COVER PAGE

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title:

Evaluating the Efficacy of Topical Insulin for the Restoration of Ocular Surface Interface in Dry Eye Disease: A Randomized Controlled Clinical Trial

ClinicalTrials.gov Identifier	NCT06939959
Document Type	Study Protocol and Statistical Analysis Plan (SAP)
Document Version	Version 1.0
Document Date	13 June 2026
Ethics Approval	IREB Approval No: 579/DME/KMC
Study Site	Khyber Teaching Hospital (KTH), Peshawar, Pakistan
Study Design	Prospective, Randomized, Participant-Masked Controlled Trial
Phase	Phase II/III Clinical Trial
Intervention	Topical Insulin 1 U/mL vs. Preservative-Free Artificial Tears (CMC 0.5%)
Primary Outcome	Proportion achieving OSDI ≤20 at 12 weeks
Follow-up Duration	12 weeks (assessments at baseline, 4, 8, and 12 weeks)
Total Enrollment	128 participants (64 per arm)
Regulatory Framework	Declaration of Helsinki (2013 revision); CONSORT 2010 guidelines

Confidential — For Registry Submission Only

1. Background and Rationale

Dry eye disease (DED) is a chronic, multifactorial disorder of the ocular surface characterized by loss of tear film homeostasis, accompanied by tear film instability, hyperosmolarity, inflammation, and neurosensory abnormalities. The TFOS DEWS III Digest (2025) identifies meibomian gland dysfunction as the predominant driver of evaporative disease. With a global symptomatic prevalence of approximately 11.6%, DED represents a major and growing burden in ophthalmic practice, increasing with age, digital screen exposure, and metabolic comorbidity.

Conventional therapies, including artificial tears, provide symptomatic lubrication but do not address underlying epithelial dysfunction or restore ocular surface homeostasis. Advanced pharmacological agents such as cyclosporine A and lifitegrast are limited by delayed onset, tolerability concerns, and cost, restricting accessibility in resource-constrained settings.

Insulin receptors and IGF-1R are expressed on corneal epithelial cells, keratocytes, and within the tear film. Receptor activation stimulates the PI3K-Akt-mTOR cascade, promoting epithelial proliferation, migration, and goblet cell differentiation. In diabetic patients, chronic hyperglycaemia depletes tear insulin levels and impairs lacrimal gland and corneal epithelial function, representing a population with particular therapeutic need.

Prior studies by Burgos-Blasco et al. and Atikah et al. have demonstrated improvements in corneal staining, tear film parameters, and inflammatory biomarkers with topical insulin. However, existing evidence is limited by small sample sizes, design heterogeneity, and lack of data from South Asian populations. No well-designed RCT has been conducted in Pakistan, where the burden of diabetes-associated DED is substantial and access to advanced therapies is limited.

2. Study Objectives

2.1 Primary Objective

To evaluate whether topical insulin (1 U/mL), administered four times daily for 12 weeks, is superior to preservative-free carboxymethylcellulose 0.5% artificial tears in achieving clinically meaningful symptomatic improvement (OSDI ≤20) in patients with dry eye disease.

2.2 Secondary Objectives

- To compare improvement in aqueous tear production (Schirmer test) between the insulin drops group (IDG) and artificial tears group (ATG) over 12 weeks.
- To compare improvement in tear film stability (fluorescein TBUT) between groups over 12 weeks.
- To assess the longitudinal trajectory of improvement in Schirmer test, TBUT, and OSDI at 4, 8, and 12 weeks.
- To evaluate the safety and tolerability profile of topical insulin via anterior segment examination at each study visit.

3. Study Design

3.1 Overview

This is a prospective, randomized, participant-masked, parallel-group controlled clinical trial conducted at the Outpatient Department of Ophthalmology, Khyber Teaching Hospital (KTH),

Peshawar, Pakistan. The study was conducted in accordance with CONSORT 2010 guidelines and adheres to the tenets of the Declaration of Helsinki (2013 revision). Ethical clearance was granted by the Institutional Review and Ethics Board (IREB; Approval No: 579/DME/KMC). Written informed consent was obtained from all participants prior to enrolment.

