Clinical Trial: Effects of Carboxymethylcellulose Artificial Tears on the Eye Microbiome

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Study Protocol and Statistical Plan 3/31/2022

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• Co-Investigator: Elizabeth Dawson

• Co-Investigator: Gary Wang, M.D., Ph.D.

• Supervisor: Walter Allan Steigleman, M.D.

University of Florida IRB-01

Protocol

1. Title:

Effects of Carboxymethylcellulose Artificial Tears on the Eye Microbiome: A Randomized, Controlled, Double-Blind Study

2. Investigators:

- Principal Investigator: Yujia Zhou
- Co-Investigator: Elizabeth Dawson
- Co-Investigator: Gary Wang, M.D., Ph.D.
- Supervisor: Walter Allan Steigleman, M.D.

3. Abstract:

Carboxymethylcellulose (CMC), a common component in artificial tears, modifies the gut microbiome when ingested. We will identify its effects on the eye microbiome of adult UF eye clinic patients in this randomized, controlled, double-blind study. Effects of CMC on the eye microbiome are unknown and have implications for ocular disease and artificial tear choice. Participants will be given either CMC artificial tears or artificial tears without CMC, complete symptom surveys, and undergo conjunctival swabs. Investigators will conduct chart reviews, bacterial genome sequencing, and analyze microbiomes using Shannon's diversity index and principal coordinate analysis on R statistical packages.

4. Background:

Carboxymethylcellulose (CMC) is a thickener frequently included in FDA-approved artificial tears. A study by Chassaing et al. in 2021 entitled “Randomized Controlled-Feeding Study of Dietary Emulsifier Carboxymethylcellulose Reveals Detrimental Impacts on the Gut Microbiota and Metabolome” demonstrated that CMC ingestion alters the gut microbiome. The eye also has a microbiome distinct from other organ systems. The ocular microbiome influences eye diseases including dry eye disease and keratitis, as summarized in an article entitled “Microbes of the human eye: Microbiome, antimicrobial resistance and biofilm formation” by Ranjith et al. in 2021. Interventions such as contact lens wear can change the diversity and composition of eye bacteria, as demonstrated in an article entitled “Changes in the Eye Microbiota Associated with Contact Lens Wearing” by Hakdong et al. in 2016.

Therefore, we hypothesize that the use of CMC in artificial tears may affect the eye microbiome and influence ocular diseases. Similar studies on microbiome composition used bacterial ribosomal RNA sequencing and statistical analysis with existing R packages such as phyloseq and DADA2. A UF investigator, Rachel Newsome, recently conducted a microbiome study entitled “The gut microbiome of COVID-19 recovered patients returns to uninfected status in a minority dominated United States cohort” at the University of Florida in 2021. For these reasons, we are conducting a similar study on the use of artificial tears containing CMC compared to control artificial tears without CMC. Results of this study will lead to a better understanding of the risks and benefits associated with CMC artificial tears for eye lubrication and dry eye disease.

5. Specific Aims:

We aim to examine whether carboxymethylcellulose in artificial tears changes the eye microbiome and identify which bacterial genera are enriched.

6. Research Plan:

Before Study Enrollment

1. Staff creates Excel key and labels with identifiers
2. Screen clinic schedule for inclusion and exclusion criteria
3. Study staff assigns and labels cardboard boxes with numerical identifiers, then attaches spare labels
4. Principal investigator fills boxes with control or CMC artificial tears, consent copies, and patient instructions, then seals the boxes with tape
5. Store boxes on-site for staff

Initial Study Schedule

1. Participant checks into clinic and receives clinical workup
2. Dr. Steigleman approaches, recruits, and enrolls patient.
3. Staff ensures patient meets criteria at time of visit
4. Participant signs consent form
5. Staff gives a random box to the participant and attaches box label to informed consent form
6. Participant completes survey 1
7. Licensed staff collect 1st swab
8. Participant has a normal clinic visit and is scheduled to return

