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Re: revision JCTR-D-15-01121

Dear Dr. van Golen,

Thank you for giving us an opportunity to resubmit a revised version of our manuscript entitled "Epidemiological factors contributing to hyponatremia following traumatic brain injury."

We have addressed all comments of the reviewers using the track changes function in Word (attached as supplementary material not for publication). Moreover, every modification or rebuttal of the reviewer's comments is detailed per comment below in red italics.

We are grateful for the useful comments of the reviewers, as a result of which the paper has been considerably improved.

On behalf of the authors, kindest regards,

Michal Heger

REVIEWER COMMENTS

Reviewer #1:

The authors have attempted to tackle an interesting clinical question, namely identifying some of the epidemiologic risk factors associated with hyponatremia in the setting of traumatic brain injury. This is an interesting Clinical Investigation, but there are some concerns as to the methodology used and the conclusions reached. The manuscript could also benefit from some small grammatical and syntax improvement.

We are grateful for your commentary and suggestions, which we have addressed to the fullest extent as indicated below for every one of your comments. The language and terminology has been further polished in accordance with your suggestions.

Abstract:

The Background needs to be more robust. It should include 2 more sentences: 1 relating to the general epidemiology of TBI and another relating to the incidence of hyponatremia in these patients as found in the literature (already included in the main Introduction).

We have changed the Background of the abstract and added new information to the Introduction accordingly:

"Traumatic brain injury (TBI) is a common injury treated at the neurosurgery department. The incidence is 3‰ and the mortality rate is 25%. The incidence of hyponatremia following TBI is 33%. Hyponatremia is one of the main causes for disability and/or death in TBI patients. This study investigated the epidemiology of hyponatremia following TBI."

The Results should include P values for all of the multivariate factors which the authors examined, both those that were significantly associated with hyponatremia and those that were not.

The present study observed that the age ($p>0.05$), gender ($p=0.347$), and whether or not the patient undergoes craniotomy ($p=0.492$) are not correlated with the occurrence of hyponatremia. The type of injury ($p=0.031$), Glasgow score lower than 8 ($p<0.001$), the incidence of hydrocephalus ($p<0.01$) and basal skull fracture ($p<0.001$) are correlated with the occurrence of hyponatremia.

The missing statistics were added to the text.

Introduction:

Please include a working hypothesis at the end of the Introduction for what the authors believed they would find in their analysis, and justification for such hypothesis.

The following text was added to the last paragraph of the introduction:

"We hypothesized that age, gender, type of injury, Glasgow score after the injury, and whether or not basal skull fracture had occurred are related to the occurrence of hyponatremia after brain injury."

The justification for the hypothesis is simply that at this point we do not know what the interconnectedness is between these variables and hyponatremia. That is why a retrospective study is the most suitable approach.

Materials and Methods:
I do not see any comment related to IRB or Ethics Board approval for undertaking this retrospective data gathering and analysis. Please include a statement as to whether this was done, or a waiver of such approval was granted.

The following IRB statement was added to the text:

"The study was approved by the institutional review board and medical ethics committee of the First Affiliated Hospital, Henan University of Science & Technology on 20 August 2009 under professional license number 20064111041030519751014965 4."

Moreover, a copy of the approval letter has been included for verification purposes.

Was the determination of hyponatremia based on a single blood draw during the first week of hospitalization or on multiple blood draws? Please be more specific as to how hyponatremia was determined.

The text was modified accordingly:

Blood samples were collected according to the hospital's standard blood collection procedure for patients with TBI. Blood was drawn three times from each patient, namely on the day of admission, on the third day after admission, and on the seventh day after admission. The hyponatremia diagnosis was made when the serum sodium level was lower than 135 mmol/L. More specifically, a serum sodium level of 130-135 mmol/L was considered mild hyponatremia, 120-130 mmol/L was considered moderate hyponatremia, and <120 mmol/L was considered severe hyponatremia. Serum sodium levels were determined by routine clinical chemistry.

