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Acetaminophen Adducts Detected in Serum of Pediatric Patients with Acute Liver Failure

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Abstract

Objectives—Previous studies in patients with acute liver failure identified acetaminophen (APAP) protein adducts in the serum of 12% and 19% of children and adults, respectively, with acute liver failure of indeterminate etiology. This report details APAP adducts testing in a subset (n=393) of patients with varied diagnoses in the Pediatric Acute Liver Failure Study Group (PALFSG).

Methods—Serum samples were available from 393 participants included in the PALFSG registry. Adducts measurement was performed using validated methods. Participants were grouped by diagnostic category as known APAP overdose, known other diagnosis and indeterminate etiology. Demographic and clinical characteristics and participant outcomes were compared by adducts status (positive or negative) within each group.

Results—APAP adducts testing was positive in 86% of participants with known APAP overdose, 6% with other known diagnoses and 11% with an indeterminate cause of liver failure. Adducts positive participants were noted to have marked elevation of serum alanine aminotransferase and aspartate aminotransferase coupled with total serum bilirubin that was significantly lower than adducts negative patients. In the indeterminate group, adducts positive patients had different outcomes than adducts negative patients (p=0.03), spontaneous survival was 16/21 (76%) in adducts positive versus 75/169 (44%) in adducts negative patients. Prognosis did not vary by adducts status in patients with known diagnoses.

Conclusions—Further study is needed to understand the relationship of APAP exposure, as determined by the presence of APAP adducts, to the clinical phenotype and outcomes of children with acute liver failure.

Keywords

Biomarkers; Indeterminate Hepatitis; Drug Induced Liver Injury

Introduction

Acetaminophen (APAP) overdose is a common cause of drug poisoning in the United States and in many industrialized nations. In children, APAP-induced acute liver failure (ALF) may comprise 14% of all cases of ALF in this age group.[1] Toxicity is associated with both single dose exposure exceeding 100 mg/kg as well as chronic APAP exposure of 2 or more days with reported daily APAP doses within the therapeutic range for children. [2] Establishing a diagnosis of APAP overdose as the cause of pediatric acute liver failure

(PALF) is based primarily upon clinical history, which is dependent upon patient and parent recall of drug dose and administration times. APAP levels are typically obtained as a screening measure in patients with ALF when the dosing history is inconclusive for an acute APAP overdose, but interpretation of results requires a broader clinical context. For example, APAP levels are frequently within the therapeutic range, and thus, inconclusive, when clinical presentation to the medical facility is delayed or exposure has been chronic. [3,4]

The toxicity of APAP is largely attributed to metabolism of the drug. [5] Following use of recommended doses, APAP undergoes conjugation to produce metabolites eliminated by the kidney. A small portion of the drug is oxidized through CYP P450 in the liver to form the metabolite N-acetyl para-quinoneimine (NAPQI), which is highly reactive and under normal circumstances is detoxified by hepatic glutathione. Following large single dose exposure, the conjugation capacity of the liver is exceeded and hepatic glutathione stores are depleted allowing NAPQI to selectively bind to cysteine amino acids in proteins. [6] As a result, normal hepatocellular function is disrupted and large amounts of APAP protein adducts, which are released from hepatocytes upon hepatocyte lysis, can be detected in the serum. [7]

In an earlier report, APAP protein adducts were detected in 12.5% of 64 children with ALF of indeterminate etiology. [8] Since the publication of this original series, an additional 286 children with ALF of indeterminate etiology were enrolled in the Pediatric Acute Liver Failure Study Group (PALFSG). In this report, we expand upon that initial analysis by describing summary data for measurement of adducts in an expanded cohort, and now include patients with all diagnoses in the analysis.

