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Hypocalcaemia as a Prognostic Factor of Mortality and Morbidity in Moderate and severe Traumatic Brain Injury and its role with Protein S-100b

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Abstract

Introduction: The effects of traumatic brain injury (TBI) can result in severe disability or death and have an important social and economic impact. Its annual direct and indirect costs amount to roughly 2.5 billion euros. Our objective was to evaluate whether hypocalcaemia of serum ionized calcium (defined as <1.10 mmol/L (4.5 mg/dL) is a prognostic factor for mortality and morbidity (defined as GOS ≤ 3) in early moderate and severe TBI.

Material and methods: Prospective study from January 2014 to December 2015. Patients were between 16 and 87 years old and had a Glasgow Coma Scale (GCS) of 3-13 points following TBI, with demonstrable intracranial lesions in cranial computed tomography (CT).

Results: We recruited 61 patients who suffered moderate and severe TBI with a median age of 42 years old (range: 17 to 86). Forty-three (70.50%) male 18 (29.50%) female. Twenty-one (34.42%) patients had a GOS ≤ 3 and 40 (65.58%) a GOS >3. We found a significant statistical difference (0.009) in ionized serum calcium, Protein S-100b (0.002), IL 6 (0.007) and Haemoglobin (0.011) on day three of admission between GOS ≤ 3 and >3 (disability/death). The best logistic regression model included: age, absent pupillary reactivity, hypocalcaemia of ionized serum calcium (<1.10 mmol/L), Protein S100 b and IL6 on day three.

Conclusion: Hypocalcaemia looks like being an epiphenomenon of several factors. It seems to play a role as prognosticator, however not as leverage point for therapy.

Keywords: Depression; Neuro-feedback; Level of attention

Introduction

The effects of traumatic brain injury (TBI) can result in severe disability or death [1,2] and have an important social and economic impact [1]. The annual direct and indirect costs of TBI amount to roughly 2.5 billion euros [1,3]. MRI performed within the first 8 days after head injury was found to be a reliable predictor of death and moderate/severe disability for patients in coma and on ventilation following TBI, depending on the location of the lesion [4]. Apart from imaging signs, other easy-to-assess early predictors of outcome are lacking. As such, there is an on-going effort to identify biological markers that are closely related to clinical symptoms in order to better predict the outcome after TBI.

The recent adoption of high throughput technologies and a change in focus from the identification of single to multiple markers has fostered new optimism in this direction [5-7]. Different markers have been studied, particularly bivalent cations such as magnesium (Mg^{2+}) and calcium (Ca^{2+}) [5-7]. Of these, calcium seems to play a more important role in TBI [5,8]. A study performed in 2010 compared statins (cholesterol-lowering drugs) as inductors of an anti-inflammatory effect, promoting recovery after moderate/severe TBI. The results showed that patients with a lower serum calcium level on day 3 died earlier than those patients who had a normal serum calcium level on day 3 after TBI. This led to the development of an ambispective study including a total of 122 Mexican patients suffering from moderate/severe TBI, which showed that hypocalcaemia on day 3 seemed to be a reliable predictor of mortality after TBI, reaching significant levels (P=0.026) [6]. However, morbidity was not assessed. Our objective was to evaluate whether hypocalcaemia of serum ionized calcium (defined as <1.10 mmol/L (4.5 mg/dL) is a prognostic factor for mortality and morbidity (defined as GOS ≤ 3) in early moderate and severe TBI.

Material and Methods

We developed a prospective study from January 2014 to December 2015. Patients were between 16 and 87 years old and had a Glasgow Coma Scale (GCS) of 3 to 13 points following TBI [7], with demonstrable intracranial lesions in cranial computed tomography (CT). Patient recruitment in the Evangelic Hospital, Oldenburg, Germany was performed with permission from the ethical committee of the Carl von Ossietzky University with the number: Drs.21/4/2014

Patients with the following characteristics were excluded:

- TBI older than 3 days
- Intake of medication, conditions or diseases affecting calcium metabolism (such as hyperparathyroidism, acute pancreatitis, massive blood transfusion, and treatment with hydrochlorothiazide.
- Haemorrhages in the brainstem as an isolated finding
- Previous treatment in another clinic
- Pregnancy
- Hyperphosphatemia (>1.32 mmol/L)

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- Hypomagnesemia (<0.61 mmol/L)
- Alcoholism
- Hypoalbuminemia at admittance
- Prior disability due to TBI.

