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Plasma renalase levels are associated with the development of acute pancreatitis

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Abstract

Background/objectives: Severe acute pancreatitis is associated with significant morbidity and mortality. Identifying factors that affect the risk of developing severe disease could influence management. Plasma levels of renalase, an anti-inflammatory secretory protein, dramatically decrease in a murine acute pancreatitis model. We assessed this response in hospitalized acute pancreatitis patients to determine if reduced plasma renalase levels occur in humans.

Methods: Plasma samples were prospectively and sequentially collected from patients hospitalized for acute pancreatitis. Two forms of plasma renalase, native (no acid) and acidified, were measured by ELISA and RNLS levels were compared between healthy controls and patients with mild and severe disease (defined as APACHE-II score ≥7) using nonparametric statistical analysis.

Results: Control (33) and acute pancreatitis (mild, 230 (76.7%) and severe, 70 (23.3%) patients were studied. Acidified RNLS levels were lower in pancreatitis patients: Control: 10.1 μg/ml, Mild 5.1 μg/ml, Severe 6.0 μg/ml; $p < 0.001$. Native RNLS levels were increased in AP: Control: 0.4 μg/ml, Mild 0.9 μg g/ml, Severe 1.2 μg/ml $p < 0.001$; those with severe AP trended to have higher native RNLS levels than those with mild disease ($p = 0.056$). In patients with severe AP, higher APACHE-II scores at 24 h after admission correlated with lower acid-sensitive RNLS levels on admission ($r = -0.31$, $p = 0.023$).

Conclusion: Low plasma acidified RNLS levels, and increased native RNLS levels are associated with AP. Additional studies should assess the clinical correlation between plasma RNLS levels and AP severity and outcomes.

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Conflicts of interest

G. Desir is a named inventor on several issued patents related to renalase discovery and therapeutic use. Renalase is licensed to Bessor Pharma, and G. Desir holds an equity position in Bessor and its subsidiary Personal Therapeutics. Bessor Pharma was not involved in the preparation of this manuscript or influenced its content.

Keywords

Pancreatitis; Renalase; Severity

1. Introduction

Acute pancreatitis (AP) is among the most common causes of hospitalization among gastrointestinal diseases in the United States, with an increasing global incidence of about 34 per 100,000 person-years [1,2]. Although the overall mortality rate related to pancreatitis is 0.8% and decreasing, pancreatitis-related morbidity remains high and relates to disease severity [3]. The incidence of the severe disease varies among studies but is about 20% of hospitalized patients [4]. Early serious complications include persistent organ failure and infection; long-term complications include endocrine/exocrine pancreatic insufficiency [1,4].

The early identification of patients with severe AP could influence disease management [5]. Scoring systems such as Ranson's criteria, Glasgow criteria, APACHE II, and CT severity index have been used in clinical settings. Other predictors of severe acute pancreatitis have included C-reactive protein level, serum trypsinogen activation peptide, procalcitonin, and blood urea nitrogen, among others that have been examined with variable success [4,6]. Identifying prognostic factors related to disease mechanisms could influence therapy.

Renalase (RNLS) is a novel plasma flavoprotein produced in the kidneys and other tissues with pro-survival and anti-inflammatory effects [7]. Treatment with recombinant RNLS is protective in acute experimental injuries, including those to the kidney, heart, and pancreas [7-9]. In the cerulein model of acute pancreatitis in mice, recombinant RNLS treatment after inducing disease reduced AP severity while genetic deletion of RNLS in mice resulted in more severe pancreatitis [8]. In this study, total plasma RNLS levels (measured by immunoblot) decreased dramatically within an hour after inducing pancreatitis and rebounded above baseline levels during disease recovery [8].