Second Study Schedule

1. Participant checks into clinic and receives clinic workup 1 week after initial visit
2. If participant does not have box labels, the informed consent form label is consulted to associate surveys and data
3. Participant completes survey 2
4. Staff ensures that both surveys are completed and recorded
5. Licensed staff collects 2nd swab
6. Participant completes a normal clinic visit and has thereafter completed the study

Tasks Throughout Study Duration

1. Freeze and transport samples to lab for temporary storage
2. Lyse, amplify, and sequence samples, then store sequences
3. Analyze sequences with R statistical packages
4. Monitor for harms in clinic at visit and treat accordingly
5. Relevant chart review and record data for each participant
6. Statistical analysis of sampled microbiomes by R packages

- **Recruitment:** During the spring of 2022, 80 patients of the UF Oaks Eye Clinic who meet inclusion criteria will be identified by study staff prior to their visit by chart review using the Epic electronic medical record and clinic scheduler on a secure encrypted UF computer. Up to 20 participants are expected to drop out over the course of this study. When patients have completed their clinical workup, Dr. Steigleman will approach patients without exclusion criteria to ascertain willingness/interest in the study, then recruit them. Study staff will give willing participants the appropriate informed consent form and counsel participants for consent to enroll in a private examination room. Completed consent forms will labelled with the participant's randomly selected numerical identifier as described below and stored securely in the UF Oaks Eye Clinic Office.
- **Participant Criteria:** This study includes adults above age 18 of either sex who can self-administer artificial tears and are scheduled to return for clinical follow-up in 1 week. This study excludes individuals with active eye infections, have prosthetic eyes, are immunocompromised, or are diagnosed with autoimmune diseases or malignant neoplasms about the eye. Individuals who take immunomodulatory therapy, steroids, antibiotics, medicated eyedrops, or are already using CMC artificial tears within 1 week of the study will also be excluded. These criteria maximize the number of self-consenting participants while minimizing participants whose compliance, conditions, or medications may change the eye microbiome.
- **Research Intervention:** Participants will be given either artificial tears without CMC (control) or commercial artificial tears containing CMC (treatment) and told that they may have received either CMC or a placebo. Both are FDA-approved for over-the-counter use and for treatment of dry eye. Both interventions are standard therapies for dry eye disease. Prospective participants have the option to not enroll in the study, and the alternative is to complete a normal clinic visit. Individuals for whom artificial tears do not constitute standard therapy are excluded from this study.
- **Assignment:** This study design will include an experimental control, random assignment, and double blinding of staff and participants. The principal investigator will create a securely saved Excel spreadsheet with unique randomly generated numerical identifiers keyed to either the control group receiving artificial tears without CMC or the treatment group receiving CMC artificial tears. The principal investigator will then create plain cardboard boxes with the identifier on removable adhesive labels. Boxes will contain a copy of the informed consent form, participant instructions, and 21 plastic vials of either artificial tears without CMC or CMC artificial tears as assigned on the identifier spreadsheet. Patients will be blinded to their assigned intervention group. Study staff other than the principal investigator, who are blinded to box contents, will give participants a randomly selected box and an electronic survey (PDF) on an encrypted clinic computer in their private examination room. Labels will be placed on consent forms and identifiers will replace personal identifying information on electronic surveys.
- **Data Collection:** Electronic surveys will contain the Ocular Surface Disease Index and a box for entry of the patient's unique identifier. After completion of the survey, licensed physicians serving as study staff will swab the participant's right and left eye conjunctiva with a single cotton tip applicator, seal the applicator in a sample

tube, attach a label, and freeze it for storage in dry ice. The participant will complete a normal clinical schedule afterwards and will be scheduled to return in 1 week. Upon 1 week follow-up, participants will be swabbed again and complete a second survey. The second survey will be identical to the first survey but contain an additional question quantifying eye drop use to measure compliance. Patients will complete a normal clinical schedule afterwards. Study staff will correlate identifiers to patients using the informed consent forms and record ophthalmic history, ophthalmic medications, age, gender, tobacco usage, diabetes status, and race by chart review during this study.

