STUDY PROTOCOL WITH STATISTICAL ANALYSIS PLAN

Comparison of TLC (Total Leucocyte Count), Neutrophils and Monocytes in Oral Squamous Cell Carcinoma and Pre-malignant Disorders

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Official Title: Comparison of TLC (Total Leucocyte Count), Neutrophils and Monocytes in Oral Squamous Cell Carcinoma and Pre-malignant Disorders

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1. Document Control

Item	Details
Official study title	Comparison of TLC (Total Leucocyte Count), Neutrophils and Monocytes in Oral Squamous Cell Carcinoma and Pre-malignant Disorders
Study design	Analytical cross-sectional study
Study setting	Department of Oral Surgery, Dow University of Health Sciences, Ojha Campus and CHK
Study duration	Six months after approval from the Ethical Review Committee (ERC)
Target sample	100 participants: 50 OSCC and 50 pre-malignant/OPMD participants
Sampling	Non-probability purposive sampling
Primary outcomes	TLC, absolute neutrophil count (ANC), absolute monocyte count (AMC)
Statistical software	IBM SPSS Statistics version 27.0
Protocol version/date	Version 1.0 / 26 September 2026

2. Study Synopsis

This analytical cross-sectional study will compare selected peripheral hematological parameters—total leukocyte count (TLC), absolute neutrophil count (ANC), and absolute monocyte count (AMC)—between patients with histologically confirmed oral squamous cell carcinoma (OSCC) and patients with histologically confirmed pre-malignant oral disorders/OPMDs. The supplied synopsis describes these parameters as accessible indicators of systemic inflammatory and immune response. The study will recruit 100 participants, equally divided into two groups (50 OSCC and 50 OPMD participants), over six months following ethical approval. Routine CBC records and histopathology reports will be used, with peripheral blood smear examination for validation of differential leukocyte counts.

3. Background and Rationale

Oral squamous cell carcinoma is described in the supplied synopsis as a major public health concern and the most prevalent malignancy affecting the head and neck region. Tobacco use, alcohol consumption and HPV infection are identified as important risk factors. Oral pre-malignant disorders may precede malignant transformation and may be associated with molecular and inflammatory changes.

The study rationale is based on the observation that hematological parameters, particularly TLC, neutrophils and monocytes, may reflect systemic inflammation and immune responses associated with cancer progression. The supplied synopsis cites previous work reporting higher TLC, absolute neutrophil and monocyte counts in OSCC compared with pre-malignant lesions. Comparing these parameters between the two groups may help characterize differences in inflammatory and hematological profiles.

The study therefore aims to compare the mean values of neutrophils, monocytes and TLC in patients with OSCC and patients with pre-malignant conditions.

4. Objectives

Primary objective: To compare the mean value of neutrophils, monocytes and total leucocyte count in oral squamous cell carcinoma and pre-malignant conditions.

5. Study Design and Setting

Design: Analytical cross-sectional study.

The supplied synopsis states that data will be collected at a single point in time to evaluate and compare the selected hematological parameters between the two study groups.

Setting: Department of Oral Surgery, Dow University of Health Sciences, Ojha Campus and Civil Hospital Karachi (CHK).

Study period: Six months after approval from the Ethical Review Committee (ERC), with data collection also subject to the stated CPSP requirements in the synopsis.

6. Study Population

The study population will comprise adults aged 18–70 years with histologically confirmed OSCC or histologically confirmed pre-malignant oral disorders who provide informed consent.

7. Study Groups

- Group 1 (OSCC): 50 participants with histologically confirmed oral squamous cell carcinoma.
- Group 2 (Pre-malignant/OPMD): 50 participants with histologically confirmed pre-malignant oral disorders.

