It is important to investigate CECD in this population to assess whether South Africans with glaucoma who require cataract surgery are at risk of corneal decompensation post operatively.

In light of the fact that people of African descent have thinner CCT than other populations, it will be valuable to assess whether there is any correlation between low CCT and endothelial density. It will also be valuable to assess whether there is an inverse relationship between CECD and IOP, as has been shown in other populations. This investigation will be novel; South African people are known to have thinner CCT than other populations and there is not yet normative data on CECD in South Africans, nor is there published data on CECD in South Africans with glaucoma.

3. Research question

Is the corneal endothelial cell density of black South Africans with glaucoma lower than that of black South African controls with healthy eyes?

The rationale for this study is that this type of study has never been undertaken in this population. Other populations have been investigated and patients with glaucoma have been shown to have lower corneal endothelial cell counts than healthy controls. Black South Africans have been shown to have different corneal thickness and rates of progression of glaucoma – it will be of value to assess whether corneal endothelial cell density differs between people with glaucoma and healthy controls in this population.

4. Research aim

The aim of this study is to establish if primary open angle glaucoma is associated with a lower CECD than a healthy cohort of African patients.

5. Research objectives

- To measure and compare the CECD of South African people with glaucoma with that of healthy controls.

6. Research methods

a. Design

This research project will be a prospective case-control study which will compare cases of people with glaucoma to controls who have healthy eyes.

b. Study site

The study will be done at St John Eye Hospital in Diepkloof, Soweto in Johannesburg. This is a tertiary facility which is part of the Chris Hani Baragwanath Academic Hospital complex of facilities and is staffed by specialist ophthalmologists, ophthalmology registrars in training and medical officers working exclusively in the discipline of ophthalmology. The hospital provides care under all of the sub-specialties of ophthalmology and serves the population of Soweto as well as being a referral centre for the South and West Rand areas of Johannesburg and North West Province.

c. Study populations

There will be two groups of people invited to participate in the study:

- Patients known with primary open angle glaucoma who are being managed for this disease at St John Eye Hospital who self-identify as black South African people. This group will be referred to as “patients with glaucoma”
- People with healthy eyes, with no evidence of glaucoma or other ocular diseases, who self-identify as black South African people. This group will be referred to as “healthy controls”.

Inclusion criteria

Patients with glaucoma will be included based on the following criteria:

- Age of 18 years or older
- South African
- Black ethnicity
- Confirmed diagnosis of primary open angle glaucoma under management at St John Eye Hospital using one or more topical agents

Healthy controls will be included based on the following criteria:

- Age of 18 years or older
- South African
- Black ethnicity
- Normal intra-ocular pressure (10 - 21mmHg) in the study eye
- Clinically normal ocular examination including normal gonioscopy and normal optic disc in the study eye
- Absence of other ocular pathologies in the study eye

Exclusion criteria

Patients with glaucoma will be excluded based on the following criteria:

- Co-existing ocular disease including corneal pathology or significant cataracts which prevent assessment of the optic disc in the study eye
- Previous surgical or laser management of glaucoma in the study eye
- Previous cataract surgery in the study eye

- Contact lens use

Healthy controls will be excluded based on the following criteria:

- Co-existing ocular disease including corneal pathology or significant cataracts which prevent assessment of the optic disc in the study eye
- Previous intraocular surgery or laser in the study eye
- Contact lens use

Sampling

Convenience sampling will be used to recruit participants who are known to, and attending St John's eye hospital.

Sample size has been calculated by referencing previous studies done on the same topic in other populations. These studies were adequately powered to achieve statistical significance. Sample size has been calculated using a Cohens D of 0.475, which is derived from a mean (+SD) CECD of 2692 (+358) and 2253 (+291) in non-glaucomatous and glaucomatous eyes respectively^{2,15,16,18,21}. Using a power of 80% and alpha of 5%, the minimum sample size needed for a one-tailed t-test, with 1:1 sampling is 112.

- Patients with glaucoma who are known to St John Eye Hospital will be invited to participate on days which they attend for routine appointments.
- People with healthy eyes (nursing staff, medical students, people accompanying patients to St John Eye Hospital) will be invited to participate in the study so as to recruit the group of age matched healthy controls.