The patients were admitted to the emergency room and treated according to the guidelines for advanced trauma life support (ATLS). Once the patients were stabilized, blood samples for hematic biometry, blood chemistry, and serum electrolytes (sodium, potassium, calcium, ionized calcium) and arterial blood gases were taken [7].

The routine treatment included a crystalloid solution, gastric protector, analgesic and sedative in case of agitation. For intubation purposes, we used Propofol plus rocuronium. Clinical variables at hospital admittance consisted of age, sex, seizures and pupillary reaction assessment. We measured respiratory and cardiac frequency, as well as arterial systolic, diastolic and mean arterial pressures [7]. The Brain Trauma Foundation guidelines were followed in the treatment of all patients.

For statistical analysis, we utilized the program JMP-7 [9]. An analysis of descriptive statistics, obtaining the measures of central tendency and dispersion of all the variables was completed. For comparative analysis, we used the Student's t-test for normally distributed continuous variables and the Wilcoxon/Kruskal-Wallis test for non-normally distributed continuous variables. For categorical variables, the chi-squared test was applied, and for tables with boxes less than 5, the Fisher's exact test was utilized. Statistical significance was indicated by a value of $p<0.05$. We calculated hazard ratios (HR) with confidence intervals (CI) of 95%. We conducted logistic regression analysis with the variables that showed a significant difference ($p<0.05$) in the bivariate analysis. In the final model, these variables were expressed with HR (CI 95%). The number of patients to be treated was calculated by the method of Peduzzi et al. [10].

Results

We recruited 61 patients who suffered moderate and severe TBI and fulfilled the inclusion criteria with a median age of 42 years old (range: 17 to 86). Of the studied patients, 43 (70.50%) were male and 18 (29.50%) were female. Twenty-one (34.42%) patients had a GOS ≤ 3 and 40 (65.58%) had a GOS>3. A total of 14 patients did not fulfil the inclusion criteria and thus were excluded. The demographic and clinical variables, basal pH levels and number of days in the intensive care unit are shown in Table 1. Inferring that different TBI pathologies imply different outcomes, the characteristics of both groups were equal (not shown in the table).

Evaluation of the hospitalization variables revealed that most of them were not significantly different, with the exception of age ($p<0.001$) and those known to be risk factors associated with poor prognosis, namely Glasgow Coma Scale at discharge ($p=0.041$), ICU days ($p=0.002$), mean arterial pressure ($p=0.015$), pH on day 3 ($p=0.022$), and pupillary reactivity ($p=0.003$). In our study, there were no differences in the demographic and clinical variables (Table 1) nor in the blood cellularity measures and serum electrolyte levels at hospital admittance (Table 2). The comparison of the Glasgow Coma Scale at discharge between the two groups revealed statistical significance ($p<0.001$); this is explained by the poor prognosis of the second group (Table 1).

We found a statistically significant difference ($p=0.009$) in the ionized serum calcium levels, protein S-100b ($p=0.002$), IL 6 ($p=0.007$) and haemoglobin ($p=0.011$) on the third day of admission between the

	GOS ≤ 3 (n=21)	GOS > 3 (n=40)	p-Value
Gender (M/ F)	15/6	28/12	0.907§
Age (years)†	65.5 (27-86)	47.5 (17-80)	0.001‡
Glasgow Coma Scale at admittance*	8 ± 3	9 ± 3	0.445‡
Glasgow Coma Scale at discharge*	9 ± 4	14 ± 1	<0.001‡
ICU days*	16.9 ± 13.1	8.6 ± 7.1	0.002‖
Mean arterial tension (mmHg)*	109.6 ± 13.4	117.7 ± 11.3	0.015‖
Cardiac frequency*	100 ± 21.8	97.5 ± 18.9	0.640‖
Respiratory frequency*	18.4 ± 15.3	14.5 ± 1.5	0.120‖
pH day 0*	7.40 ± 0.09	7.38 ± 0.07	0.402‖
pH day 3*	7.45 ± 0.06	7.42 ± 0.04	0.022‖
pH day 7*	7.42 ± 0.08	7.42 ± 0.05	0.921‖
Isochoria			
Yes	15/21(71.4%)	33/39[a] (84.6%)	
No	6/21(28.6%)	6/39[a] (15.4%)	0.231¶
Pupillary reactivity			
Yes	11/21 (52.3%)	34/40 (77.5%)	
No	10/21 (47.7)	5/40 (32.5%)	0.003¶

[a] Due to a penetrating knife wound at the eye n=1 patient was not taken into account for isochoria.
§ Fisher's exact test. † Median (ranges). ‡Wilcoxon ranges. * Mean ± Standard Deviation (SD).
‖ Student's t-test. ¶ Chi-squared test.

