Serum bilirubin level correlates with mortality in patients with traumatic brain injury

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Abstract
As a catabolic product of hemoglobin, bilirubin has been confirmed playing an important role in the development of various central nervous system disease. The aim of this study is to explore the correlation between serum bilirubin level and mortality in patients with traumatic brain injury (TBI).

Patients admitted with traumatic brain injury (TBI) in our hospital between January 2015 and January 2018 were enrolled in this study. Clinical and laboratory data of 361 patients were retrospectively collected to explore the independent risk factors of mortality.

The comparison of baseline characteristics showed that non-survivors had lower Glasgow Coma Scale (GCS) (P < .001) and higher level of serum total bilirubin (TBIL) (P < .001) and direct bilirubin (DBIL) (P < .001). We found that only GCS (P < .001), glucose (P < .001), lactate dehydrogenase (LDH) (P = .042) and DBIL (P = .005) were significant risk factors in multivariate logistic regression analysis. GCS and DBIL had comparable AUC value (0.778 vs 0.750, P < .05) on predicting mortality in TBI patients. The AUC value of the combination of GCS and DBIL is higher than the single value of these two factors (P < .05). Moreover, predictive model 1 consisted of GCS, glucose, LDH and DBIL had the highest AUC value of 0.894.

DBIL is a significant risk factor of mortality in TBI patients. Assessing the level of DBIL is beneficial for physicians to evaluate severity and predict outcome for TBI patients.

Abbreviations: AKI = acute kidney injury, ARDS = acute respiratory distress syndrome, AUC = area under the ROC curve, CI = confidence intervals, DBIL = direct bilirubin, EMR = electronic medical record, GCS = Glasgow Coma Scale, GOS = Glasgow Outcome Score, LDH = lactate dehydrogenase, OR = odds ratio, ROC = receiver operating characteristic curve, TBI = traumatic brain injury, TBIL = total bilirubin, WBC = white blood cell.

Keywords: bilirubin, marker, prognosis, traumatic brain injury

1. Introduction
It is estimated that 69 million people could suffer traumatic brain injury (TBI) annually in the world.[1,1] TBI has become the leading cause of mortality and disability in young population.[2] The enormous burden to public health and social economy caused by the high mortality and morbidity of TBI makes it necessary to explore pathophysiological mechanism and discover novel predictive markers.

Heme, the prosthetic group of hemoglobin, myoglobin and cytochromes, can be catabolized to biliverdin, carbon monoxide and iron by the rate-limiting heme oxygenase-1 (HO-1).[3] And biliverdin is subsequently transformed to bilirubin by cytosolic biliverdin reductase.[4] The heme catabolic pathway has been confirmed playing an important role in the development of various central nervous system diseases including ischemic stroke, aneurysmal subarachnoid hemorrhage, neuroblastoma and Alzheimer’s.[5–9] In addition, previous study showed that serum bilirubin would increase resulting from activation of HO-1, which constitutes a defense response to oxidative stress after suffering TBI.[10] As an end product of heme degradation, bilirubin is actually characterized as antioxidant, anti-inflammatory and cytoprotective.[11] Based on these properties, some studies were designed to explore the relationship between serum bilirubin level and patients' prognosis in several clinical settings including ischemic stroke, myocardial infarction, diabetes and gastric cancer.[11–14] A study found that elevated bilirubin was independently associated with increased severity and worse prognosis.[15] Moreover, bilirubin was recently proved to be an efficient marker of hemorrhagic transformation in patients undergoing mechanical thrombectomy.[16]

The prognostic role of bilirubin in TBI patients has not been confirmed. We designed this study to evaluate the correlation between serum bilirubin level and injury severity and mortality of patients.
2. Materials and methods

2.1. Subjects

This study was performed in West China hospital. Patients admitted with TBI in our hospital between January 2015 and January 2018 were enrolled in this study. The confirmed diagnoses of TBI were based on findings of computed tomography (CT) and/or magnetic resonance imaging (MRI). The exclusion criteria included: admitted to our hospital 24 hours after suffering trauma; hospitalized in our hospital less than 24 hours; lacking of records of required data; complicated with other neurological diseases, hematological system diseases, hepatic and renal dysfunction, cardiovascular diseases, metabolic diseases, cancer. A total of 361 patients was finally included in this study. This study obtained the approval of the ethics committee of West China hospital. We got written informed consent from patients or their legal representatives.

2.2. Data collection

Basic characteristics and vital signs in admission of each patient were recorded in electronic medical record (EMR) system by emergency workers. Laboratory tests were obtained by analyzing the blood specimen on the first day of admission. Experienced nurses evaluated Shock state during hospitalization. Coagulopathy was defined as meeting any of the following tests: platelet count < 100 x 10^9/L, international normalized ratio (INR) > 1.25, prothrombin time (PT) > 14 seconds and activated partial thromboplastin time (APTT) > 36 seconds. Acute kidney injury (AKI) was confirmed based on the KIDGO criteria and acute respiratory distress syndrome (ARDS) was diagnosed according to Berlin criteria. Glasgow Outcome Score (GOS) was scored at discharge. Records of these clinical and laboratory variables were collected from EMR system.