Materials and Methods

Pediatric Acute Liver Failure Study Group

Data and samples were collected through the PALFSG, a multi-center, longitudinal study that includes a serum repository. [1] Initiated in 1999, the registry includes data on children (under the age of 18) with ALF, collected from 24 participating centers in the United States, Canada and United Kingdom. Enrollment criteria for the PALFSG, included acute liver injury combined with either severe coagulopathy (International Normalized Ratio [INR] greater than 2.0 or prothrombin time [PT] greater than 20 seconds) or encephalopathy with moderate coagulopathy (INR ≥ 1.5 or PT ≥ 15 seconds). Patients with known chronic liver disease were excluded. Written, informed consent was obtained from the participant's parent/legal guardian before participation in the study. The PALFSG protocol was approved by the individual Institutional Review Boards of participating centers. After enrollment, demographic, clinical, and diagnostic data were recorded daily for up to 7 days. Outcomes (death, liver transplantation, hospital discharge) within 21 days of enrollment were recorded for all participants in the registry. Diagnostic and clinical evaluations were performed under the direction of the attending physician at the clinical site and constituted the local standard of care. A final diagnosis was assigned by the primary investigator at the clinical site as summarized previously. [1] Diagnostic criteria for acute APAP toxicity in the PALFSG included a toxic serum APAP level by Rumack nomogram or a history of an acute ingestion of 100 mg/kg within a 24-hour period. [2,8] Data were transmitted to a central data coordinating center where data editing and quality control procedures occurred.

If clinical care permitted, a single daily serum sample was collected with the first morning blood draw following enrollment and daily for up to seven days, or until death, liver transplantation, or hospital discharge. Following centrifugation, aliquoted serum samples were frozen at −80°C at the site and batch-shipped to the research bio-repository for storage. The frequency and volume of serum collected for research purposes was dependent upon patient weight, hemoglobin, and daily volume of blood required for diagnosis and patient management. Given these patient safety restrictions, research samples were not available at all potential time points for all PALFSG participants. This analysis was performed in serum samples from the PALFSG registry. Previously reported results for 104 patients, 64 patients with indeterminate ALF, 10 with APAP toxicity and 30 with other specific diagnoses are included in this analysis. [8]

Measurement of Serum APAP Adducts Concentrations

Serum samples (100–500 μL each) from subjects were analyzed, without knowledge of clinical parameters, final diagnosis, or outcomes, for APAP protein adducts using a previously reported and validated method. [9,10] In a previous report, receiver operator curve analysis established a toxicity threshold of ≥1.1 nmol/mL of APAP protein adducts in serum (sensitivity, 96.8%; specificity, 95%) in patients with APAP overdose and an alanine aminotransferase (ALT) value >1000 IU/L. [9] For the purposes of this analysis, adducts values ≥ 1.0 nmol/mL are defined as positive. We chose the > 1.0 nmol/mL cut-off to allow us to be consistent with the recent Acute Liver Failure Study Group publication examining adducts in adults. [11] Also, this cut-off has been found to yield similar sensitivity and specificity as the previously reported 1.1 nmol/mL threshold (James personal communication). For the 104 patients enrolled between 1999 and 2004, also included in the initial report, [8] quantitative adducts levels below 1.0 nmol/mL are not reported due to limitations in the sensitivity of the earlier assay. [10] The median and interquartile range for adducts levels are reported for the 289 participants with serum tested using the most recently published method which includes a more efficient proteolytic digestion step allowing for more precise quantification below the cut-off value. [9,11] In the APAP overdose group, the median values were calculated from the first sample tested for each patient.

Statistical Analysis

Demographic data, encephalopathy grade, and laboratory values were determined on day of enrollment. If unavailable, they were imputed from values three days or less before enrollment. Percentages were reported for demographic characteristics, final diagnosis and outcomes, if categorical, and medians and interquartile ranges were reported for continuous laboratory values. Pearson chi-square tests of association were used to test differences in proportions between those who were adducts positive versus adducts negative. McNemar chi-squares were used to test associations between measurement thresholds from the same samples. Exact tests were used when cells were sparse. Wilcoxon rank-sum tests were used to test for differences in distributions between those with different adducts status for continuous variables. P-values less than 0.05 were used to determine statistical significance. Analyses were conducted using SAS Statistical Software (SAS Institute, Cary, NC).