Table 1: Demographic and clinical variables.

	GOS ≤3 (n=21)	GOS>3 (n=40)	p-Value
Day 0			
Total leukocytes (× 10^3/µL) *	10.1 ± 3.6	10.7 ± 4.1	0.599‖
Hematocrit (%) *	35 ± 6.2	36 ± 7.4	0.615‖
Hemoglobin (g/dL) *	12.3 ± 2.5	12.4 ± 1.9	0.877‖
Sodium (mmol/ L) *	140 ± 3.4	140.3 ± 3.7	0.439‖
Potassium (mmol/L) *	3.9 ± 0.5	3.9 ± 0.6	0.689‖
Calcium (mmol/L) *	2.2 ± 0.2	2.2 ± 0.1	0.394‖
Ca^{++} ion (mmol/L) *	1.18 ± 0.2	1.15 ± 0.1	0.337‖
Glucose (mg/dL) *	160.5 ± 66.5	139.3 ± 47.6	0.157‖
Protein S-100 (ng/dL) *	0.4 ± 0.5	0.3 ± 0.2	0.169‖
IL6 (ng/dL) *	185 ± 242	96.1 ± 183.3	0.117‖
Magnesium (mmol/L) *	0.8 ± 0.1	0.8 ± 0.1	0.479‖
Phosphate (mmol/L) *	0.8 ± 0.3	0.9 ± 0.2	0.098‖
Day 3			
Total leukocytes (X 10^3/ µL) *	9.7 ± 3.6	10 ± 4.7	0.825‖
Hematocrit (%) *	27.2 ± 7.9	30.3 ± 9.3	0.196‖
Hemoglobin (g/dL) *	9.4 ± 1.9	10.8 ± 2.1	0.011‖
Sodium (mmol/L) *	135 ± 30.3	141 ± 4	0.223‖
Potassium (mmol/L) *	4.1 ± 0.6	4 ± 0.4	0.488‖
Calcium (mmol/L) *	2.1 ± 0.2	2.1 ± 0.1	0.264‖
Ca^{++} ion (mmol/L) *	**1.09** ± 0.10	**1.16 ± 0.06**	**0.009‖**
Glucose (mg/dL) *	146.2 ± 36.7	135.8 ± 31	0.250‖
Protein S-100 (ng/dL) *	1.2 ± 0.1	0.1±0.05	0.002‖
IL6 (ng/dL) *	131.5 ± 202.5	39 ± 44.2	0.007‖
Magnesium (mmol/L) *	0.8 ± 0.1	0.8 ± 0.1	0.188‖
Phosphate (mmol/L) *	0.9 ± 0.3	0.8 ± 0.3	0.405‖
Day 7			
Total leukocytes (X 10^3/µL) *	10 ± 3.6	9.6 ± 4.9	0.800‖
Hematocrit (%) *	27.8 ± 7.2	31.9 ± 8	0.075‖
Hemoglobin (g/dL) *	9.8 ± 1.7	11.2 ± 2.7	0.020‖
Sodium (mmol/ L) *	139 ± 6.2	135.7 ± 22.3	0.548‖
Potassium (mmol/L) *	4.2 ± 0.4	3.9 ± 0.6	0.039‖
Calcium (mmol/L) *	2.1 ± 0.2	2.2 ± 0.1	0.058‖
Ca^{++} ion (mmol/L) *	1.18 ± 0.2	1.17 ± 0.08	0.694‖

Glucose (mg/dL) *	152.6 ± 30	130.5 ± 44.3	0.067‖
Protein S-100 (ng/dL) *	0.1 ± 0.04	0.08 ± 0.07	0.095‖
IL6 (ng/dL) *	36.6 ± 24.1	25.3 ± 21.4	0.082‖
Magnesium (mmol/L) *	0.9 ± 0.1	0.8 ± 0.1	0.739‖
Phosphate (mmol/L) *	0.9 ± 0.2	1 ± 0.3	0.163‖
* Mean ± Standard Deviation (SD); ‖ Student's t-test.			

