STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title

Impact of Anemia on Time to Resolution of Diabetic Ketoacidosis and Hospital Outcomes in Children: A Single-Center Retrospective Cohort Study

Brief Title

Hemoglobin Levels and Resolution Time of Diabetic Ketoacidosis in Pediatric Patients

Unique Protocol ID

ADUPEDDKAHB2025

NCT Number

Pending

Sponsor

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Document Date

January 22, 2026

1. Study Design

This is a single-center, retrospective, observational cohort study conducted in a tertiary pediatric emergency department and pediatric intensive care unit.

2. Background and Rationale

Diabetic ketoacidosis (DKA) remains one of the most serious acute complications of diabetes mellitus in children and adolescents. Despite the availability of standardized treatment protocols, the time required for biochemical resolution of DKA varies considerably across patients.

Hemoglobin (Hb) is a major non-bicarbonate buffer in human physiology and plays a critical role in acid–base homeostasis beyond its primary function as an oxygen carrier. Reduced hemoglobin concentration may impair buffering capacity and tissue oxygen delivery, potentially contributing to prolonged metabolic acidosis during DKA. Despite this strong physiologic rationale, the relationship between anemia and time to DKA resolution has not been systematically investigated in pediatric populations.

This study aims to investigate whether lower hemoglobin levels are associated with a longer time to biochemical resolution of DKA in children. In addition, we will standardize hemoglobin by age and sex using hemoglobin z-scores to minimize confounding by physiologic variation across childhood and adolescence.

3. Study Objectives and Hypotheses

Primary Objective

To evaluate the association between hemoglobin levels measured within 0–24 hours after biochemical resolution of diabetic ketoacidosis (DKA) and the time required to achieve

biochemical resolution of DKA. Primary exposure will be Hb z-score; absolute Hb will be assessed in sensitivity analyses.

Secondary Objectives

- To assess the association between post-resolution hemoglobin levels and total hospital length of stay.
- To assess the association between post-resolution hemoglobin levels and pediatric intensive care unit (PICU) length of stay.
- To explore the relationship between admission hemoglobin and hematocrit levels, as surrogate markers of dehydration severity, and clinical outcomes.

Hypotheses

Null Hypothesis (H0):

- Hemoglobin level measured after biochemical resolution of DKA is not associated with the time to resolution of DKA.

Alternative Hypothesis (H1):

- Lower hemoglobin levels measured after biochemical resolution of DKA are independently associated with a longer time to resolution of DKA.

4. Study Population

Inclusion Criteria

- Age 1–18 years
- Diagnosis of DKA according to ISPAD criteria
- First documented DKA episode

- Availability of hemoglobin measurement within 0–24 hours after biochemical resolution of DKA
- Documented time of DKA treatment initiation and biochemical resolution

Exclusion Criteria

- Hyperosmolar hyperglycemic state (HHS) or mixed DKA–HHS
- Known hemoglobinopathies (e.g., thalassemia major, sickle cell disease)
- Evidence of active hemolysis documented in medical records
- Blood transfusion administered before or during the DKA treatment period
- Missing hemoglobin measurement within the predefined post-resolution window

5. Definitions

DKA Resolution

Biochemical resolution of diabetic ketoacidosis (DKA) is defined by the simultaneous achievement of the following criteria:

- Venous pH ≥ 7.30, and
- Serum bicarbonate ≥ 15 mmol/L

Primary Outcome

Time to DKA resolution: Time, measured in hours, from initiation of DKA treatment to biochemical resolution of DKA, as defined above.

Time Frame: From initiation of DKA treatment until biochemical resolution, assessed up to 7 days.

Secondary Outcomes

Total hospital length of stay: Time, measured in days, from hospital admission to hospital discharge.

Time Frame: From hospital admission until hospital discharge, assessed up to 30 days.

Pediatric intensive care unit (PICU) length of stay: Time, measured in days, from PICU admission to PICU discharge.

Time Frame: From PICU admission until PICU discharge, assessed up to 30 days.6.

Data Collection

Data will be extracted retrospectively from electronic medical records using a standardized data collection form. Collected variables will include:

- Demographic characteristics: age and sex
- Admission clinical and laboratory findings: including venous pH, serum bicarbonate, blood glucose, and hematocrit
- Serial blood gas and biochemical measurements obtained during DKA management
- Hemoglobin and hematocrit values measured at admission and within 0–24 hours after biochemical resolution of DKA
- Treatment-related timelines: time of DKA treatment initiation and time of biochemical resolution
- Clinical outcomes: time to DKA resolution, PICU length of stay, and total hospital length of stay

To ensure independence of observations and minimize bias related to recurrent events, only the first documented DKA episode per patient will be included in the analysis.

