Peer Review File

Article information: https://dx.doi.org/10.21037/tp-2025-439

Reviewer A

This interesting paper retrospectively investigates relationship between urine pH, serum phosphate levels and serum ions in patients with infantile epileptic spasms syndrome (IESS) and their response to ACTH treatment. There are concerns:

(1) While the onset of spasms is between 1-24 moths, if it occurs before 3 moths of age, other developmental epileptic encephalopathies (DEE) should be considered.

Reply 1: We agree with the view that spasms occurring before 3 months of age need to be differentiated from other developmental and epileptic encephalopathies (DEE). In the Methods and Discussion sections, we supplemented the specific diagnostic criteria for infantile epileptic spasms syndrome (IESS) with reference to the relevant diagnostic standards of the International League Against Epilepsy (ILAE) to ensure the diagnostic accuracy of the included cases, and elaborated on the results of this part.

Changes in the text: See Page 7, line 98-99. See Page 12-13, line 222-238.

(2) Lines 37-36 require a reference(s).

Reply 2: We have modified our text as advised.

Changes in the text: See Page 4, line 37-38.

(3) The Introduction completely omits smultiple clinical studies of Shaun Hussain and coll., who clearly showed that time to onset of ACTH treatment is critical for outcome.

Reply 3: We have incorporated the research of this team in the section "Factors Influencing the Therapeutic Response to ACTH" within the Introduction, thereby laying a more solid literature foundation for the subsequent research hypotheses.

Changes in the text: See Page 4, line 48.

(4) Lines 46-7, the sentence is unclear.

Reply 4: We have modified our text as advised.

Changes in the text: See Page 4, line 44-48.

(5) Acid-base balance can significantly affect seizure susceptibility with alkaline pH being proconvulsant and acidic pH having anti-seizure effects. Please look up Aram and Lodge, early studies of Traynelis, also early Velisek.

Reply 5: We have reviewed the relevant literature and added the corresponding content.

Changes in the text: See Page 4-5, line 52-54.

(6) Line 60, unclear O’Callaghan…

Reply 6: We have modified our text as advised.
Changes in the text: See Page 5, line 63-64.

(7) Line 63, "factors assessing disease prognosis, efficacy of treatment, etc" are commonly called biomarkers. The authors should introduce the term and use it throughout the manuscript.
Reply 7: We have modified our text as advised.
Changes in the text: See Page 4, line 48.

(8) Looking on the Introduction and authors' buildup leading to hypothesis – it may be possible that ACTH-induced pH adjustment is the culprit in its anticonvulsant effects in the IESS.
Reply 8: We would like to thank the reviewing experts for their in-depth interpretation and supplementation of the research hypotheses. This speculation is highly consistent with our research direction.

(9) In the inclusion criteria, the authors should clearly restate the diagnostic criteria of IESS used in this particular study.
Reply 9: We have modified our text as advised.
Changes in the text: See Page 6, line 81-83.

(10) It is very difficult to speak about patient gender (a psychosocial construct) at the age of infantile spasms. Sex is much better term here.
Reply 10: We have modified our text as advised.
Changes in the text: See Page 6, line 92.

(11) Line 89, the reviewer doubts that the patients were able to record anything – caregivers maybe, health care providers, possibly.
Reply 11: We have modified our text as advised.
Changes in the text: See Page 6, line 96-97.

(12) Lines 92-93 should be explained better as per the comment #1.
Reply 12: We agree with the view that spasms occurring before 3 months of age need to be differentiated from other developmental and epileptic encephalopathies (DEE). In the Methods and Discussion sections, we supplemented the specific diagnostic criteria for infantile epileptic spasms syndrome (IESS) with reference to the relevant diagnostic standards of the International League Against Epilepsy (ILAE) to ensure the diagnostic accuracy of the included cases, and elaborated on the results of this part.
Changes in the text: See Page 7, line 98-99. See Page 12-13, line 222-238.

(13) Regarding ACTH treatments, US are using practice parameters that did not find any difference between high-and low- dose of ACTH treatment. Hence, low-dose ACTH is currently preferred unless corticosteroids are used.

Reply 13: We have reviewed the relevant literature and revised the content accordingly.
Changes in the text: See Page 7, line 111-112.

(14) Natural sources of ACTH should be specified here, not only referenced. Is there any carrier for the ACTH (hydroxy methylcellulose, gelatin, or anything else?).
Reply 14: We have modified our text as advised.
Changes in the text: See Page 7, line 114-118.