Given the protective effects of RNLS and changes observed in the plasma levels in experimental AP models, we hypothesized that plasma RNLS levels could be a marker of human disease. Recently, enzyme-linked immunosorbent assay (ELISA) testing of RNLS identified two distinct plasma forms of RNLS; that detected after transient acidification of plasma (designated “acidified”) and another detected without plasma acidification, (designated native RNLS) [9,10]. These two forms have also been referred to as total and free plasma RNLS, respectively, elsewhere [10]. In most subjects, the acid-sensitive fraction is 10-20-fold greater than the fraction detected without acidification. Though differences between the two forms detected by ELISA remain unclear, here we examined both forms because they correspond to distinct clinical features of various diseases such as chronic renal disease, acute myocardial injury, COVID19, and pancreatic cancer [10-12]. In the current pilot study, we assessed whether these two forms of plasma RNLS corresponded to AP presence and severity in hospitalized patients.

2. Methods

2.1. Plasma RNLS collection and measurement

Blood from hospitalized patients and healthy controls was collected in heparin-containing tubes. After 60 min at room temperature and centrifugation, the supernatants were aliquoted and stored at −80 °C. Samples were thawed, and plasma RNLS levels were measured by enzyme-linked immunosorbent assay (ELISA) [10]. Here we refer to two forms of plasma RNLS with distinct chemical and clinical behaviors [10]. Native RNLS was defined as RNLS measured by ELISA in non-acid treated, native plasma. Acidified RNLS was defined as RNLS measured by ELISA after briefly acidifying and then neutralizing the plasma before the ELISA assay. After transient plasma acidification the estimated renalase level increased in virtually all samples, presumably by exposing a hidden binding site for the detection antibody. Percent native RNLS was calculated as native RNLS divided by total RNLS (the sum of native RNLS and aRNLS).

2.2. Clinical data collection

Clinical data, including acute pancreatitis severity assessed by the APACHE-II criteria, were collected from the clinic's documentation records, anonymized, and processed. The Clinical Investigation Committee approved the protocol of University Medicine Greifswald, Greifswald, Germany (Prozyt, Registration No. Reg-Nr. III UV 91/03), and the Human Investigation Committee at VA Connecticut Healthcare, West Haven, CT (1582982–3). Clinical data relating to patient age, sex, etiology, and other AP parameters, and the clinical outcome were unavailable for this cohort.

2.3. Statistical analysis

Severe acute pancreatitis was defined as patients with APACHE-II scores ≥7 at 24 or 48 h after admission. All other patients were considered to have mild acute pancreatitis. Native RNLS and acidified RNLS levels on admission were compared in healthy blood donors and patients with mild and severe acute pancreatitis using nonparametric statistical analysis. Correlation studies for the admission APACHE-II scores and admission plasma RNLS level used the Spearman correlation and a two-tailed analysis. Sensitivity analysis was conducted for severe AP defined as patients with APACHE-II scores ≥8 at 24 or 48 h after admission. A p-value of ≤0.05 was considered statistically significant. All data analyses used the SPSS Version 24 (Chicago, IL, USA).

3. Results

3.1. Patient cohort

There were 34 non-hospitalized blood donor controls and 300 patients hospitalized with acute pancreatitis. Clinicopathologic characteristics are described in Table 1. Of the patients hospitalized for acute pancreatitis, 230 (76.7%) had mild disease, and 70 (23.3%) had severe acute pancreatitis as determined by an APACHE-II score of 7 or above at either 24 or 48 h after admission. Average hospital length of say was 11.8 days. The median APACHE-II score among patients with acute pancreatitis was 4.0 (IQR 2.0–6.0) 24 h after admission and

4.0 (IQR 2.9–6.0) 48 h after admission. There was no in hospital mortality among patients in this cohort.