Table 2: Chemistry variables and blood cellularity at 0, 3rd and 7th day.

Parameter	HR	Lower 95 %	Upper 95%	p value
Pupillary reactivity	12.4	9.42	15.38	0.0004
Hypocalcaemia of serum ionized calcium (< 1.10 mmol/L) on third day	3.14	2.49	3.78	0.05
Age in years	8.45	6.48	10.42	0.0036
Protein S-100 b	5.00	3.88	6.12	0.025
IL-6	4.60	3.65	5.67	0.032

Table 3: Best logistic regression (Glasgow Outcome Score ≤ 3).

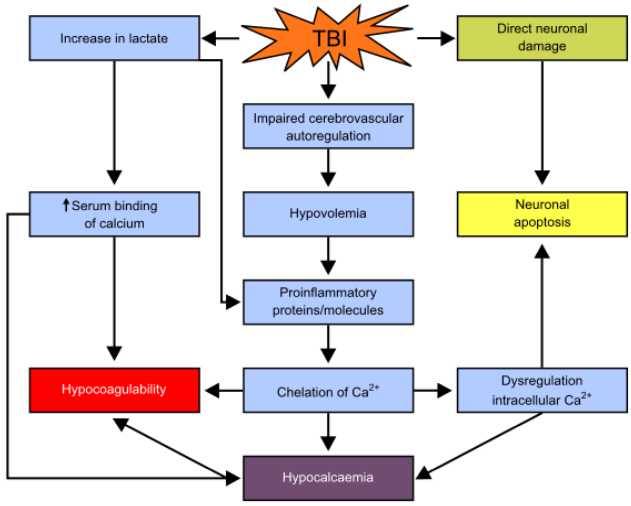

Figure 1: Proposed pathophysiological mechanism for hypocalcaemia following TBI.

groups: GOS ≤ 3 and >3 (disability/death) (Table 2). In the haemoglobin levels on the seventh day we also found a statistically significant difference (p=0.020) and potassium on day 7 (p=0.039) between groups (Table 2). We calculated a hazard ratio (HR) of 3.14 (95% CI: 2.49-3.78) (p=0.05) for the association of hypocalcaemia of serum ionized calcium (<1.10 mmol/L) on third day and the Disability/Death group. The best logistic regression model included: age, absent pupillary reactivity, hypocalcaemia of ionized serum calcium (<1.10 mmol/L), protein S-100b and IL6 on day 3. These variables substantiated poor Glasgow Outcome Scores (Table 3).

In our model, we have attempted to analyse all the significant variables in order to establish which variables influence the dependent variable, Glasgow Outcome Score. It appears that in the included cases, age, pupillary reactivity, hypocalcaemia of serum ionized calcium, protein S-100b and IL 6 on day three following trauma were significant factors in our study. However, in comparison to our initial results, haemoglobin on day 3 and potassium on day 7 were non-significant and thereby potential confusing factors.

Discussion

In this prospective study, the importance of calcium regarding prognosis in mortality and morbidity after moderate-severe TBI could be assessed. The serum levels of ionized calcium on day 3 were supplemented with protein S-100b and IL6 also on day 3 as part of the theory that calcium is diminished due to neuro- and systemic inflammation. Neuro-inflammation is likely to influence cerebral oedema, as shown in (Figures 1 and 2) [11-13]. As an indirect sign, patients with poor outcomes had impaired pupillary reactivity, also detected in our study as poor prognosis in the logistic regression model. The latter is a crucial clinical sign for intracranial elevated pressure due to cerebral oedema accompanying an imminent risk of cerebral herniation/cerebral ischemia, which again correlates with dismal prognosis [6,7]. Lactate is also produced under these circumstances because of disturbances in the aerobic mitochondrial use of ATP, with further exacerbation of hypocalcaemia through calcium-lactate chelation [12,14,15].