3.2 Masking

This was a participant-masked trial. A second investigator, not involved in outcome assessment, prepared topical insulin according to a written standardised compounding protocol and dispensed both interventions in identical unlabeled single-use droppers of equal volume, ensuring that participants remained unaware of their treatment allocation throughout the 12-week follow-up. The principal investigator, who conducted all clinical assessments, was not masked to treatment allocation. To mitigate the resultant risk of detection bias, all objective measurements (Schirmer test and TBUT) were independently rechecked by a second colleague clinician who was unaware of the study hypothesis; any discrepancy exceeding 1 mm or 1 second was resolved by a third reading and consensus.

4. Study Population

4.1 Eligibility Criteria

Inclusion Criteria

- Age ≥18 years, either sex
- Schirmer's test (without anaesthesia) <10 mm at 5 minutes
- Fluorescein tear break-up time (TBUT) <10 seconds
- Ocular Surface Disease Index (OSDI) score >20
- Willingness and ability to comply with study procedures and follow-up schedule
- Provision of written informed consent

Exclusion Criteria

- Active ocular infection at screening
- Severe ocular surface disease unrelated to DED, including cicatricial pemphigoid, Stevens–Johnson syndrome, or graft-versus-host disease
- Ocular surgery within the preceding 6 months
- Known hypersensitivity to insulin or any excipient in the study formulations
- Current systemic insulin therapy
- Inability to comply with follow-up schedule or provide informed consent

4.2 Sample Size Justification

Sample size was calculated using the two-proportion z-test formula:

$$n = (Z\alpha/2 + Z\beta)^2 \times [P_1\ (1-P_1\ ) + P_2\ (1-P_2\ )] \div (P_1\ -P_2\ )^2$$

Based on expected response rates of 63% for artificial tears and 85% for topical insulin (as reported by Azmi and Bastion), using a two-sided α = 0.05 and 80% power (Zα/2 = 1.96, Zβ = 0.84), the minimum calculated sample size was 55 participants per group. To account for an anticipated attrition rate of 10–15%, a total of 128 participants (64 per group) were enrolled.

5. Randomisation and Allocation Concealment

5.1 Randomisation Sequence

Participants were allocated 1:1 to the IDG or ATG using blocked randomisation with variable block sizes (blocks of 4 and 6). The allocation sequence was generated by an independent biostatistician using validated statistical software prior to the commencement of enrolment.

5.2 Allocation Concealment

The randomisation sequence was concealed in sequentially numbered, opaque sealed envelopes prepared by the independent biostatistician. Envelopes were opened in sequence at the point of participant allocation by the dispensing investigator, who was not involved in outcome assessment. This mechanism ensured allocation concealment was maintained throughout the recruitment phase.

6. Interventions

6.1 Group I: Artificial Tears Group (ATG) — Control

- Formulation: Preservative-free carboxymethylcellulose (CMC) 0.5% artificial tears
- Dose: One drop per eye, four times daily
- Duration: 12 weeks
- Packaging: Identical unlabeled single-use droppers (equal volume to IDG)

6.2 Group II: Insulin Drops Group (IDG) — Intervention

- Formulation: Topical insulin 1 U/mL, prepared by diluting commercially available human regular insulin (100 U/mL) in sterile isotonic saline
- Compounding: Prepared according to a written standardised compounding protocol by the dispensing investigator
- Dose: One drop per eye, four times daily
- Duration: 12 weeks
- Storage: Refrigerated at 2–8°C; participants in the IDG were counselled on appropriate storage
- Packaging: Identical unlabeled single-use droppers (equal volume to ATG)

6.3 Washout and Concomitant Medications

All pre-existing topical DED medications were discontinued with a minimum one-week washout period before baseline assessment. No other topical ophthalmic medications were permitted during the study period unless medically necessary, in which case this was documented as a protocol deviation.

7. Outcome Measures

7.1 Primary Outcome

Measure	Definition
OSDI Response	Proportion of participants achieving clinically meaningful symptomatic improvement, defined as OSDI score ≤20 at 12 weeks. Assessed using the validated 12-item Ocular Surface Disease Index questionnaire.