8. Operational Definitions

Term	Operational definition
Oral pre-malignant disorders (OPMDs)	A clinical group consisting of patients with clinically identifiable oral lesions, specifically oral submucous fibrosis, lichen planus, discoid lupus erythematosus and epidermolysis bullosa, with definitive histopathological confirmation as specified in the supplied synopsis.
Oral submucous fibrosis	Clinically characterized by mucosal blanching, loss of tissue elasticity, burning on eating spicy food and progressive restriction of mouth opening (inter-incisal distance <35 mm), with histopathological confirmation by subepithelial collagen deposition, hyalinization and inflammatory infiltrates.
Epidermolysis bullosa	An inherited blistering disorder defined for this study by documented mucocutaneous fragility, chronic oral mucosal vesicles/bullae or scarring, with prior genetic and/or histopathological confirmation.
Discoid lupus erythematosus	A chronic autoimmune condition defined by chronic, well-demarcated oral discoid lesions and

	histopathological confirmation as described in the synopsis.
Oral squamous cell carcinoma	A malignant neoplasm arising from squamous epithelial cells lining the oral cavity, with diagnosis confirmed histopathologically.
Total leukocyte count (TLC)	Total number of white blood cells per microliter of peripheral blood, assessed using an automated hematology analyzer from a 3 mL EDTA venous blood sample collected prior to surgical intervention. Leukocytosis is defined in the synopsis as TLC >11,000/µL.
Absolute neutrophil count (ANC)	Absolute number of mature neutrophils per microliter of peripheral blood, calculated as total WBC count × percentage of neutrophils.
Absolute monocyte count (AMC)	Absolute number of monocytes per microliter of peripheral blood, calculated as total WBC count × percentage of monocytes.

9. Eligibility Criteria

9.1 Inclusion Criteria

- Patients aged 18–70 years.
- Patients with histologically confirmed OSCC or pre-malignant disorders.
- Patients who provide informed consent.

9.2 Exclusion Criteria

- Patients with active systemic infections or inflammatory conditions.
- Patients undergoing chemotherapy or radiotherapy.
- Patients with known hematological disorders.

10. Recruitment and Sampling

Non-probability purposive sampling will be used because recruitment requires participants with specific confirmed diagnoses. The planned sample comprises 100 participants, with 50 participants in each study group.

11. Sample Size Determination

The supplied synopsis states that sample size was calculated using PASS 2021. Monocyte values from the cited study were used: OSCC 3.6122 ± 1.44 and pre-malignant group 3.34 ± 1.30321. A two-independent-sample t-test framework was used with 80% power and a 95% confidence level. The stated calculated minimum sample size was 100 participants (50 per group), with an estimated population size of 50 per group using finite population correction (FPC).

The synopsis additionally cites Jerrold H. Zar, Biostatistical Analysis, Second Edition, page 134, as a book reference for the sample-size/FPC rationale.

12. Study Procedures and Data Collection

Eligible participants will be recruited after fulfilment of inclusion criteria and informed consent. Demographic and clinical variables will be recorded on a structured proforma, including age, gender, occupation, smoking history, alcohol consumption, betel quid/areca nut use, HPV exposure history, duration of symptoms, lesion site, clinical presentation and final histopathological diagnosis (OSCC versus OPMD subtype).

Routine CBC records and histopathology reports from the Department of Surgery, Dow University of Health Sciences, Ojha Campus and Civil Hospital Karachi will be utilized as described in the synopsis.

TLC, neutrophil and monocyte counts will primarily be obtained from automated hematology analyzers as part of routine CBC testing. A peripheral blood smear will additionally be examined for validation. Smears will be stained with Leishman stain, and a qualified pathologist will review the smears and confirm differential leukocyte counts. Differential leukocyte count may also be performed manually.