Results

Study Population

The PALFSG enrolled 986 participants between December 1999 and October 2010. Of these, a convenience sample of 393 were selected based on availability of serum for adducts testing; 190 with indeterminate diagnosis, 145 with established diagnosis other than APAP toxicity and 58 with APAP toxicity. Twelve patients with APAP toxicity due to a single dose exposure had daily serial adducts level measurements. Table 1 describes their clinical characteristics.

Participants with Acetaminophen Overdose Diagnosis

Among 58 participants with a final diagnosis of APAP overdose and samples available for adducts testing, 42 had a history of single dose ingestion, 10 had multiple dose exposure and in 6 the number of doses could not be determined. Patients with APAP ingestion were enrolled between 1 to 6 days following ingestion, with 93 % enrolling within 4 days or less of the ingestion. Only 12% were less than 10 years of age and 79% were female with 91% non-Hispanic, Table 2. Eighty-six percent of the samples tested (37/42 single dose, 8/10 multiple dose and 5/6 unknown dose) had adducts levels ≥ 1.0 nmol/ml. The median level for APAP adducts in participants with APAP overdose was 4.8 (Q1–Q3: 2.4–9.6) nmol/ml. No significant differences were detected in adducts levels between single and multiple dose groups. Over half (5/8) of the participants with negative adducts results were adolescents with a single ingestion. The sample (for adducts analysis) for 5 of the 8 participants was obtained on the second day following study enrollment. The interval of ingestion to enrollment was two days or more in 5 participants and unknown in 3 participants. Serum APAP levels were available for 45/58 APAP overdose participants, with 6/7 (86%) of adducts negative and 21/38(55%) of adducts positive patients having a level of < 10 mg/L at the time of testing, $p < 0.0001$. Two participants in the APAP overdose cohort died and three were transplanted.

Serial testing for adducts levels on 3 or more sequential days was available for 12 patients with known APAP toxicity, see trend lines in Figure 1-Supplemental. A typical pattern of rapid clearance during the first two days following presentation was exhibited by most and the fall in adducts levels paralleled decreasing ALT values.

Participants with Indeterminate Diagnosis

Adducts levels ≥ 1.0 nmol/mL were detected in samples from 21 of 190 (11%) participants with indeterminate etiology, with a median level of 3.07 (Q1–Q3: 2.61–6.93) nmol/ml in the 16 specimens with quantitative levels. Adducts positive participants were less likely to be Hispanic ($p=0.012$), Table 3. Ninety percent of participants with adducts levels ≥ 1.0 nmol/mL had a recent history of APAP use as compared to 41% of adducts negative participants ($p<0.0001$). Among the 21 adducts positive patients, 3 received N-acetylcysteine (NAC) as participants in a randomized trial [12] and 6 received NAC on a clinical basis as determined by their physicians. History of exposure to NAC was either unknown (n=1) or negative (n=11) in the remaining patients. There was no significant difference in 21 day outcomes between the 9 patients that received NAC and the 12 patients

that did not have documented NAC therapy, p=0.77. Table 4 shows laboratory parameters at presentation and 21 day outcomes. Participants with adducts ≥ 1.0 nmol/mL had significantly higher hepatic transaminase levels and creatinine values, but significantly lower total and direct bilirubin. In addition, adducts positive participants were more likely to present with Grade III-IV hepatic encephalopathy (p=<0.0001). Despite higher encephalopathy grades at presentation, participants with adducts ≥ 1.0 nmol/mL were less likely to die or undergo liver transplantation (p=0.03) with 16/21 (76%) versus 75/169 (44%) spontaneous survivors in the adducts positive and negative groups, respectively.