(15) The ACTH treatment paradigm should be described in detail.
Reply 15: We have modified our text as advised.
Changes in the text: See Page 7, line 106-107.

(16) Better explanation is needed for the group of "acquire structural abnormalities". Were those head traumas?
Reply 16: In this study, acquired structural abnormalities mainly refer to those caused by conditions such as infection and hypoxic-ischemic encephalopathy (HIE), and do not include factors related to traumatic brain injury.

(17) It would be important to describe, what was the first seizure type in children not presenting with spasms as the first seizure.
Reply 17: Given that the first seizure of children with the condition typically does not occur in a hospital setting, we can only determine whether it is a focal or generalized seizure based on the caregiver's description. Detailed classification is not possible, so these seizures are collectively referred to as non-spasmodic seizures.

(18) Line 154: First spasm or first seizure? What was the lead time in either group?
Reply 18: We have modified our text as advised.
Changes in the text: See Page 10, line 171-174.

(19) Lines 157-159 – was there a significant difference? If yes, please state so and provide the appropriate statistics.
Reply 19: We have modified our text as advised.
Changes in the text: See Page 10, line 177-178.

(20) Lines 160-162 – please provides average/median pH levels for both groups.
Reply 20: We have modified our text as advised.
Changes in the text: See Page 10, line 179-180.

(21) Lines 164-165 may indicate that the early occurrence of seizures/spasms might have been linked to a different DEE.
Reply 21: In the and Discussion sections, we supplemented the specific diagnostic

criteria for infantile epileptic spasms syndrome (IESS) with reference to the relevant diagnostic standards of the International League Against Epilepsy (ILAE) to ensure the diagnostic accuracy of the included cases, and elaborated on the results of this part.
Changes in the text: See Page 7, line 98-99. See Page 12-13, line 222-238.

(22) What was the positive outcome for ACTH treatme – an electroclinical resolution? Please be as specific as possible.
Reply 22: We have made detailed supplements to the relevant content.
Changes in the text: See Page 8, line 133-136.

(23) Lines 170-171 – Please see Hussain papers (above #3).
Reply 23: We have incorporated the research of this team in the section "Factors Influencing the Therapeutic Response to ACTH" within the Introduction, thereby laying a more solid literature foundation for the subsequent research hypotheses.

(24) Also lines 170-171, please identify the relationships: e.g., lower urine pH were associated with better outcome…..
Reply 24: We have modified our text as advised.
Changes in the text: See Page 11, line 189-195.

(25) Line 181 OR, not 0R.
Reply 25: We have modified our text as advised.
Changes in the text: See Page 11, line 203.

(26) Lines 178-179 – how did the pH convert AFTER treatment?
Reply 26: Due to objective reasons, we were unable to obtain urine samples after treatment and only analyzed the relationship between pre-treatment urine pH value and therapeutic efficacy. Furthermore, previous studies have shown that ACTH treatment can increase the urine pH value.
Changes in the text: See Page 15, line 281-283.

(27) In the Discussion, a special care should be focused on the references as they are non-standard.
Reply 27: We have modified our text as advised.

(28) Line 233 clearly deserves a reference.
Reply 28: We have modified our text as advised.

(29) This reviewer do not think that the temporal lobe epilepsy has anything in common with IESS (line 246).
Reply 29: We have modified our text as advised.

(30) Lines 261-263 deserve appropriate references, see above #5.

Reply 30: We have modified our text as advised.
Changes in the text: See Page 14, line 273.

(31) The Discussion should be easily shortened by ½ page.
Reply 31: We have modified our text as advised.

Reviewer B
This is a study that analyzes a significant number of patients, which adds relevance to its findings. We propose the following modifications to address some limitations:

1. Efficacy was not assessed using Video-EEG, so subclinical seizures may have gone undetected. It would also be of interest to evaluate changes in EEG patterns. It should be verified whether any patients underwent a VEEG follow-up after one month.
Reply 1: We collected video electroencephalograms (vEEG) on the 7th and 14th days of treatment. However, due to objective reasons, we were unable to collect the electroencephalogram (EEG) one month after treatment, and this has been explained in the Limitations section.
Changes in the text: See Page 16, line 308-309.

2. The use of different methods for assessing urinary pH reduces the reliability of the results.
Reply 2: The determination of urine pH value in this study was conducted using the colorimetric method, and reagents from the same batch were used throughout the process to ensure the reliability of the data. Furthermore, this study is a retrospective study, so it is not possible to further supplement the experimental content.