3.2. Acute pancreatitis and plasma RNLS levels

In our cohort, the control median acidified RNLS was 10.1 μg/ml, and 3.8% was native RNLS, whereas, for pancreatitis patients, the corresponding values on admission were 5.3 μg/ml and 17.7% (Table 1 and Fig. 1A&B). The findings suggested that AP causes a significant decrease in acidified plasma RNLS levels but an increase in native RNLS levels. To determine whether admission plasma RNLS related to severity, RNLS values were analyzed in mild and severe disease. Patients with AP (either mild or severe) had significantly lower acidified RNLS levels compared to controls (Control: 10.1 μg/ml (IQR 8.2–12.6 μg/ml), Mild: 5.1 μg/ml (IQR 3.9–10.4 μg/ml), Severe: 6.0 μg/ml (IQR 4.7–7.2 μg/ml); $p < 0.001$, Fig. 1A&B). However, those with severe disease had higher native RNLS levels compared to controls (Control: 0.4 μg/ml (IQR 0.3–0.7 μg/ml), Mild: 0.9 μg/ml (IQR 0.5–1.6 μg/ml), Severe: 1.2 μg/ml (IQR 0.7–2.2 μg/ml); $p < 0.001$, Fig. 1C&D). The percent native RNLS levels were also higher in AP patients (Control: 3.8% (IQR 2.5%–8.6%), Mild: 17.5% (IQR 8.6%–31.5%), Severe: 18.2% (IQR 9.3%–27.1%); $p < 0.001$). Compared to patients with mild AP, patients with severe AP tended to have higher native RNLS levels than those with severe disease ($p = 0.056$; Fig. 1C). There were no statistically significant differences in acidified RNLS and percent native RNLS levels between patients with mild and severe AP ($p = 0.193$ and $p = 0.901$, respectively). However, higher APACHE-II scores 24 h after admission in patients with severe AP correlated with lower acidified RNLS levels on admission ($r = -0.31$, $p - 0.023$, Fig. 2C). Overall APACHE-II scores at 24 or 48 h after admission did not correlate with levels of either form of plasma RNLS (Fig. 2A-B and 2B & D, respectively).

Finally, we examined whether changes in levels of acidified RNLS from 0 to 24 h predicted APACHE-II scores between 24 and 48 h. We found that changes in initial acidified RNLS levels from 0 to 24 h were significantly different based on delayed changes in the APACHE-II score. (Worsening: median 6.1 μg/ml, No Change: median 4.9 μg/ml, Improving: median 5.5 μg/ml, $p = 0.049$, Table 2). There was a trend towards differences in acidified RNLS levels among patients with severe AP who had changes in their APACHE-II scores (Worsening: median 4.6 μg/ml, No Change: median 5.9 μg/ml, Improving: median 5.7 μg/ml, $p = 0.049$, Table 2).

4. Discussion

Our study examined plasma levels of two forms of RNLS by ELISA in AP patients and observed correlations between these values and the presence of disease. Patients with AP had significantly lower acidified RNLS levels and higher native RNLS levels on admission than healthy control individuals. This study used the APACHE-II criteria to classify AP severity. Though not ideal, these criteria are similar in their ability to other risk stratification scores, such as the Ranson and BiSAP scores, in predicting patients who may develop severe disease early in their disease course [13]. Additionally, patients with severe AP trended towards higher native RNLS levels than patients with mild AP. The findings suggest that

like our experimental murine AP model, human AP is associated with an overall decrease in plasma renalase levels. This observation could be relevant to preclinical data suggesting that administering recombinant RNLS can reduce the severity of acute experimental pancreatitis [8,14].

The patterns of acidified RNLS and native RNLS plasma levels differed among patients with AP. Though the biologic functions of acidified RNLS and native RNLS detected by ELISA remain unknown, we reported that these forms can provide informative biomarker information in a disease-specific manner. For example, elevated native RNLS plasma levels have been associated with mortality in malignancies, chronic kidney disease, and congestive heart failure, whereas reduced plasma acidified RNLS correlates with a poor prognosis for COVID-19.[10] Prior data suggest that RNLS administration reduces pancreatitis injury in both mild [8] and severe experimental pancreatitis (FG, GD, TK unpublished data). Whether reduced plasma acidified RNLS levels in acute pancreatitis could lead to therapeutic interventions requires more exploration.

