STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title

Febrile Seizures in Children: Association of Thyroxine (T4), Epinephrine, and Norepinephrine Levels With Seizure Characteristics and Hospital Outcomes—A Prospective Controlled Observational Study

Brief Title

Neuroendocrine Response in Pediatric Febrile Seizures

Unique Protocol ID

ADUPEDFSTEN

NCT Number

Pending

Sponsor

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1. Study Design

This is a single-center, prospective, observational case–control study conducted in a tertiary pediatric emergency department.

Participants are not assigned to any intervention. All clinical management follows standard care.

2. Background and Rationale

Febrile seizures are the most common seizure type in childhood and occur in the context of systemic febrile illness. Although generally benign, febrile seizures represent an acute physiological stress event. Seizure activity may activate the sympathetic–adrenal system and influence the hypothalamic–pituitary–thyroid axis, resulting in measurable changes in circulating catecholamines and thyroid hormones.

Despite this physiological rationale, the magnitude and clinical implications of the neuroendocrine response during febrile seizures have not been systematically evaluated. In particular, it remains unclear whether acute hormone levels are associated with seizure duration, recurrence, or short-term hospital outcomes.

This study aims to evaluate the association between acute-phase serum thyroxine (T4), epinephrine, and norepinephrine levels and seizure characteristics and hospital outcomes in children presenting with febrile seizures.

3. Study Objectives and Hypotheses

Primary Objective

To evaluate the difference in acute-phase serum T4, epinephrine, and norepinephrine levels between children presenting with febrile seizures and age-matched febrile children without seizures.

Secondary Objectives

• To assess the association between acute hormone levels and seizure duration.

• To assess the association between acute hormone levels and total hospital length of stay.

• To assess the association between acute hormone levels and pediatric intensive care unit (PICU) admission.

Hypotheses

Null Hypothesis (H0):

Acute neuroendocrine hormone levels do not differ between febrile seizure patients and febrile controls and are not associated with seizure characteristics or hospital outcomes.

Alternative Hypothesis (H1):

Acute neuroendocrine hormone levels differ between febrile seizure patients and febrile controls and are associated with seizure characteristics and hospital outcomes.

4. Study Population

Inclusion Criteria

• Age 6 months–5 years

• Fever documented at presentation or reported within the preceding 24 hours

• Case group: febrile seizure during febrile illness

• Control group: febrile illness without seizure

• Written informed consent obtained from parent/legal guardian

Exclusion Criteria

• Suspected or confirmed central nervous system infection

• Prior diagnosis of epilepsy or history of afebrile seizures

• Seizures attributable to significant metabolic derangement

• Known thyroid disease or thyroid medication use

• Known adrenal disorders

• Major chronic neurologic disorders

• Inability to obtain blood samples within protocol-defined time windows

5. Definitions

Febrile Seizure

A seizure occurring in a child aged 6 months to 5 years associated with fever, without evidence of intracranial infection or other defined causes.

Primary Outcome

Difference in acute-phase serum T4, epinephrine, and norepinephrine levels between febrile seizure patients and febrile controls.

Time Frame: Baseline (during the emergency department presentation)

Secondary Outcomes

Seizure duration (minutes)

Time Frame: Baseline (during the index febrile seizure event)

Total hospital length of stay (days)

Time Frame: From hospital admission through discharge (up to 7 days)

PICU admission (yes/no)

Time Frame: During the index hospitalization (up to 7 days)

6. Data Collection

Data will be collected prospectively using a standardized case report form.

Collected variables include:

• Demographics: age, sex

• Temperature at sampling

• Seizure type (simple/complex)

• Seizure duration (minutes)

• Seizure recurrence

• Treatments administered

• Length of hospital stay

• PICU admission

• Serum T4, epinephrine, norepinephrine levels

In the febrile seizure group, blood samples will be obtained within 30 minutes after seizure cessation during the emergency department visit. In the control group, samples will be obtained during the febrile episode.

7. Statistical Analysis Plan

Analysis Population

All enrolled participants with available acute-phase hormone measurements will be included in the primary analysis.

Planned sample size: approximately 120 participants.

Exposure Definition

Primary exposure variables:

• Serum T4 level

• Serum epinephrine level

• Serum norepinephrine level

Hormone levels will be analyzed as continuous variables.

Outcome Measures

Primary:

Group status (febrile seizure vs febrile control)

Secondary:

• Seizure duration

• Hospital length of stay

• PICU admission

Descriptive Analysis

Continuous variables will be evaluated for distribution using histograms and Q–Q plots.

Normally distributed variables: mean ± SD

Non-normally distributed variables: median (IQR)

Categorical variables: frequency and percentage

Primary Analysis

Acute hormone levels will be compared between groups using:

• Independent samples t-test (if normally distributed)

• Mann–Whitney U test (if non-normal)

Effect sizes will be reported.

Multivariable Analysis

To evaluate independent associations, multivariable regression models will be constructed adjusting for:

• Age

• Sex

• Temperature at sampling

Linear regression will be used for continuous outcomes.

Logistic regression will be used for PICU admission.

Model assumptions will be assessed.

Secondary Analyses

• Correlation between hormone levels and seizure duration

• Regression analysis for hospital length of stay

• Logistic regression for PICU admission

Sensitivity Analyses

Stratification by seizure type (simple vs complex)

Separate evaluation of each hormone marker

Inclusion of treatment exposure variables in adjusted models

Missing Data

The extent and pattern of missing data will be evaluated.