Potential participants will be informed about the study and given an information sheet which explains the details of the study (Appendix A). Potential participants who are willing to participate in the study will be asked to give written consent (Appendix B). For each group, the following will then be done: history (age, sex, province, home language, co-morbidities, date of diagnosis of glaucoma*, current medications for glaucoma*, previous interventions for glaucoma*) will be taken and

examination (visual acuity (VA), intraocular pressure (IOP), vertical cup:disc ratio, gonioscopy grading) will be done on all patients. In addition, the following non-contact special investigations will be done: central corneal thickness, keratometry readings, retinal nerve fibre layer thickness on ocular coherence tomography, and spectral microscopy to assess CECD as well as related endothelial cell parameters including pleomorphism and mean cell area. These history, examination and special investigation finding will be captured as per Appendix C.

* denotes parameters on patient history only relevant to patients with glaucoma

Only one eye per patient will be used for the study. The eye will be selected using a computer generated randomised number sequence. If the selected eye cannot be used for some reason, the fellow eligible eye will be used.

Both groups (patients with glaucoma and healthy controls) will be age stratified into bands of 10 years in order to be able to compare groups.

d. Measurement tools

Corneal endothelial cell parameters and central corneal thickness will be measured with the TOMEY EM-4000 Specular Microscope. Keratometry readings will be done with a Nidek Optical Biometer. Retinal nerve fibre layer thickness will be assessed with Heidelberg Optical Coherence Tonometer. All of the above machines perform their measurements without making contact with the eye.

e. Data collection

The following information will be collected and captured as in Appendix C:

Demographic information (age, sex, home language, province of origin)

Which eye was examined

Medical information (co-morbidities, date of diagnosis of glaucoma*, current medications for glaucoma*, previous interventions for glaucoma*)

Visual acuity

Intraocular pressure

Vertical cup:disc ratio

Corneal endothelial cell properties: endothelial cell density, average cell size, coefficient of variance of cell size, standard deviation, minimum and maximum size

* denotes parameters on patient history only relevant to patients with glaucoma

f. Data management

Appendix C will serve as a data collection sheet

Microsoft Excel will be used to capture and securely store all data. The computer which the data is entered onto will be password protected and kept locked up at all times. Patient names, file numbers and other potentially identifying details will not be captured in appendix C or entered into the Excel document.

o Data analysis

Statistical analysis will be performed in the following manner:

- descriptive statistics (mean and standard deviation; median and interquartile range) will be used to describe continuous variables
- categorical variables will be described in terms of proportions
- differences between the groups will be assessed using Chi-squared and t-tests
- statistical significance will be accepted with a p-value of <0.05
- statistical analysis will be performed using STATA version 16 (Stata, College Station, Texas, USA)

g. Time schedule (including Gantt chart)

The study will commence once approval is granted by the post-graduate and ethics committee as well as Chris Hani Baragwanath Academic Hospital. The study is expected to take 6 months to complete.

The data collection for this study will be done by the principal investigator as soon as permission to commence the study is granted. The data will be collected during the course of patients attending the clinic for their booked follow up appointments or from willing participants escorting patients to the hospital. Data collection for each individual participant is expected to take approximately 20 minutes. The period of collection of data for the 112 required participants is expected to be 6 months.

	May '23	Jul '23	May - Jun '23	Aug '23 – Jan '24	Feb '24	Mar – Apr '24	May '24
Protocol Submission							
Present protocol							
Await ethics approval							
Data collection							
Data analysis							
Write up							
Submit for publication							

h. Ethical considerations

- **Participant autonomy**

Autonomy of all potential participants will be respected. Everyone invited to participate in the study will have explanation of the voluntary nature of the study explained verbally as well as in written form (Appendix A). Once a person has consented to participate in the study, their autonomy will be respected by them being allowed to withdraw at any point.