Participants with Specific Diagnosis

There were 145 participants with other specific diagnoses, (Supplemental Table). In this group, 8 (6%) had adducts ≥ 1.0 nmol/mL, with a median level 1.70 (Q1–Q3: 1.44–4.40) in the 5 specimens with quantitative levels. The distribution of adducts positive patients was as follows: infectious hepatitis (1/27), ischemic liver or veno-occlusive disease (2/13), Hemophagocytic Lymphohistocytosis (1/5), fatty acid oxidation (FAO) defects (2/2), cocaine exposure (1/1) and sepsis (1/1). All other patients were negative including other metabolic diseases (n=27), autoimmune hepatitis (n=29), drug-induced liver injury (n=13), and neonatal iron storage disease (n=10). In contrast to the indeterminate group, participants with adducts ≥ 1.0 nmol/mL with a specific diagnosis were more likely to be Hispanic (p=0.02). There were no other significant demographic differences. Although adducts positive participants were more likely to have a history of acetaminophen exposure (50% versus 26%, p<0.0001), 4 of 8 adducts positive participants had no documented history of acetaminophen exposure elicited. None of the adducts positive patients participated in the NAC trial and 1 patient received NAC per the direction of the attending physician. History of NAC exposure was negative in the remaining 7 patients. The adducts positive participants with specific diagnoses had higher hepatic transaminase levels and lower median total and direct bilirubin at presentation, but differences were less pronounced than in the indeterminate group, Table 5. There were no differences in creatinine or coagulation status at presentation. The level of encephalopathy at presentation and at maximal level did not vary by adducts status and adducts status did not affect 21 day outcomes.

Discussion

This study confirms and extends a previously published report examining the potential clinical utility of APAP protein adducts as a biomarker of APAP toxicity in children with ALF. [8] In the present study, APAP protein adducts were detected in 86% of patients with documented APAP overdose, 6% of those with a confirmed other diagnosis and 11% of children with ALF of indeterminate etiology. Adducts positive participants in both the other specific diagnoses and indeterminate diagnosis groups had higher liver enzymes and lower total bilirubin levels than adducts negative patients, a biochemical pattern that closely resembled that of known APAP cases. While patient outcomes in the specific diagnosis group did not vary significantly by adducts status, adducts positive patients from the indeterminate group were less likely to die or to require liver transplantation, despite having higher levels of encephalopathy at presentation. It was also notable that adducts positive patients had higher serum creatinine levels than adducts negative patients. Higher rates of

spontaneous survival for indeterminate ALF patients with positive APAP adducts were previously reported in the adult literature. [11] Although findings are not adequate to support current use of adducts testing for individual patient decisions, it is possible adducts determination might become useful as part of a broader biomarker panel to predict outcomes and guide transplant decisions.

Adducts testing has proven a valuable tool to confirm APAP induced liver injury which has a period of latency in the immediate time period after APAP overdose. In our study, 86% of patients with known APAP liver injury tested positive. Among those that tested negative, samples appeared to have been obtained later in the course of toxicity, at a point in time at which liver injury was likely resolving. In previous studies conducted in adults with ALF secondary to APAP, peak adducts measurements were observed to occur around 72 to 96 hours following acute single dose ingestion of APAP. The elimination half-life of adducts in adults with APAP-induced ALF was 1.73 days, [9] while the median elimination half-life of adducts in children and adolescents presenting to hospital emergency departments with acute APAP toxicity, but without liver failure was 1.47 days. [13] Regardless of degree of liver injury, adducts elimination half-lives are prolonged compared to the parent drug, APAP. The APAP elimination half-life is approximately 2–3 hours following low dose exposure, and may be as prolonged as 18 hours in APAP overdose cases with encephalopathy. [9] Even if adducts clearance in children is more rapid than adults due to either developmental changes associated with endogenous protease function and/or differences in co-morbidities, adducts are eliminated more slowly than the parent drug.

While adducts testing can identify a sub-group of patients with previously unrecognized APAP induced liver injury, the relative contribution of APAP to other causes of ALF remains undefined. In situations where a clear etiology was established, examples from this cohort include shock/ischemia and fatty acid oxidation defects, APAP protein adducts testing may identify patients with a multi-factorial etiology. The additive impact of APAP toxicity may change the natural progression of injury in patients with a singular etiology, making prognosis more difficult to predict. [14]