If missing data are less than 10%, complete-case analysis will be performed.

If ≥10%, multiple imputation will be considered under the assumption of missing at random.

Statistical Significance

All tests will be two-sided.

A p-value < 0.05 will be considered statistically significant.

Statistical analyses will be performed using SPSS or equivalent validated statistical software.

8. Ethical Considerations

Ethical approval has been obtained from the Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee (Approval No: 2025/332).

The study will be conducted in accordance with the Declaration of Helsinki.

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 scientific meetings.

Informed Consent Form (ICF)

PLEASE READ CAREFULLY!!!

You are being invited to allow your child to participate in a research study.

Before deciding whether to participate, it is important that you understand

why the research is being conducted and what participation will involve.

Please read the following information carefully and feel free to ask

any questions you may have.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to investigate the relationship between febrile seizures and serum levels of thyroxine (T4), epinephrine, and norepinephrine in children with fever.

Additionally, the study aims to evaluate whether these hormone levels are associated with seizure duration, seizure recurrence, length of hospital stay, and the need for admission to the pediatric intensive care unit.

WHO CAN PARTICIPATE IN THIS STUDY?

Your child may participate in this study if:

Your child is between 6 months and 5 years of age

Your child presents to the pediatric emergency department with fever or a febrile seizure

You provide written informed consent for participation

Your child cannot participate if they have:

A known chronic disease (such as diabetes, heart disease, kidney disease, or chronic lung disease)

A known neurological disorder (such as epilepsy, cerebral palsy, or developmental delay)

Known immunodeficiency or adrenal gland disorders (e.g., congenital adrenal hyperplasia)

WHAT PROCEDURES WILL BE PERFORMED?

During your child's evaluation in the pediatric emergency department, blood tests may be performed as part of routine clinical care.

For the purposes of this study, an additional 2 mL blood sample will be collected at the same time as routine blood sampling.

You may also be asked a few questions about your child's medical history by the research physician.

WHAT ARE MY RESPONSIBILITIES AS A PARTICIPANT?

You have no specific responsibilities related to participation in this research study.

HOW MANY PARTICIPANTS WILL BE INCLUDED IN THIS STUDY?

Approximately 120 children will participate in this study.

HOW LONG WILL THE STUDY LAST?

The total duration of the study is expected to be approximately one year.

HOW LONG WILL MY CHILD PARTICIPATE?

Participation in this study will take approximately 15–20 minutes during the emergency department visit.

WHAT ARE THE POSSIBLE BENEFITS OF PARTICIPATING?

This study may help improve understanding of the biological mechanisms involved in febrile seizures in children. The results may contribute to future research and may help improve clinical management of febrile seizures.

However, your child may not receive a direct medical benefit from participating in this study.

WHAT ARE THE POSSIBLE RISKS?

The risks associated with participation are minimal.

The additional blood sample may cause:

mild pain at the needle site

bruising

rarely, local infection

No other risks are expected.

ARE THERE ANY MEDICATIONS OR FOODS THAT SHOULD NOT BE USED DURING THE STUDY?

There are no medications or foods that are restricted during participation in this study.

UNDER WHAT CONDITIONS MAY MY CHILD BE WITHDRAWN FROM THE STUDY?

Your child may be withdrawn from the study if:

you decide to withdraw consent

required information cannot be obtained

the research team determines participation is no longer appropriate

WILL MY CHILD RECEIVE ANY TREATMENT DURING THE STUDY?

No experimental treatment will be administered as part of this study. All medical care will follow standard clinical practice.

WHAT HAPPENS IN CASE OF HARM?

No harm related to this study is expected. In the unlikely event of a problem, appropriate medical care will be provided.

WHO SHOULD I CONTACT IF I HAVE QUESTIONS?

If you have any questions about the study, you may contact:

Dr. Murat Ayar

Aydın Adnan Menderes University

Phone: +90 506 489 73 72

WILL I HAVE TO PAY FOR ANYTHING?

All examinations, laboratory tests, and procedures related to this research will not result in any cost to you or your insurance provider.

IS THERE ANY PAYMENT FOR PARTICIPATION?
You will not receive any payment for participating in this study.

VOLUNTARY PARTICIPATION

Participation in this research is entirely voluntary. You may refuse to allow your child to participate or withdraw your consent at any time without affecting your child's medical care.

If you withdraw from the study, your child’s medical data will not be used for research purposes.

CONFIDENTIALITY

All personal and medical information collected during the study will be kept confidential. Identifying information will not appear in any publications resulting from this research.

Authorized regulatory authorities and ethics committees may review study records if necessary.

CONSENT STATEMENT

I have read and understood the information provided above. I have had the opportunity to ask questions, and all my questions have been answered. I voluntarily agree to allow my child to participate in this study.

A signed and dated copy of this consent form will be provided to me.

PARENT OR LEGAL GUARDIAN (FOR PARTICIPANTS UNDER LEGAL GUARDIANSHIP)		Signature
Name and Surname:		
Address:		
Telephone & Fax:		
Date:		

Authorized Research Investigator		Signature
Name and Surname:		
Date:		

Witness (if required)		Signature
Name and Surname:		
Position / Title:		
Date:		