- **Participant confidentiality**

Confidentiality will be maintained by not collecting identifying data (names, folder numbers) of study participants in any of the data collection. Each participant will be allocated a unique number which will be used on the data collection sheet and in the capturing of the data in Excel. There will be a page which contains the file numbers of patients along with the unique study numbers and this will be kept separate from the collected data – it will be used if needed for cross checking and will be stored in a locked cupboard.

- **Beneficence**

The study is of potential benefit to all participants. Patients known with glaucoma may have glaucomatous disease progression detected during the study. Participants without glaucoma may have other ocular conditions detected – if this is the case they will be referred for management at St John Eye Hospital. In a broader sense, this study may be beneficial on a population level. It will contribute data on a topic on which there is a dearth of evidence.

- **Non-maleficence**

There are no potential negative patient outcomes anticipated during the study. The special investigations which will be

conducted do not require contact with the ocular surface and this the risk for trauma and/or infection are avoided. The testing is not physically or psychologically demanding.

i. Costs and required funding

○ **Stationery and consumables**

The anticipated stationery requirements are copies of the patient information sheets, informed consent documents and data collection forms. One of each of the above will be required per participant. The cost of the printing and copying will be covered by the principal investigator.

○ **Publication costs**

Should the research be accepted for publication in a Department of Higher Education and Training (DoHET) approved journal, application for reimbursement of the publication fee will be made to the Wits School of Clinical Medicine MMed Fund.

7. Reporting plans

The goal of this research project is to publish a report on the results of the study in a DoHET accredited medical journal. This publication will also be submitted to Wits University in order to be awarded a Masters in Medicine. The primary investigator will also apply to present the research findings at conferences (both general ophthalmology conferences and glaucoma specific conferences).

8. Role players and investigators

Principal investigator: Dr C Dold

Registrar, Department of Neurosciences, Division of Ophthalmology,
University of the Witwatersrand / Chris Hani Baragwanath Academic
Hospital (St John Eye Hospital)

Professor I Mayet

Consultant Ophthalmologist, Head of Clinical Unit at St John Eye Hospital,
University of the Witwatersrand

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10. Appendices

Appendix A – Participant information sheet

University of the Witwatersrand

Study Title: Comparison of corneal endothelial cell density between South Africans with glaucoma and healthy controls

Hello, my name is Dr Catherine Dold. I am a doctor working in eye care at St John Eye Hospital which is part of Chris Hani Baragwanath Academic Hospital. I am supervised by Professor Ismail Mayet.

I am doing a research study on a topic called corneal endothelium. Research is a way of trying to learn new things about illnesses. In this study I am measuring the corneal endothelium of people who have a disease called glaucoma and people with healthy eyes. Corneal endothelium is a layer of cells that lines the cornea (the clear portion of the eye); these cells help to keep the cornea clear and able to see. I am going to compare healthy people and people with glaucoma to

see whether the corneal endothelium of these two groups are different. In future this might help to know which patients are at risk of further corneal problems.

You have been asked to be part of this study either because you have glaucoma OR you have healthy eyes with no other problems and so you will be used as the healthy group to compare to the group of patients with glaucoma.

Glaucoma can cause blindness. For this reason patients with glaucoma are given medication, sometimes offered surgery, and are reviewed in eye clinics frequently. When the corneal endothelium of Caucasians (white people) with glaucoma is measured, it can show a risk of corneal problems following various eye surgeries. There has not been research to show whether this is the case in black South African people. We would like to look at whether corneal endothelial properties are different in black South African people with healthy eyes and those with glaucoma.

Your decision to take part in this study is voluntary. If you decide that you would like to take part in the study, you will be asked to sign a form confirming that you give your consent and that you understand the study. If you decide that you would not like to take part, all of your routine care will continue and you will not be at a disadvantage.

Steps to being involved in the study

- You will sign a consent form
- You will be asked questions about your demographics and medical history
- Your vision will be tested (on a reading chart)
- You will be examined at a machine called a slitlamp which is the microscope that eye doctors use to routinely examine patients
- Measurements will be done on one of your eyes using three other machines – the machines won't touch your eye or hurt you

- The entire process will take approximately 20 minutes
- No follow up will be required

Risks of being involved in the study: There are no risks to the examinations and special investigations which you will go through as a participant in this study. The machines you will be measured on and tested with will not touch your eye or cause any pain.