3. A table should be included describing the etiologies, both structural and genetic.
Reply 3: The etiologies of Infantile Spasms Syndrome (IESS) are diverse, with more than 200 types identified. Disclosing specific etiologies has no significant impact on the results of this study, and the number of cases corresponding to certain individual etiologies may be extremely small. Additionally, our data is currently undergoing further exploration, and detailed elaboration will be provided in subsequent research.

4. Language should be improved (e.g., use "novel avenue", and "intellectual disability" instead of "mental retardation", etc.).
Reply 4: We have modified our text as advised.

5. The main limitation lies in the hypotheses presented in the conclusions. It is not clearly explained why lower phosphorus levels are associated with a better response, especially when the discussion states that hypophosphatemia is linked

to a higher risk of seizures. Similarly, regarding urinary acidification levels: although the study suggests that alkalosis is associated with better response, this contradicts what is reported in the existing literature.
Reply 5: We have re-reviewed the relevant literature and provided explanations for this part of the content.
Changes in the text: See Page 14-15, line 261-285.

Reviewer C

The retrospective study included 186 children with IESS who received ACTH treatment, aiming to evaluate short-term outcomes. The authors found that a shorter time lag to treatment was associated with a better response to ACTH. Additionally, they observed that the responsive group had lower pre-treatment serum inorganic phosphate and higher urinary pH levels. Although these findings seem interesting, it is difficult to determine whether they are co-incidental or truly associated with IESS. The authors noted this point in the limitations section.
I have the following comments:

Major points:
Did the authors have access to post-treatment phosphorus and urinary pH levels? If so, please analyze the results of post-treatment phosphorus and urinary pH in both groups.
Reply: Due to objective reasons, we were unable to obtain urine samples after treatment and only analyzed the relationship between pre-treatment urine pH value and therapeutic efficacy. Furthermore, this study is a retrospective study, so it is not possible to further supplement the experimental content.

Did they conduct post-treatment EEG to determine the electroclinical response? This is important because approximately 30% of children with IESS who show no clinical epileptic spasms could still have subtle ongoing epileptic spasms detectable on a post-treatment EEG.
Reply: We collected video electroencephalograms (vEEG) on the 7th and 14th days of treatment. However, due to objective reasons, we were unable to collect the electroencephalogram (EEG) one month after treatment, and this has been explained in the Limitations section. Furthermore, this study is a retrospective study, so it is not possible to further supplement the experimental content.

Minor points:
The manuscript should be reviewed by a native English speaker for fluency and clarity. Please use “epileptic spasms” instead of just “spasms” to ensure consistency with the ILAE classification throughout the manuscript.
Reply: We have modified our text as advised.

Other comments:

Abstract

Line 13 no-responsive group according to the clinical outcomes after treatment. It seems there is a typo with “no-responsive group”. It should be “non-responsive group”.

Reply: We have modified our text as advised.

Changes in the text: See Page 2, line 11.

Line 17-18 Additionally, more patients experienced spasms after the age of 3 months. This sentence is unclear. Please clarify what the authors mean? I suggest rewriting the results section of the abstract for better clarity.

Reply: We have modified our text as advised.

Changes in the text: See Page 2, line 15-17.

Conclusion: The age of spasm onset and its etiology may help predict the efficacy of ACTH treatment. The abstract’s result does not mention etiology. Please revise the abstract to reflect this. Also, feel free to use IESS and ACTH in this section, as you have already defined these abbreviations earlier.

Reply: We have modified our text as advised.

Changes in the text: See Page 2, line 17-19.

Introduction

Glucocorticoids, adrenocorticotropic hormone (ACTH), and vigabatrin (VGB) are the first-line therapeutic agents for IESS. The current consensus recommends ACTH as the preferred first-line therapy for children with IESS.

I disagree with this statement. Hormonal therapy (either ACTH or prednisolone) and vigabatrin are the first line treatments for IESS, with hormonal therapy being preferable for non-TSC associated with IESS. Please revise this section.

Few studies on long-term outcomes showed that only a quarter of patients achieve a favorable long-term cognitive outcome, while a third of children remain simply seizure-free.

I suggest removing “simply”.

Reply: We have modified our text as advised.

Changes in the text: See Page 4, line 37-38.

However, not all factors ACTH treatment-related can be explained based on the existing studies. I think this sentence is unclear. Please expand on this point for better clarity.

Reply: We have modified our text as advised.