Our study has several limitations: 1) Patients were from a single institution, which may introduce bias in the type of patients included in this study, 2) This study has a limited sample size and a limited number of individuals with severe AP and detailed timecourse derived measures, 3) The access to clinical data to assess sociodemographic and clinical outcomes of patients and other biometrics that might impact the course of AP in this study was limited. Given limitations to clinical data, determination of pancreatitis severity could only be based on APACHE-II scores 24 and 48 h after admission. Some studies have found that APACHE-II scores are not the most effective measure for pancreatitis severity [13]. Future studies including the etiology of AP, other disease biomarkers, demographic data and detailed time-courses are required to make further conclusions.

In summary, we found that patients with AP present with lower acidified RNLS levels and higher native RNLS levels compared to healthy controls. Native RNLS levels trended higher in patients with more severe AP. This study is a preliminary examination of the association between plasma RNLS, overall AP, and AP severity. Whether these patterns have clinical significance requires further evaluation. Although our preliminary study suggests a possible relationship between AP and RNLS, additional studies in a replication cohort are required, including additional data with a more detailed time course, other severity measures, and a longer-term follow-up to establish the clinical significance of these changes.

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Abbreviations:

RNLS	renalase
AP	acute pancreatitis

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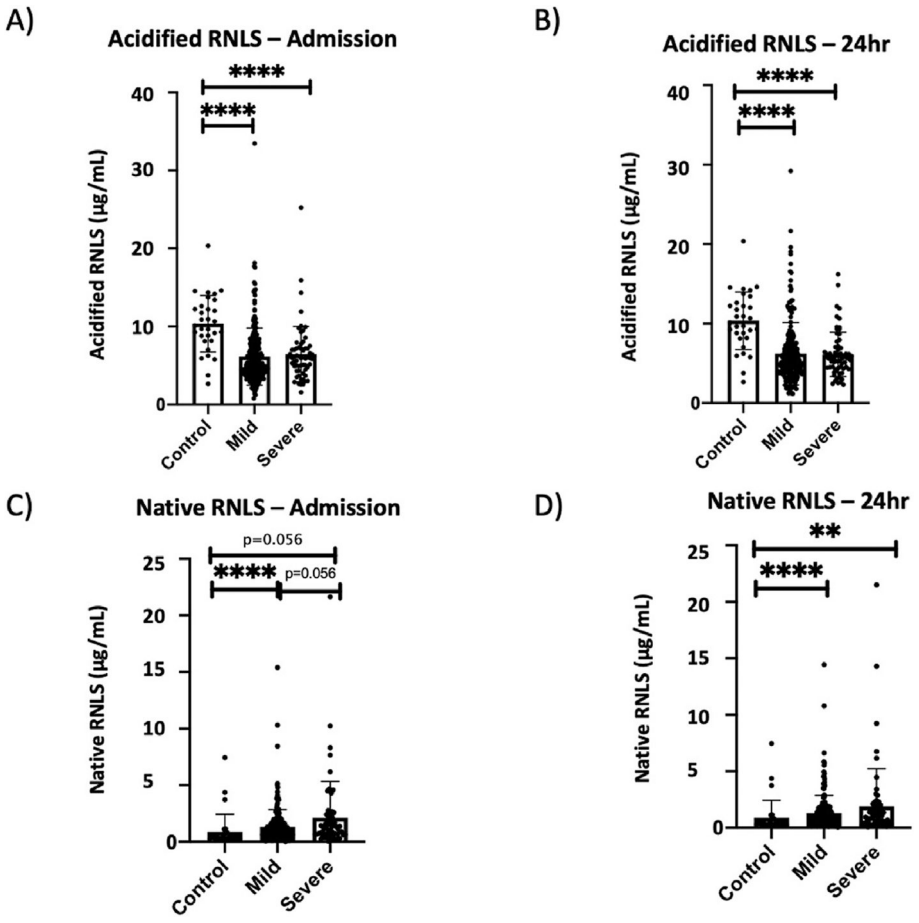