7.2 Secondary Outcomes

Outcome	Measurement Method	Time Points
Schirmer Test (mm)	Standard 5×35 mm filter paper strips without anaesthesia. The eye with worse readings at baseline selected for analysis.	Baseline, 4, 8, 12 weeks
TBUT (seconds)	Slit-lamp cobalt-blue illumination after standardised fluorescein strip application; mean of three consecutive readings.	Baseline, 4, 8, 12 weeks
OSDI Score (continuous)	Validated 12-item questionnaire yielding a 0–100 scale score; higher scores indicate worse disease.	Baseline, 4, 8, 12 weeks

7.3 Safety Outcomes

At each visit (baseline, 4, 8, and 12 weeks), anterior segment examination assessed for adverse effects including: corneal infiltrates, conjunctival hyperaemia, and signs of hypersensitivity. All adverse events were recorded and classified by severity (mild, moderate, severe) and relatedness to study treatment.

8. Statistical Analysis Plan (SAP)

Version 1.0 | Software: IBM SPSS v23.0 | Two-sided α = 0.05 throughout

8.1 Analysis Populations

Population	Definition
Intent-to-Treat (ITT)	All randomised participants regardless of protocol adherence or completeness of follow-up. Primary population for all confirmatory analyses.
Per-Protocol (PP)	Participants who completed ≥80% of prescribed doses and attended all scheduled visits without major protocol deviations. Sensitivity analysis only.
Safety Population	All randomised participants who received at least one dose of study medication.

8.2 Handling of Missing Data

- The primary ITT analysis uses Last Observation Carried Forward (LOCF) imputation for participants lost to follow-up.
- Sensitivity analysis using complete-case analysis (per-protocol population) will be performed to assess the robustness of findings.
- The proportion and pattern of missing data by treatment arm will be reported; if differential missingness is detected, multiple imputation will be considered as an additional sensitivity analysis.

8.3 Baseline Characteristics

Baseline demographic and clinical characteristics will be summarised descriptively for each treatment arm and for the overall cohort:

- Continuous variables: expressed as mean ± standard deviation (SD) or median (interquartile range) depending on distribution.
- Categorical variables: expressed as absolute frequencies and percentages.
- Normality of continuous variables will be assessed by the Shapiro-Wilk test.
- Baseline comparability will be assessed by independent samples t-test (or Mann-Whitney U if non-normal) for continuous variables and Chi-square test (or Fisher's exact test if expected cell count <5) for categorical variables. No formal hypothesis testing for baseline balance is intended; these tests serve only descriptive purposes.

8.4 Primary Outcome Analysis

Outcome: Proportion achieving OSDI ≤20 at 12 weeks (ITT population)

- Test: Chi-square test for the comparison of proportions between IDG and ATG at 12 weeks.
- Relative risk (RR) with 95% confidence intervals will be calculated.
- Odds ratio (OR) with 95% confidence intervals will be calculated.
- Absolute risk reduction (ARR) will be calculated as: ARR = P(ATG response) − P(IDG response).
- Number needed to treat (NNT) will be calculated as: NNT = 1/ARR.
- The mean OSDI difference between groups at 12 weeks will be assessed using an independent samples t-test, with the 95% CI for the difference reported.
- Significance threshold: two-sided p ≤0.05.

8.5 Secondary Outcome Analyses

Longitudinal repeated-measures analysis (Schirmer test, TBUT, OSDI continuous score)

- Method: Repeated measures analysis of variance (ANOVA) with within-subject factor Time (baseline, 4, 8, 12 weeks) and between-subject factor Group (IDG vs. ATG).
- Sphericity assumption: tested using Mauchly's test. If violated, degrees of freedom will be corrected using the Greenhouse-Geisser correction.
- The primary term of interest is the Group × Time interaction, which tests whether the trajectory of change over time differs between groups.
- Effect sizes will be reported using partial eta-squared (η^2): small = 0.01, medium = 0.06, large = 0.14.
- Post-hoc pairwise comparisons between time points within each group will use Bonferroni correction.