Changes in the text: See Page 4, line 44-48.

O'Callaghan FJK's showed that higher serum calcium was significantly associated with a favorable ACTH response and positive long-term outcomes. The ICISS trial led by O’Calllaghan did not mention serum calcium levels. Please check this reference and revise as needed.

Reply: We have modified our text as advised.
Changes in the text: See Page 5, line 63-64.

On the one hand, ACTH can influence serum Ca2+ levels in children with IESS. On the other hand, serum Ca2+ levels play an important role in IESS treatment with ACTH as a factor for assessing disease prognosis.
Please rewrite these statements for better clarity.
Reply: We have modified our text as advised.
Changes in the text: See Page 5, line 64-67.

Methods
The general development of IESS patients before the onset of epilepsy was effectively assessed before the consultation, considering any form of developmental lag in mental or motor functioning as abnormal.
This should be specific about the methods used to assess developmental delay. Did they use a formal test or any tools?
Reply: In China, regular child health check-ups are conducted at fixed intervals, during which experienced physicians assess children's developmental milestones. We used the results of the last check-up before the child's visit as the basis for our analysis. Regrettably, there is currently no unified assessment scale available in this context.

All patients were treated with slow intravenous ACTH infusion once daily for 2 weeks at ≥ 2.5 U/kg/day. I wonder that all patients received IV ACTH as in-patient or out-patient basis.
Reply: All patients completed their treatment during hospitalization.
Changes in the text: See Page 7, line 107.

Statistical analysis
While “multivariate” and “multivariable” analysis are often used interchangeably, it’s important to consider how many outcomes are included in the model. In this study, there was a single outcome (response to ACTH treatment) used to identify any variables associated with the outcome. I suggest using the term “multivariable” regression model.
Reply: Thank you for your professional clarification on the distinction between "multivariate" and "multivariable" analysis. This point has helped us correct the potential ambiguity in terminology use. We fully agree with your suggestion: since our study focuses on a single outcome (response to ACTH treatment) and aims to identify variables associated with it, the term "multivariable" regression model is more accurate and rigorous. In the revised manuscript, we will uniformly revise the relevant terminology to "multivariable" regression model to ensure consistency with the study design and academic expression standards. We greatly appreciate this valuable guidance, which has further improved the precision of our manuscript.

Results
The first seizure type was not a seizure type of epileptic spasm in 36 children (19.4%).
I suggest rewriting this as "In 36 (19.4%) children, other seizure types were present before epileptic spasms."
Reply: We have modified our text as advised.
Changes in the text: See Page 9, line 159-160.

and 145 children (14%) had a combination of other seizure forms -> I am not sure what the authors mean "a combination of other seizure forms" please clarify it.
Reply: Given that the first seizure of children with the condition typically does not occur in a hospital setting, we can only determine whether it is a focal or generalized seizure based on the caregiver's description. Detailed classification is not possible, so these seizures are collectively referred to as non-spasmodic seizures.

The paragraph begins "The outcome of ACTH treatment was assessed by one-way logistic regression analyses, showing significant associations for several independent factors, including…."
This paragraph is a key finding of the paper, and I recommend rewriting it for improved readability and coherence.
Reply: We have modified our text as advised.
Changes in the text: See Page 11, line 188-195.

Discussion
Line 214 early-onset epileptic encephalopathy, such as Ohtahara syndrome, Early Myoclonic Encephalopathy (PME),.. -> This appears to be a typo of EME not PME?
Reply: We have modified our text as advised.

The paragraph begins with, "One of the characteristic electroencephalogram (EEG) patterns of IESS is hypsarrhythmia…" This section is interesting and may relate to their findings regarding phosphate and urine pH. However, a rewrite for better clarity and flow is recommended.
Reply: We have modified our text as advised.
Changes in the text: See Page 14-15, line 261-285.

Overall, the discussion regarding phosphate and urine pH is well-presented. The authors also noted the limitations of these findings. I suggest rewriting this section more concisely and precisely to make it easier for readers to follow.
Reply: We have re-reviewed the relevant literature and provided explanations for this part of the content.
Changes in the text: See Page 14-15, line 261-285.

Tables

I recommend removing the “Statistic column” in table 1 for improve readability. Additionally, please revise and remove underscores __ from the variable names in Tables 1-3. For example, “Treatment_age” should be “age of treatment” and “Age_of_seizure_onset” should be “Age of seizure onset”, etc.
Reply: We have modified our text as advised.