The small sample size is a limitation of this paper. In our centre, according to local records, there is an annual average of 40 patients who could fulfil the inclusion criteria. In this case, 14 eligible patients were excluded, giving a total of 75 patients, almost reaching the expected 80 patients in 2 years of recruiting. Therefore, we consider our sample to be representative of the studied population. GCS values appeared to be decisive in terms of the impact on outcome following TBI. However, this value is prone to considerable variability between observers. This variability is mainly seen in inexperienced health care workers who have little contact with patients suffering from neurological disorders. Another aspect that could have incurred errors is that the mean time until arrival at the shock room in our institution was 2 hours. In this period, the value of the Glasgow Coma Scale in critical patients could change very quickly, coupled with inexperienced health care workers assessing patients with neurological disorders in pre-admission care [16]. Age is a well-known factor for unfavourable outcome in patients suffering TBI [2,3,17]. Nevertheless, serum ionized calcium in our study was an independent finding regarding age.

Radiological findings in TBI include supra-/infratentorial lesions and diffused axonal injury (DAI), serving as prognostic factors. In addition to the direct impact on brain tissue, the resulting brain oedema and impaired autoregulation of adequate delivery of oxygen in the already insulted neuronal cells causes neuro-inflammation with the release of IL-6 and the binding of protein S-100 to calcium, leading to hypocalcaemia [17,18].

A higher rate of hypocalcaemia has already been reported in other critically ill patients. The underlying pathologies are various [19]. Most commonly, this phenomenon has been seen in sepsis syndrome [20]. However, the severity and incidence of hypocalcaemia in non-septic

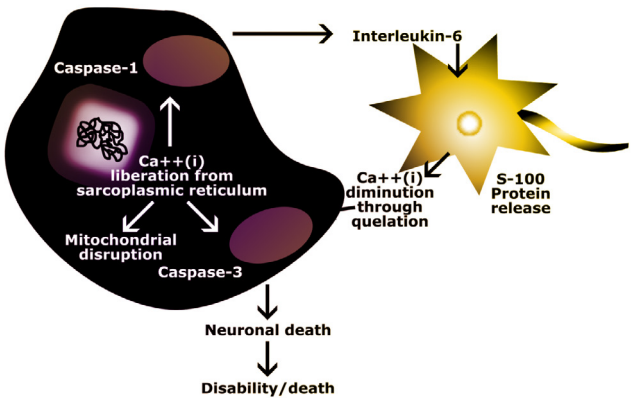

Figure 2: Representation at cellular level of the proposed pathophysiological mechanism after TBI in which calcium may be involved.

but critically ill patients have not been well examined. Some authors have studied this phenomenon showing it to be frequent in critically ill adults and to be associated with mortality and severe organ dysfunction in children. They described a correlation with severity of illness such as sepsis and burns, but not with a specific illness per se [21,22].

Based on the results of the present study, the link between protein S-100b and the diminution of calcium in its active form (ionized calcium) in this pathological status (neuronal oxygen deficit with cerebral vessel dysregulation after TBI) could be addressed as follows:

1. This protein (S-100b) is liberated to the extracellular space from the injured neurons [13].

2. Subsequently elevated binding from protein S-100b with calcium occurs [23,24].

3. The latter causes hypocalcaemia and consequent activation of neuronal apoptosis [25].

4. This finally leads to neuronal loss manifested in disability and even death, as shown in Figures 1 and 2. [1,2,26].

According to our observations, we assume that hypocalcaemia is a marker for the depth of brain damage as a result of a cascade of various pathologic mechanisms such as direct mechanical trauma, neuro-inflammation, altered vessel-autoregulation and hypoxia. Hypocalcaemia appears to be an epiphenomenon of several factors. However, it seems to play a role as prognosticator; nevertheless, it has not been studied as a therapeutic target.

Conclusion

Serum levels of ionized calcium on day 3 are easy to assess and could be useful for the prediction of mortality and disability in patients with moderate and severe TBI in terms of a marker for depth of TBI. The proposed pathophysiological mechanism is the diminution of serum ionized calcium due to chemical binding together with proinflammatory proteins/molecules like protein S-100b, leading to a cellular influx of ionized calcium with consequent apoptosis. Further studies should be performed in order to clarify the role of calcium in TBI regarding the ongoing pathophysiological process. In the future, it could be decisive in terms of diagnosis and even treatment.

Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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