2.3. Statistical analysis

Kolmogorov-Smirnov test was used to confirm the normality of variables. Normal distribution variables were expressed as mean ± standard deviation whereas non-normal distribution variables were expressed as median (interquartile range). Categorical variables were expressed as numbers (percentage). We performed Student’s t test and Mann-Whitney U test to compare the difference of normal distribution variables and non-normal distribution variables, respectively. And χ² test was used to compare the difference of categorical variables.

We performed multivariate logistic regression analyses to find risk factors of in-hospital mortality. Spearman rank correlation test was utilized to analyze the relationship between two variables. The predictive value of factors and models was evaluated by drawing receiver operating characteristic curves (ROC). Z test was performed to compare the predictive value of different factors and models.

A P value < .05 was considered to be statistically significant. SPSS 22.0 Windows software (SPSS, Inc, Chicago, IL) was used for all statistical analyses.

3. Results

3.1. Comparison of baseline characteristics based on survival state

The mortality of included patients was 50.14% (176/351). We divided patients into two groups based on their survival state. Compared with survivors, non-survivors had higher heart rate (P < .001) and lower temperature (P = .043) in admission (Table 1). The GCS score was significantly lower in non-survivors (8 vs 5, P < .001). The comparison of laboratory tests showed that non-survivors had higher level of white blood cell (WBC), glucose, lactate dehydrogenase (LDH), total bilirubin (TBIL), direct bilirubin (DBIL) and indirect bilirubin (IDBIL). And the level of leukocyte, platelet, cholesterol, albumin, hemoglobin and prothrombin time (PT) was lower in non-survivors. In addition, the incidence of shock (15.9% vs 45.4%, P < .001), coagulopathy (14.8% vs 55.7%, P < .001), AKI (5.7% vs 36.9%, P < .001) and ARDS (25% vs 42%, P = .001) were significantly higher in non-survivors.

3.2. Multivariate logistic regression analysis of risk factors associated with mortality

We performed multivariate logistic regression analysis to explore independent risk factors of mortality in TBI patients. After adjustment, we found that only GCS (P < .001), glucose (P < .001), LDH (P = .042) and DBIL (P = .005) were statistically significant risk factors of mortality (Table 2).

3.3. Correlation between serum direct bilirubin level and GOS

The Spearman rank correlation test was utilized to analyze the relationship between serum direct bilirubin level and GOS. Results showed that correlation coefficient r was −0.459 (P < .001) (Fig. 1).

3.4. Prognostic value of risk factors and predictive model

The ROC curves (Fig. 2) showed that the area under ROC curve (AUC) value of GCS was 0.778 with a sensitivity and specificity of 0.543 and 0.892 (Table 3). The AUC value of DBIL was 0.750 with a sensitivity and specificity of 0.665 and 0.703. GCS and DBIL had comparable value in predicting outcome of TBI patients (Z = 0.7763, P > .05). In addition, the AUC value of two-factors model combing GCS with DBIL was 0.843, which was higher than ROC of GCS (Z = 2.0303, P < .05) and DBIL (Z = 2.8352, P < .05). Finally, the four-factors model 1 consisted of GCS, glucose, LDH and DBIL was more efficient than two-factors model in predicting outcome with AUC of 0.894 (Z = 1.9912, P < .05).

4. Discussion

In this study, non-survivors had higher serum level of TBIL, DBIL, and IDBIL compared with survivors. And level of DBIL was inversely associated with 30-day GOS which indicated that DBIL was valuable in evaluating severity and predicting outcome in TBI patients.

As previous studies indicated, heme catabolic metabolism could play a key role in the development of various kinds of brain injury. The end-product of heme degradation including bilirubin, carbon monoxide and ferrous iron are all involved in the pathophysiological process. Carbon monoxide, which are usually considered as poisonous molecule, actually could attenuate neuronal death, neural inflammation and cerebral vasospasm. Moreover, a recent study indicates that carbon monoxide is beneficial to maintain circadian rhythm which in
The accumulated heme derived from pathways above mentioned would induce the expression of HO-1 which in turn promotes the production of bilirubin. Then, free bilirubin would be oxidized to biliverdin by reactive oxygen substances. With sufficient biliverdin reductase, the biliverdin would be reduced to bilirubin again. The circulation between bilirubin and biliverdin actually constitutes an important part of antioxidant barrier and hence accumulate significantly in blood. The free bilirubin in blood could be glucuronized into DBIL by liver enzymes.