13. Study Variables and Outcomes

Variable	Type	Source/measurement	Role
Study group (OSCC vs OPMD)	Categorical	Histopathological diagnosis	Grouping variable
TLC	Continuous	Automated hematology analyzer; cells/μL	Primary outcome
ANC	Continuous	Calculated from TLC × % neutrophils	Primary outcome
AMC	Continuous	Calculated from TLC × % monocytes	Primary outcome
Age	Continuous	Clinical record/proforma	Potential confounder
Gender	Categorical	Clinical record/proforma	Potential confounder
Smoking status	Categorical	History/proforma	Potential confounder
Betel quid/areca nut use	Categorical	History/proforma	Potential confounder
Clinical and histopathological diagnosis	Categorical	Clinical/histopathology record	Eligibility/grouping

14. Data Management and Quality Control

Participant information will be recorded on a structured data collection proforma. Laboratory values will be transcribed from routine CBC reports and validated, where applicable, by peripheral smear review. Histopathological diagnosis will be used to confirm eligibility and study-group allocation.

Data should be checked for completeness before analysis. Participant identifiers should be handled according to institutional ethical and confidentiality requirements; the supplied synopsis does not specify a particular coding or anonymisation procedure, so the institution-approved procedure should be inserted before submission.

15. Statistical Analysis Plan (SAP)

15.1 Analysis population

The analysis population will include recruited participants who meet the eligibility criteria and have the required group diagnosis and relevant hematological measurements available. The final handling of missing data should be documented in the analysis report.

15.2 Statistical software

IBM SPSS Statistics version 27.0 will be used for statistical analysis.

15.3 Descriptive analysis

Quantitative variables (TLC, ANC and AMC) will be assessed for distribution. Normally distributed variables will be summarized using mean and standard deviation (SD); non-normally distributed variables will be summarized using median and interquartile range (IQR). Categorical variables, including gender, smoking status and betel quid use, will be summarized using frequencies and percentages.

15.4 Assessment of normality

The Shapiro–Wilk test will be used to assess normality of quantitative outcome distributions. Distributional assessment should also consider the observed data and, where appropriate, graphical inspection.

15.5 Primary between-group analysis

For normally distributed TLC, ANC and AMC values, the independent-samples t-test will compare the OSCC and pre-malignant groups. For non-normally distributed outcomes, the Mann–Whitney U test will be used.

15.6 Categorical variables

Categorical variables will be compared between groups using the Chi-square test. Fisher's exact test will be used where expected cell counts are less than 5.

15.7 Confounding and effect modification

Age, gender, smoking status and betel quid use will be recorded as potential confounding variables/effect modifiers. Their distribution will be compared between groups. The supplied synopsis specifies stratified analysis where significant imbalance is found, to evaluate the effect on TLC, neutrophil count and monocyte count.

15.8 Significance level

A two-sided p-value of ≤0.05 will be considered statistically significant for the planned analyses.

15.9 Missing data and deviations

The supplied synopsis does not specify a formal missing-data imputation strategy or multiplicity adjustment. Before database lock, the investigator/statistician should document the approach used for missing outcome data and whether any deviations from this SAP occurred. Any post-hoc analysis should be identified as such in the final report.

16. Ethical Considerations

The study will be initiated only after approval from the relevant Ethical Review Committee (ERC)/human subjects protection review board, as applicable. Written informed consent will be obtained from participants before inclusion.

The protocol states that routine CBC and histopathology records will be utilized. Blood sampling, where performed for study purposes, should follow the institution-approved consent and safety procedures. Confidentiality of participant information will be maintained according to institutional requirements.

17. Study Duration and Participant Engagement

The overall study data-collection period is six months following ethical approval. The supplied synopsis does not specify an exact duration of individual participant engagement in hours/days. If a clinical-trial registry requires a participant-level duration, this should be entered based on the approved consent/protocol rather than estimated.

18. Dissemination and Data Use

Study findings will be analyzed and reported in accordance with institutional and ethical requirements. Any dissemination should preserve participant confidentiality.

19. Protocol Amendments

Any substantive protocol amendment that affects study conduct, eligibility, procedures, outcomes, or statistical analysis should be documented with its version/date and submitted for ethics review where required. The registry submission should use the most recent human-subjects-protection-board-reviewed version applicable to all study locations.

20. References

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