Fig. 1. Acute pancreatitis has lower acidified RNLS levels and higher native RNLS levels on admission compared to controls

(A) Admission acidified RNLS levels in control patients and patients with mild and severe acute pancreatitis defined as an APACHE II score ≥8; (B) 24-h Acidified RNLS levels in control patients and patients with mild and severe acute pancreatitis; (C) Admission NRNLS levels in control patients and patients with mild and severe acute pancreatitis; (B) 24-h native RNLS levels in control patients and patients with mild and severe acute pancreatitis; ns = $p > 0.05$, One asterisk = $p \leq 0.05$, Two asterisk = $p \leq 0.01$, Three asterisk = $p \leq 0.001$, Four asterisk = $p \leq 0.0001$.

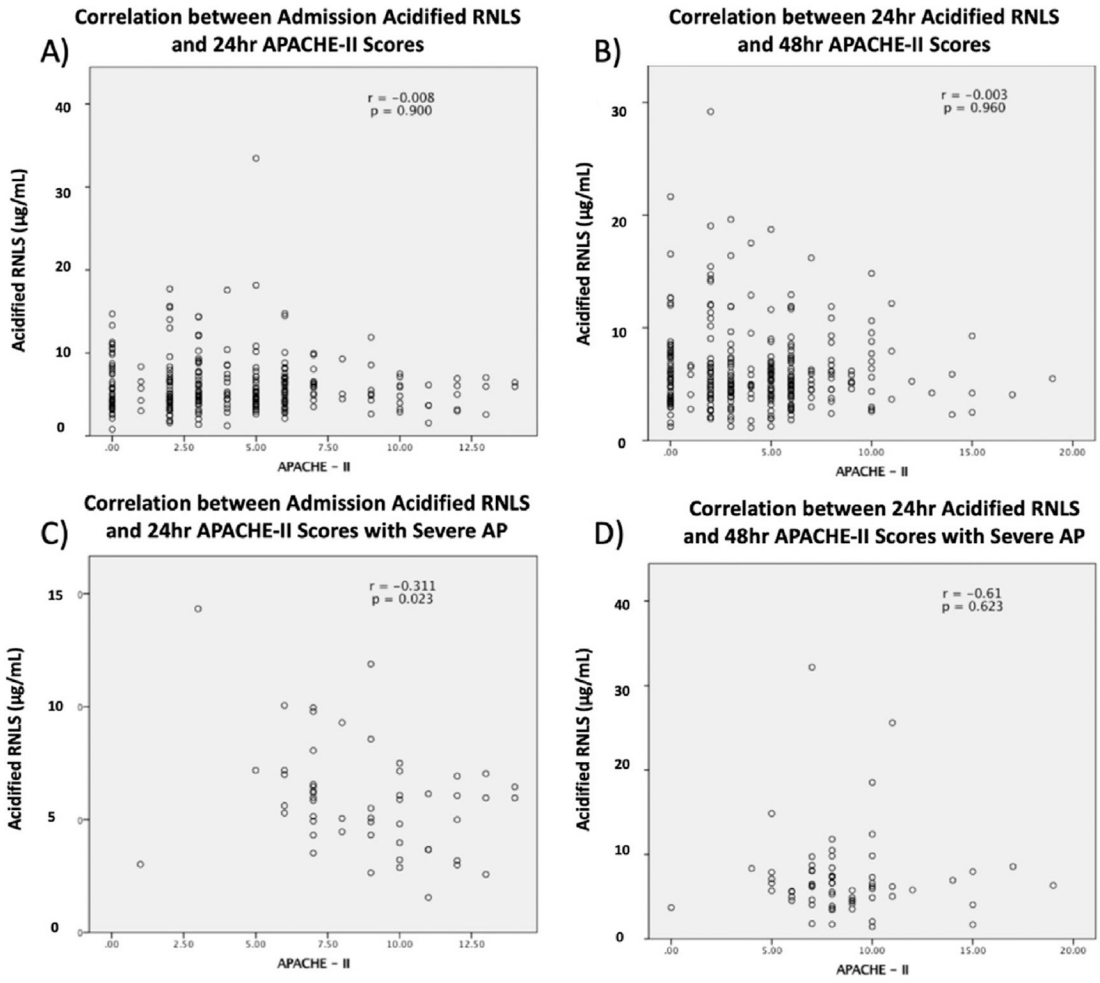

Fig. 2. Low Acidified RNLS levels do not correlate with APACHE scores

(A) No correlation with admission acidified RNLS and 24-h APACHE II scores; (B) No correlation with 24 h-acidified RNLS levels and 48-h APACHE II scores; Correlation calculated using Spearman correlation coefficient.

Table 1

Clinicopathologic characterizations of patients with and without acute pancreatitis.

Factor	Control; n (%) (n = 33 patients)	Acute Pancreatitis; n (%) (n = 300 patients)
Acute Pancreatitis Characteristics		
Acute Pancreatitis Severity; n (%)		
Mild		230 (76.7%)
Severe		70 (23.3%)
APACHE Score; median (IQR)		
24 h		4.0 (2.0–6.0)
48 h		4.0 (2.0–6.0)
Acidified RNLS; median (IQR)		
Baseline (Controls)	9286.9 (4214.5–21313.7)	