Patients who tested positive for adducts were significantly more likely to have a history of APAP exposure to dosages below the generally accepted toxicity threshold. Although drug exposure history is more easily obtained for children with ALF than adults since parents are available to provide dosing details, reporting of over-the-counter medication exposure can be highly inaccurate. Even when focused questions are asked regarding APAP exposure, parents may be unaware of APAP as an ingredient in over-the-counter medications. [15–17] Adducts status did not vary by age, although multiple previous publications have suggested infants are at higher risk for inadvertent overdosing of APAP due to confusion with labeling of APAP products designed for infants. [18] Since these samples were collected during the last ten years, initiatives to improve package labeling and raise public awareness of the content of multi-drug over-the-counter preparations and safe dosing guidelines may have reduced this risk. However, these findings emphasize the importance of eliciting a detailed and accurate history of APAP exposure in evaluation of any liver injury.

NAC ameliorates or prevents liver injury following acute APAP overdose. [4] NAC has also been tested as a therapy in PALF not due to acute APAP overdose. [12] In that randomized trial, NAC did not improve overall one year survival among PALF participants, and a sub-analysis revealed patient survival with native liver at one year was significantly better among participants who received placebo than those who received NAC treatment. This study included 21 patients with an indeterminate diagnosis that received NAC either clinically or as participants in a trial. There were no apparent differences in their outcomes, but the small sample would only have allowed for observation of a moderate to large effect size. Randomized studies examining safety and efficacy of NAC therapy in adducts positive children are necessary to determine if adducts status should direct NAC utilization in this population.

An important limitation is serum samples were not available for all participants in the PALF cohort. Previous reports from our study group detail probable causes for incomplete sample collection with patient size and volume limits on blood sampling for research being important obstacles.[19] In choosing a convenience cohort, we thus limit generalizability of our findings to young infants since blood samples were less likely to be available for children under 12 months of age.

Conclusion

In summary, we report testing for APAP protein adducts in a large, multi-center cohort of children with ALF. Among children with ALF of indeterminate cause, those that were adducts positive and had a clinical phenotype that included high liver enzymes and low serum bilirubin were highly significantly different from patients who were adducts negative. These adducts positive patients also had a better prognosis. Adducts were identified within a small percentage of patients with established diagnoses. In these patients, adducts were associated with a similar clinical phenotype, but adducts status was not associated with prognosis. These data suggest occult APAP toxicity may play a role in approximately 10% of children where a distinct diagnosis for ALF cannot be established. These findings have future implications for management of children with ALF of indeterminate cause since adducts status is related to prognosis in this group.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The PALFSG investigators are listed as supplementary material in the Appendix.

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Table 1

Clinical Characteristics of Participants (n=393)

	Adduct Sample n=393
	n(%)
Age (years)	
< 1	79(20.1)
1–2	57(14.5)
3–9	103(26.2)
10–17	154(39.2)
Gender	
Male	192(48.9)
Female	201(51.1)
Race	
White	295(76.0)
African American	55(14.2)
Other	38(9.8)
Unknown	5
Final Diagnosis	
Acetaminophen Toxicity	58(14.8)
Metabolic	27(6.9)
Autoimmune	29(7.4)
Infection	26(6.6)
Indeterminate	190(48.4)
Other*	63(16.0)
Outcome at 21 days	
Death without transplantation	44(11.2)
Transplantation	115(29.3)
Spontaneous Survival	234(59.5)

* Common other diagnoses included hemophagocytic syndrome (5), shock/ischemia (8), neonatal iron storage (10), non-acetaminophen drug induced (13), and venoocclusive disease (5).

Table 2

Characteristics and Outcomes of Participants (n=58) with a Final Diagnosis of Acetaminophen Toxicity

Age (Years)	**n(%)**
<1	2(3)
1–2	1(2)
3–9	4(7)
10–17	51(88)
Gender	
Female	46(79)
Ethnicity	
Hispanic or Latino	5(9)
Race	
White	44(77)
African American	5(9)
Other	8(14)
Hepatic Encephalopathy at Enrollment	
Grade II or less	51
Grade III or IV	7
Laboratory Value Median (25%– 75%)	
Alanine Aminotransferase IU/L (n=55)	5305(3371–7771)
Aspartate Aminotransferase IU/L (n=58)	4053(2022–7605)
Total Bilirubin mg/dL (n=58)	2.2(1.2–3.1)
Direct Bilirubin mg/dL (n=51)	0.6(0.3–1.2)
International Normalized Ratio (n=53)	2.3(1.7–3.7)
Creatinine mg/dL (n=57)	0.7(0.6–1.1)
21 day Outcome	
Death without Transplantation	2(3.5%)
Transplantation	3(5.2%)
Spontaneous Survival	53(91.4%)