8.6 Subgroup Analyses (Exploratory)

The following post-hoc subgroup analyses will be performed on the primary outcome (OSDI ≤20 at 12 weeks). These are exploratory and hypothesis-generating in nature. The trial was not powered to detect subgroup differences; results should not be used to guide clinical practice without independent replication.

Subgroup	Categories	Statistical Test
Sex	Male vs. Female	Chi-square test within each stratum; interaction term in logistic regression

Age Group	<40 years vs. ≥40 years	Chi-square test within each stratum; interaction term in logistic regression
Diabetes Status	Diabetic vs. Non-diabetic	Chi-square test within each stratum; interaction term in logistic regression
Baseline Disease Severity	OSDI ≤32 vs. OSDI >32	Chi-square test within each stratum; interaction term in logistic regression

Note: All subgroup analyses were post-hoc and exploratory. No correction for multiple comparisons has been applied to subgroup analyses; findings are reported with uncorrected p-values and should be interpreted with caution.

8.7 Safety Analysis

- All adverse events will be summarised by treatment arm using frequencies and percentages.
- Adverse events will be tabulated by system organ class and preferred term.
- No formal statistical testing will be performed for safety endpoints given the exploratory nature and expected low event rates.

8.8 Statistical Significance

A two-sided p-value ≤0.05 was considered statistically significant throughout. No adjustment for multiple comparisons was made for secondary outcomes; these should be considered supportive evidence. Subgroup analyses were exploratory and not subject to formal multiplicity correction.

9. Schedule of Assessments

Assessment	Baseline (Week 0)	Week 4 / Week 8	Week 12
Informed Consent	✓		
Eligibility Assessment	✓		
Demographic & Medical History	✓		
Randomisation	✓		
Schirmer Test	✓	✓	✓
Fluorescein TBUT	✓	✓	✓
OSDI Questionnaire	✓	✓	✓
Anterior Segment Examination	✓	✓	✓

Adverse Event Assessment		✓	✓
Concomitant Medication Review	✓	✓	✓
Study Medication Dispensing		✓	✓

Note: 'Week 4 / Week 8' column applies to both interim follow-up visits, each of which was assessed identically.

10. Ethical and Regulatory Considerations

10.1 Ethics Approval

This study was approved by the Institutional Review and Ethics Board (IREB) of Khyber Medical College, Peshawar, Pakistan (Approval No: 579/DME/KMC). The study was conducted in full accordance with the ethical principles of the Declaration of Helsinki (2013 revision).

10.2 Trial Registration

The trial was prospectively registered at ClinicalTrials.gov under identifier NCT06939959 prior to the commencement of participant enrolment, in accordance with WHO and ICMJE registration requirements.

10.3 Informed Consent

Written informed consent was obtained from all participants prior to any study-related procedures. Consent documents were provided in the participant's preferred language. Participants were informed of their right to withdraw from the study at any time without consequence to their clinical care.

10.4 Data Confidentiality

All participant data were recorded using unique numerical identifiers. Personally identifiable information was stored separately from study data in a locked secure facility accessible only to the principal investigator. Data were entered and stored in password-protected electronic databases.

11. Acknowledged Limitations

- Single-centre design: restricts generalisability to other populations and healthcare settings.
- Assessor unmasking: The principal investigator was not masked to treatment allocation, introducing risk of detection bias. This was mitigated by independent measurement verification by a masked colleague clinician, with discrepancies resolved by consensus.
- Absence of validated adverse event instrument: minor or transient events may be under-detected.
- Heterogeneous DED population: inclusion of both diabetic and non-diabetic patients may obscure aetiologically specific treatment responses.
- 12-week follow-up: precludes conclusions regarding long-term durability of treatment effect.
- Absence of inflammatory biomarker assessment (IL-1α, IL-6, MMP-9): limits characterisation of insulin's anti-inflammatory mechanism.

- Post-hoc exploratory subgroup analyses: the study was not powered to detect subgroup differences; results should not guide clinical practice without independent replication.

12. Key References

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