The SIADH diagnostic criteria were: serum sodium level of <130 mmol/L, plasma osmolality of <270 mmol/L, a ratio of urine osmolality:plasma osmolality of >1, urinary sodium levels of >20 mmol/L or >80 mmol/24 h, no heart-, liver-, kidney-, adrenal-, and thyroid dysfunction, no skin edema or ascites, no blood pressure decrease, and no dehydration or other signs of hypovolemia.

The CSWS diagnostic criteria were: serum sodium level of <130 mmol/L when the salt intake was at a normal level, blood volume of <70 mL/kg body weight, urinary sodium of >20 mmol/L or >80 mmol/24h, increase in plasma ANP, and no heart-, liver-, kidney-, adrenal-, and thyroid dysfunction.

The authors admit that their selection of factors to examine in their logistic regression model were somewhat arbitrary. Can they give any more justification for choosing these specific factors (age, gender, injury, GCS, surgery, edema, basilar skull fracture)? Why not include weight, pre-injury sodium level, pre-injury diuretic use?

For traumatic brain injury patients, their age, gender, post-injury Glasgow score, type of injury, cerebral edema, whether basal skull fractures and other factors are often used in clinical practice. The information is objective and accurate. The weight of the patients is not routinely checked. The serum sodium level and the medical history before the injury occurred are out of our reach because the patients or their family could not provide accurate information.

Statistical analysis should be confirmed as adequate by a reviewing statistician.

This task is for the editor to decide on - we conducted the statistical analysis with the help of our in-house statistician.

Results:
The Table 1 Labels are unnecessary.

Changed accordingly, thank you.

Please include 2 additional Tables summarizing the 2nd and 3rd paragraphs of the Results (causes of hyponatremia, symptoms of hyponatremia).

Changed accordingly, thank you.

Discussion:
Page 9, 3rd paragraph: Please delete this paragraph, which is a review of basic SIADH, CSWS, and DI mechanisms

Changed accordingly, thank you.

Page 10, "Study Limitations": The first limitation that should be mentioned is the retrospective nature of this study, which lends itself to recorder and observer bias and incomplete or inaccurate data. The second limitation should be the arbitrary, and possibly incomplete, factors analyzed in the multivariate logistic regression model.

We have added the following limitations to the already existing list of limitations:

"The first limitation was the retrospective nature of the study. Although retrospective studies have advantages (e.g., analysis of multiple outcomes, suitability for addressing conditions with relatively low incidence), there are also disadvantages, the most prominent being selection bias and misclassification as a result of the retrospective processing of information."

"Finally, the factors analyzed in the multivariate regression analysis could be more expansive. However, we deliberately chose not to include more factors because these were either missing, incomplete, or unreliable (e.g., weight, pre-injury sodium level, pre-injury diuretic use) or too complex to derive on a retrospective basis because 1) the site of brain injury can be one or more than one location, or even comprise diffuse damage; 2) the extent of the injury for every site can be different; and 3) the manifestation of damage is complicated (epidural hematoma, subdural hematoma, intracerebral hematoma, brain contusion, or even the coexistence of multiple injuries)."

Reviewer #2:

Dear Editor,
I have dual impression about this paper. Very good statistic methods and poor clinical significance are combine in this manuscript. But and level of study correspond to retrospective analysis.

The claim that the clinical significance is poor is rather debatable, which is best illustrated by the comments of the other two reviewers ("this is an interesting clinical investigation" and "it is an important and relatively understudied topic").

With respect to the comment about the retrospective nature of our study, many valuable case-control studies, such as Lane and Claypon's 1926 investigation of risk factors for breast cancer, were retrospective investigations. However, we certainly do admit that most sources of

error due to confounding and bias are more common in retrospective studies than in prospective studies. For this reason, we have indicated in the text that the retrospective nature of our study is one of the shortcomings of the study (in line with the final suggestion of reviewer 1).