- **Sample Processing and Data Analysis:** Samples will be frozen at -80 degrees Celsius and transported to a BSL-2 lab at UF for processing. During processing, samples will be lysed, amplified, and sequenced for bacterial 16S RNA using protocol supplied by Illumina MiSeq. Swabs and container tubes will be destroyed after sequencing. The sequenced bacterial microbiome of each sample will then be trimmed, error-corrected, and classified using the DADA2 R package as described in the article described above (Newsome 2021). After all samples are sequenced, microbiomes will be grouped by principal coordinate analysis using the phyloseq R package. Shannon's diversity indices will be calculated using the phyloseq R package. Differences in alpha diversity before and after treatment will be tested by PERMANOVA using the vegan R package, and differences in beta diversity will be tested by ANOVA using the nlme R package.
- **Data Security:** The original consent forms with unique identifier stickers will be kept securely in a folder and stored in a locked office within the UF Oaks Eye Clinic. Surveys will be accomplished on a pdf and do not contain any participant identification aside from the study-assigned numerical identifier. All other documents and data files will be deidentified and stored on an encrypted computer within the UF IT environment. Only medical students, resident physicians, and attending physicians serving as study staff will access participant medical records on the Epic EMR using a secure encrypted computer or smartphone. This study will not have a Data Safety Monitoring Board. Dr. Steigleman will serve as oversight for this research project and will meet weekly with study staff to monitor study conduct and patient safety.

7. Possible Discomforts and Risks:
Enrolled study participants may more commonly encounter mild eye discomfort during conjunctival swabs, anxiety regarding assignment to a treatment group, short delays in clinic flow (1-2 minutes), and inconvenience of completing surveys during the study.

Rare discomforts or risks may include breach of protected patient data, eye pain, dry eye, eye infection, and eye trauma including corneal abrasions and conjunctival hemorrhage because of methods used in this study. Participants will not be charged for any time, medication, or procedure used in this study.

To minimize anxiety and medical risks, patients will receive counseling and treatment interventions in the setting of the UF Oaks Eye Ophthalmology Clinic by a licensed attending ophthalmologist or an ophthalmology resident capable of mitigating and treating such harms. The inconvenience of enrolling in the study will be mitigated by utilizing the wait time (10 to 40 minutes) during a routine clinic appointment. The conjunctival swab is a routine technique practiced by attending and resident ophthalmologists with minimal risk. Eye discomfort and pain during this procedure will be reduced with a soft cotton-tip applicator, and patients may be given routine topical anesthetics or eye lubricants after the procedure if needed. Topical anesthesia prior to swabbing and other methods of collecting microbiome samples such as eye irrigation will not be used due to their anticipated negative effect on sample quality.

8. Possible Benefits:
The participants will receive free FDA-approved treatment (artificial tears) for dry eye during the study and improved guidance on artificial tear choice after the study's conclusion based on the study results. This may not benefit all participants directly but may directly help some participants alleviate dry eye. There are no other direct benefits to participants.

To date, there is no data on whether this common artificial tear ingredient may have deleterious or favorable effects on the eye microbiome. The composition of eye microbiomes has been shown to have associations with eye diseases, including dry eye disease. This information may guide artificial tear choice in the future, as opposed to the status quo, where all brands of preservative-free artificial tears are treated as equivalent.

These benefits outweigh the minimal risk to enrolled study participants, who will be in the care of a trained ophthalmologist during the length of this study in the unlikely event that harm occurs to them. Our study methods use minimally invasive techniques to gather data and are routine in the setting in an ophthalmology clinic. We designed the study such that the risk of unreasonable harms such as billing for research time, eye infections, and patient discomfort are minimized or eliminated.

9. Conflict of Interest:
We do not have any conflicts of interest to disclose. Neither the principal investigator, co-investigators, or UF hold patents, licenses, or stock in the artificial tears used. We have no external sponsors.