Admission		5292.1 (4055.7–7435.7)
24 h		5329.5 (3908.8–7100.8)
48 h		5558.7 (4033.5–7650.4)
72 h		6905.4 (4344.1–8030.3)
Native RNLS; median (IQR)		
Baseline (Controls)	376.2 (255.1–660.4)	
Admission		989.4 (508-1-1581.8)
24 h		828.9 (472.0–1572.5)
48 h		867.4 (453.2–1578.2)
72 h		920.3 (501.9–1559.4)
Percent Native RNLS; median (IQR)		
Baseline (Controls)	3.8 (2.5–8.7)	
Admission		17.7 (8.9–29.8)
24 h		15.8 (7.9–28.5)
48 h		15.7 (7.7–28.9)
72 h		15.2 (7.7–26.6)

Table 2

Acidified RNLS levels on admission do not predict 24 h and are not associated with changes in APACHE-II scores from 24 to 48 h.

RNLS; median (IQR)	Worsening APACHE-II	No Change in APACHE-II	Improving APACHE-II	p-value
All Pancreatitis				
Acidified RNLS				
Admission				
24h	5919.7 (4306.2–7023.1)	5022.3 (3719.4–7510.2)	5420.2 (4500.4–7314.5)	0.386
	6093.9 (4731.1–6999.9)	4876.3 (3724.6–7269.6)	5544.5 (4294.5–6692.9)	**0.049**
Native RNLS				
Admission	1021.6 (510.1–2723.4)	920.4 (456.1–1469.8)	1079.0 (668.4–1487.3)	0.097
24h	933.7 (415.3–2270.5)	826.1 (450.8–1427.2)	917.0 (586.3–1869.7)	0.158
Severe Pancreatitis				
Acidified RNLS				
Admission				
24h	5919.7 (3550.9–6950.5)	5136.3 (4316.8–6443.1)	5834.6 (5035.7–7704.7)	0.420
	4555.9 (4489.1–6946.2)	5906.1 (3827.0–5127.4)	5715.5 (4106.7–8191.5)	0.067
Native RNLS				
Admission	951.0 (582.4–2640.5)	635.3 (287.4–8322.5)	1341.1 (708.1–1611.5)	0.256
24h	1104.4 (337.1–2240.3)	589.0 (259.7–931.9)	1365.1 (648.0–1957.4)	0.171