Yes, this is interesting to see strong relationship between the occurrence of hyponatremia and a GCS score < 8, presence of brain edema, and/or a basal skull fracture, statistically. But some brain damage with Hypothalamo-pituitary axis impact (edema, traumatic and aSAH, ischemia, hemorrhage and any shift of brain axis) will led to water-electrolyte disorders.

We have included this as a possible mechanistic explanation in the Discussion:

"Secondly, some forms of brain damage with hypothalamo-pituitary axis impact (e.g., edema, traumatic and aneurysmal subarachnoid hemorrhage, ischemia, and any shift in the direction and degree of the brain) will lead to water-electrolyte disorders."

Electrolyte level, onset time of disturbances, polyuria (presence/absence), edema, cerebral vasospasm (presence/absence), ICP/ CPP monitoring, central haemodynamic - these aspects need to full clinical picture for neurosurgical patients. I think that clinical part was not clarify.

This is a retrospective study. Due to the limitations of the hospital and medical care conditions at the time the patients were admitted, some of the monitoring methods such as angiography, intracranial pressure and cerebral perfusion pressure monitoring, and blood and brain dynamics could not be routinely performed. Therefore, we could not use these parameters in the study. Evidently, we are not proud of this, but at this point we can do no more than to accept our reality and work as well as possible within all possible boundaries. Nevertheless, we certainly acknowledge that these detection methods are very necessary and important for the diagnosis and treatment of patients with traumatic brain injury. Accordingly, we will conduct a more thorough study in the future if the hospital's conditions permit.

page 4. Glasgow coma scale scoring: this is one time point (within 1 hour after admition) - please clarify.

The patients that were included in the present study were admitted to the hospital within 1 h after the injury had occurred. Upon admission we assessed the Glasgow coma scale scoring of the patients and followed up according to the prognosis. Accordingly, the Glasgow coma score reflects the status of the patients upon admission to the hospital, which occurred within 1 h of the injury.

page 5. Results: 1) Hyponatremia caused by inadequate intake of sodium - is really great problem for our patients (29)??? 2) Strong diuretic therapy - please clarify. 3) frequency of CSWs and SIADH 21/6 - please confirm. 4) only «9 polyuric patients» - please confirm.

With respect to the use of diuretics, severe traumatic brain injury is often accompanied by high intracranial pressure. Based on the condition we would use mannitol plus furosemide to dehydrate and reduce intracranial pressure. This has been clarified in the manuscript.

Many patients also experience nausea, vomiting, poor appetite, which can all cause low intake of sodium, and hence hyponatremia.

To confirm: 6 cases of CSWs, 21 cases of SIADH, 9 cases of polyuria were observed during this study.

page 6. Treatment of hyponatremia: «Patients with CSWS were given blood transfusion?» - please clarify.

The main clinical manifestations of cerebral salt wasting syndrome are: hyponatremia, increased urinary sodium, and low blood volume. Severe brain injury patients often experience varying degrees of blood loss. Therefore, for treatment, the blood transfusion is often first applied to correct the hypovolemia, and thereafter hypertonic saline supplementation to correct the hyponatremia. This has been clarified in the manuscript.

page 7. Discussion: Why did you chose these clinical point for analysis such as age, gender, type of injury, surgery?

The purpose of this study was to investigate the risk factors for hyponatremia following severe traumatic brain injury, and the patient's age, gender, type of injury, surgery are all very important factors.

page 8. Mechanisms of Hyponatremia: please confirm and change First and Second your mechanisms, first - may delete.

The first mechanistic description has been deleted.

page 10. Treatment: Did you use vaptans therapy for SIADH groups?

Vaptans belong to the class of selective non-peptide arginine vasopressin (AVP) receptor antagonists. We did not use this class of drugs to treat SIADH.

Reviewer #3:

This is a retrospective analysis of TBI patients, identifying several features associated with the development of hyponatremia in this population. It is an important and relatively understudied topic, but there are some points that I would like to raise below.

Thank you very much for the useful comments.