A remarkable result was that though TBIL, DBIL, and IDBIL of non-survivors were significantly higher than non-survivors, only DBIL obviously exceeded upper limit of the normal value. With the increased production of bilirubin after TBI, the consumption of bilirubin would also increase. Owing to the strong hydrophilicity of DBIL, it could not cross the blood brain barrier and hence accumulate significantly in blood. However, unconjugated IDBIL could not accumulate too much in blood like DBIL due to good liposolubility, and be consumed by reactive oxidative species in brain. This is a reasonable explanation for the increasing DBIL above normal value and non-obvious increase of IDBIL. This fact was confirmed by the multivariate logistic regression analysis in which DBIL instead of IDBIL was a significant risk factor.
A previous study with small sample size has illustrated that plasm bilirubin could increase rapidly after TBI and then gradually decreased in the next days.\cite{10} We consider that initial increase of bilirubin is caused by hemorrhage and hematoma which might reflect the severity of injury. And the degree of subsequent decrease is actually associated with overall load of oxidative stress. In our study, the value of bilirubin on admission should represent the initial damage degree in brain and therefore correlates with the final outcome.

There were several limitations in our study. First, this was a single center study in which selection bias could not be avoided. Second, TBI involves multiple pathophysiologic processes including contusion, bleeding, edema, necrosis, etc. These processes play diversified role in the production and metabolism of bilirubin. We could not divide patients into subgroups for analysis according to their damage form such as intracranial hemorrhage, cerebral contusion and diffused axonal injury. Finally, we did not record the trend of bilirubin after injury so that the relation between changing level of bilirubin and outcome could not be analyzed.

5. Conclusion

Measuring the serum level of bilirubin is beneficial for evaluate prognosis of patients suffering TBI. The incorporation of direct bilirubin into prognostic models will improve the value on predicting outcome in TBI patients.

Acknowledgments

Written informed consent was obtained from every eligible patient or their legal representatives.

| Table 2 | Multivariate logistic regression analysis of risk factors for mortality in TBI patients. |
|---------|---------------------------------|
| Variables | OR | 95% CI | P |
| Age | 1.003 | 0.984-1.021 | .782 |
| Male | 1.234 | 0.585-2.864 | .525 |
| SBP | 1.008 | 0.991-1.026 | .343 |
| DBP | 0.982 | 0.956-1.008 | .180 |
| Heart rate | 0.996 | 0.984-1.007 | .458 |
| Temperature | 1.008 | 0.990-1.027 | .390 |
| GCS | 0.723 | 0.620-0.842 | <.001 |
| WBC | 0.988 | 0.919-1.062 | .734 |
| Neutrophil | 0.978 | 0.889-1.065 | .607 |
| Leukocyte | 0.775 | 0.447-1.343 | .363 |
| Platelet | 1.001 | 0.997-1.006 | .522 |
| Glucose | 1.216 | 1.103-1.341 | <.001 |
| Cholesterol | 0.938 | 0.623-1.413 | .759 |
| Albumin | 0.981 | 0.928-1.037 | .500 |
| Hemoglobin | 0.999 | 0.982-1.016 | .912 |
| PT | 1.048 | 0.922-1.191 | .471 |
| LDH | 1.001 | 1.000-1.003 | .042 |
| Direct bilirubin | 1.321 | 1.082-1.606 | .005 |
| Indirect bilirubin | 0.966 | 0.893-1.044 | .382 |
| Shock | 0.885 | 0.395-1.984 | .767 |
| Coagulopathy | 1.802 | 0.827-3.929 | .139 |
| Hydrocephalus | 1.398 | 0.565-3.455 | .469 |
| AKI | 2.305 | 0.909-5.947 | .079 |
| ARDS | 0.855 | 0.427-1.711 | .658 |

AKI = acute kidney injury, ARDS = acute respiratory distress syndrome, CI = confidence interval, DBP = diastolic blood pressure, GCS = Glasgow Coma Scale, LDH = lactate dehydrogenase, OR = odds ratio, PT = prothrombin time, SBP = systolic blood pressure, WBC = white blood cell.

Table 3

| Risk factor | AUC | Sensitivity | Specificity | 95% CI |
|-------------|-----|-------------|-------------|-------|
| GCS | 0.778 | 0.543 | 0.892 | 0.729-0.826 |
| DBIL | 0.750 | 0.665 | 0.703 | 0.700-0.800 |
| GCS + DBIL | 0.843 | 0.824 | 0.72 | 0.803-0.883 |
| Model 1 | 0.894 | 0.756 | 0.886 | 0.862-0.926 |

Model 1 is composed of GCS, glucose, LDH and DBIL. AUC = area under the receiver operating characteristics curve, CI = confidence interval, DBIL = direct bilirubin, GCS = Glasgow Coma Scale.

Figure 1. Box-plot of serum direct bilirubin level and GOS. The spearman rank correlation test showed that correlation coefficient r was −0.459 (P < .001).
**Author contributions**

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