7. Statistical Analysis Plan

Analysis Population

All eligible pediatric patients aged 1–18 years who meet the inclusion criteria and have available hemoglobin measurements within 0–24 hours after biochemical resolution of diabetic ketoacidosis (DKA) will be included in the analysis. To ensure independence of observations, only the first documented DKA episode per patient will be analyzed. Expected sample size: approximately ~150 patients, based on institutional case volume.

Exposure Definition

The primary exposure variable will be post-resolution hemoglobin standardized as an age- and sex-adjusted z-score (Hb z-score).

Hemoglobin z-scores will be calculated using published pediatric reference values providing mean and lower limit of normal (−2 SD) hemoglobin concentrations across age groups, with sex-specific values applied for adolescents aged 12–18 years. Because the lower limit corresponds to −2 standard deviations, the standard deviation (SD) will be derived as:

$$SD=(Mean - Lower\ Limit)/2$$

$$The\ hemoglobin\ z\text{-}score = (Hb\ measured - MeanHb\ reference) / SD\ reference$$

Anemia will be defined as Hb z-score < −2 in secondary and sensitivity analyses. Hemoglobin z-scores will be calculated using published pediatric reference values (Lanzkowsky, 2022), providing age- and sex-specific mean and −2 SD hemoglobin concentrations.

Outcome Measures

The primary outcome is time to biochemical resolution of DKA, defined as the time (in hours) from initiation of DKA treatment to achievement of venous pH ≥ 7.30 and serum bicarbonate ≥ 15 mmol/L.

Secondary outcomes include pediatric intensive care unit (PICU) length of stay and total hospital length of stay, measured in days.

Descriptive Analysis

Continuous variables will be evaluated for distributional characteristics using histograms and Q–Q plots. Normally distributed variables will be summarized as mean ± standard deviation, while non-normally distributed variables will be summarized as median and interquartile range (IQR). Categorical variables will be presented as frequencies and percentages.

Primary Analysis

The association between Hb z-score and time to DKA resolution will be evaluated using linear regression analysis, with time to resolution as the dependent variable and Hb z-score as the independent variable.

If time to resolution demonstrates right-skewed distribution, logarithmic transformation will be applied. Model assumptions, including linearity, normality of residuals, and homoscedasticity, will be assessed using standard diagnostic procedures.

Multivariable Analysis

To assess the independent association between Hb z-score and time to DKA resolution, multivariable linear regression models will be constructed. Covariates will be selected a priori based on clinical relevance and biological plausibility and will include:

- Admission venous pH
- Admission serum bicarbonate

- Admission blood glucose level
- Admission hematocrit (as a surrogate marker of dehydration/hemoconcentration)
- Presence of suspected infection (based on clinical diagnosis, inflammatory markers, and/or antibiotic initiation)

Because hemoglobin is standardized by age and sex, age and sex will not be included in the primary adjusted model to avoid redundant adjustment; however, age and sex will be added in sensitivity analyses to confirm robustness. Collinearity among covariates will be assessed using variance inflation factors (VIFs).

Secondary Analyses

Associations between Hb z-score and secondary outcomes (PICU length of stay and total hospital length of stay) will be analyzed using regression models appropriate to the distribution of each outcome. If length-of-stay variables exhibit overdispersion or skewness, generalized linear models with appropriate link functions will be considered.

Sensitivity Analyses

Sensitivity analyses will be performed to evaluate the robustness of the findings and will include:

1. Modeling hemoglobin as an absolute value (g/dL) instead of z-score
2. Categorizing hemoglobin status as anemic (Hb z-score < -2) versus non-anemic
3. Including age and sex as covariates in adjusted models despite z-score standardization
4. Excluding patients with extreme hemoconcentration (upper quartile of admission hematocrit)

Missing Data

The extent and pattern of missing data will be evaluated. If missing data account for less than 10% of observations, a complete-case analysis will be performed. If missingness is 10% or greater, multiple imputation using chained equations will be applied under the assumption of missing at random. Imputation models will include the outcome, exposure, and all covariates used in the primary analysis.

Statistical Significance

All statistical tests will be two-sided, and a p-value < 0.05 will be considered statistically significant. Statistical analyses will be conducted using R statistical software.

8. Ethical Considerations

The study will be conducted in accordance with the Declaration of Helsinki. Ethical approval will be obtained from the local institutional review board.

Due to the retrospective nature of the study and the use of routinely collected clinical data, informed consent was waived by the ethics committee.

9. Data Protection

All data will be de-identified prior to analysis and stored securely. Access will be restricted to study investigators.

10. Dissemination Plan

Results will be submitted to a peer-reviewed international journal and presented at national and international scientific meetings