Major points:

1. This is a retrospective study. In the methods section it is not clear if, for the purposes of this study, the authors reviewed the CT scans again to identify features such as basal skull fracture. Reviewing the CT scans again for this study rather than relying on for example a database of CT reports collected at the time of the patient's admission would improve the quality of the manuscript and I suggest that if this was not done, that the authors do it.

Because this is a retrospective study, we collected all of the patient's imaging data, including CT scans, and all the imaging data was analyzed.

Accordingly, we added the following text:

"The inclusion and exclusion parameters were derived from the medical database of the patients and TBI features (e.g., basal skull fracture) were reconfirmed by evaluating the patients' CT scans."

We specifically requested specialists (associate professors in related departments) to assist with the analysis.

2. Results section, page 5, second paragraph: How were the diagnoses of CSW, SIADH, etc. arrived at? What diagnostic criteria were used? I note the statement on page 6: "Patients with persistently low serum sodium levels were tested for SIADH and CSWS". This needs elaboration. Patients may in theory go from SIADH to CSW during the course of their illness. Was this explored?

As suggested by the reviewer, we have specified the criteria for the diagnosis of CSWS and SIADH in the text:

"The SIADH diagnostic criteria were: serum sodium level of <130 mmol/L, plasma osmolality of <270 mmol/L, a ratio of urine osmolality:plasma osmolality of >1, urine sodium levels of >20 mmol/L or >80 mmol/24 h, no heart-, liver-, kidney-, adrenal-, and thyroid dysfunction, no skin edema or ascites, no blood pressure decrease, and no dehydration or other signs of hypovolemia.

CSWS comprises hyponatremia and low blood volume that develop during the progression of intracranial lesions due to the loss of sodium (via renal clearance) and edema. The CSWS diagnostic criteria were: serum sodium level of <130 mmol/L after regular salt intake, blood volume of <70 mL/kg body weight, urinary sodium of >20 mmol/L or >80 mmol/24h, increase in plasma ANP, and no heart-, liver-, kidney-, adrenal-, and thyroid dysfunction."

This study focused on the risk factors in patients with severe traumatic brain injury followed by hyponatremia, whereby the diagnostic criteria were based on the standard parameters as described in literature. In addition, we did not observe SIADH developing to CSWS in our patient population (information added to text).

3. Did the authors look at the time course of the hyponatremia? Did any factors predict the length and/or severity of hyponatremia?

In this study there was no systematic monitoring of the time course of hyponatremia, but we observed that due to lack of use of diuretic drugs or sodium intake caused by hyponatremia lasted shortly with proactive correction (treatment): usually about 1 week. SIADH and SCW lasted longer, some up to six months.

4. Did the authors only look at initial CT scans? Cerebral edema may develop after the initial CT scan, particularly if it was performed very early in the course of the patient's condition. This is a potential weakness of the study if this is the case.

Patients in this study were subjected to the head CT when admitted to hospital, and rechecked 12 hours after hospitalization.

5. Could the patients be further delineated into e.g. subdural hematoma, extradural hematoma, contusions, ICH, diffuse axonal injury, etc. I appreciate the numbers will not be large. At the very least it would be useful to know exactly what forms of TBI the patients in this study had i.e. how many extradural hematoma, diffuse axonal injury patients, etc.

The main purpose of this study was to discuss the risk factors for hyponatremia after severe brain injury. The type of brain injury is just one of the factors, so the relationship between the type of traumatic brain injury and hyponatremia were not further discussed.

6. The authors describe no attempt to correlate the presence and severity of hyponatremia with outcomes. Do they have data on e.g. 30-day mortality rates?

Hyponatremia is a pathological process following severe traumatic brain injury. The prognosis of patients with severe brain injury depends on many factors such as the extent of brain injury, the extent of secondary pulmonary infection, whether there is intracranial infection, nutritional support, etc. For this study, we did not collect the relevant information of the mortality after 30 days of hyponatremia.