Benefits of being in the study: There is a chance that an eye abnormality may be detected while you are being examined. If this is the case you will receive a diagnosis and be offered management at St John Eye Hospital.

Reimbursements: You will not be paid to participate in the study. You will not be charged for any of the tests done on your eye.

Confidentiality: Your personal information will be kept confidential. No identifying data will be stored with your data which is collected. A unique participant number will be assigned to you so that your data can be stored without your identifying information.

What will happen with the information from the study? We will analyse all of the data which we collect and aim to publish this in a scientific journal. This will also be used as a submission for a Masters in Medicine degree.

Ethics: Before commencement of this study, ethical approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand has been obtained. Professor P Ruff, Chairperson of the Human Research Ethics Committee (Medical) at the University of Witwatersrand, who may be contacted via any one member of the secretariat. The telephone numbers for the Committee secretariat are 011 717 2700/1234/2656/1252 and the e-mail addresses are

Zanele.Ndlovu@wits.ac.za,

Rhulani.Mukansi@wits.ac.za,

Mapula.Ramaila@wits.ac.za and Iain.Burns@wits.ac.za

Thank you for taking time to read this document and consider your participation in this research project. Please feel free to direct any further questions to me (Catherine Dold, principal investigator) in person or using the following contact details: email cjdold@gmail.com or telephone 084 580 9077. You are also welcome to contact my supervisor, Professor I Mayet at eyemay.im@gmail.com or 084 410 5769.

Appendix B – Patient Consent Form

Title: Comparison of corneal endothelial cell density between black South Africans with glaucoma and healthy controls

Participant

I confirm that I have been informed by Dr Catherine Dold about the nature of this research project. I have been provided with a patient information sheet which I have read or have had read to me and explained in a language which I understand. I have been given opportunity to ask questions about the study. I am participating in the study freely. I understand that I can withdraw my participation at any point and that, in doing so, I will still receive the same management.

Name of participant: _____

Date: _____

Signature: _____

Researcher

I have explained research project to the participant. I confirm that I have answered the questions of the participant and that the participant has given voluntary consent.

Name of investigator: _____

Date: _____

Signature: _____

Appendix C – Data collection sheet

Age:

Participant no:

Sex: M / F

Eye examined: R / L

Home language:

Province of origin:

Medical information:

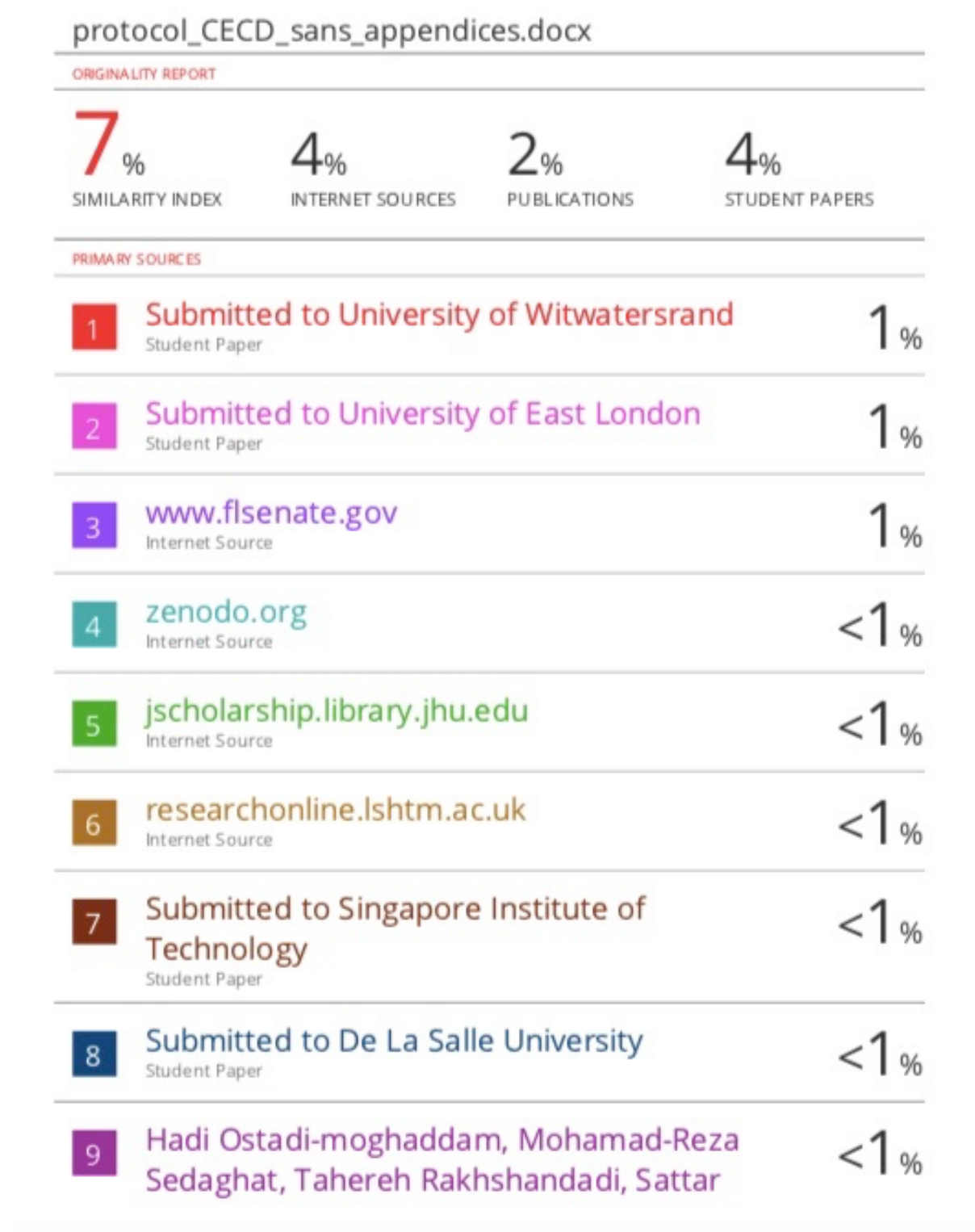
- Co-morbidities:
- *Date of diagnosis of glaucoma:
- *Current medications for glaucoma:
- *Previous interventions for glaucoma:

* denotes parameters on patient history only relevant to patients with glaucoma

Parameter	Result
Visual acuity (VA)	
Best corrected VA	
Intraocular pressure (IOP)	
Vertical cup:disc ratio	
Keratometry K1	
Keratometry K2	
Axis	
Corneal endothelial cell density	
Cell density	

Coefficient of variation (in cell area)	
Average cell size	
Standard deviation	
Maximum cell size	
Minimum cell size	
Central corneal thickness	

Appendix D – Turnitin report



Rajabi, Foroozan Narooie-Noori, Farshad Askarizadeh. "A contralateral eye study comparing characteristics of corneal endothelial cells in bilateral keratoconus patients with unilateral corneal Vogt's striae", *Journal of Current Ophthalmology*, 2018
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13 Devalla, Sripad Krishna. "Deep Learning Algorithms for Optical Coherence Tomography Images with Applications in Glaucoma", National University of Singapore (Singapore), 2022
Publication

14 www.hindawi.com <1 %
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Exclude quotes On

Exclude matches < 8 words

Exclude bibliography On

Appendix E – Plagiarism declaration



Neurology; Neurosurgery; Ophthalmology; Otorhinolaryngology (ENT)

7 York Road, Johannesburg 2193, South Africa

Telephone: 27-11-717 2774

Plagiarism declaration for written work

I, Catherine Dold, as a postgraduate student registered for a MMed at the University of the Witwatersrand declare the following:

- I am aware that plagiarism is the use of someone else's work without their permission and or without acknowledging the original source.
- I am aware plagiarism is wrong.
- I confirm that this written work is my own work except where I have stated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or if I have failed to acknowledge the ideas or writing of others.

Signature:

A handwritten signature in black ink, consisting of a large, stylized 'C' and 'D'.

Date: 27 April 2023