Table 3

Participants (n=190) with Indeterminate Etiology of Acute Liver Failure: Baseline Characteristics by Adduct Status

	Adduct Positive n=21	**Adduct Negative n=169**	***P***
	n(%)	**n(%)**	
Age (Years)			0.071
< 1	1(5)	34(20)	
1–2	6(29)	32(19)	
3–9	5(24)	63(37)	
10–17	9(43)	40(24)	
Gender			0.083
Female	13(62)	71(42)	
Ethnicity			0.012
Non-Hispanic	21(100.0)	124(73)	
Hispanic	0	45(26)	
Race			0.093
White	13(62)	128(76)	
African American	4(19)	30(18)	
Other	4 (19)	10 (6)	
Unknown	--	1	

Table 4

Outcomes of Participants (n=190) with Indeterminate Etiology

	Adducts Positive n=21	Adducts Negative n=169	*P*
	n(%)	n(%)	
Hepatic Encephalopathy at Enrollment*			<0.0001
Grade II or less	12(63)	154(93)	
Grade III or IV	7(37)	12(7)	
Maximum Hepatic Encephalopathy**			0.10
Grade II or less	12(60)	126(77)	
Grade III or IV	8(40)	38(23)	
Laboratory Value Median (Q1 – Q3)			
Alanine Aminotransferase IU/L	4619(3615–6871)	1604(726–2796)	<0.0001
Aspartate Aminotransferase IU/L	3689(2305–7146)	1785(713–3088)	0.0004
Total Bilirubin mg/dL	3.6(2.7–4.5)	15.2(8.7–20.2)	<0.0001
Direct Bilirubin mg/dL	1.9(0.8–2.4)	10.5(4.9–14.4)	<0.0001
International Normalized Ratio	3.7(2.4–4.4)	2.7(2.1–4.0)	0.17
Creatinine mg/dL	0.7(0.5–1.1)	0.4(0.3–0.7)	0.0043
21 day Outcome			0.03
Death without Transplantation	1(5)	18(11)	
Transplantation	4(19)	76(45)	
Spontaneous Survival	16(76)	75(44)	

* 2 participants in adduct positive group and 3 participants in negative group with missing data

** 1 participants in adduct positive group and 5 in adduct negative group with missing data

Table 5

Outcomes of Participants (n=145) with Specific Non- Acetaminophen Diagnoses

	Adduct Positive n=8	**Adduct Negative n=137**	***P***
	n(%)	**n(%)**	
Hepatic Encephalopathy at Enrollment*			0.25
Grade II or less	6(75)	117(89)	
Grade III or IV	2(25)	15(11)	
Maximum Hepatic Encephalopathy**			1.00
Grade II or less	6(75)	104(81)	
Grade III or IV	2(25)	25(19)	
Laboratory Value Median (Q1–Q3)			
Alanine Aminotransferase IU/L	2592 (1402–5945)	638 (113–2121)	0.007
Aspartate Aminotransferase IU/L	3461 (686–6090)	911 (214–2717)	0.04
Total Bilirubin mg/dL	2.6 (1.2–6.0)	9.2 (4.2–18.0)	0.01
Direct Bilirubin mg/dL	1.4 (0.7–2.0)	4.9 (2.0–12.4)	0.01
International Normalized Ratio	2.7 (2.0–4.1)	2.5 (2.1–3.3)	0.63
Creatinine mg/dL	0.7 (0.3–2.2)	0.5 (0.3–0.7)	0.62
21 day Outcome			0.87
Death without Transplantation	1(13)	22(16)	
Transplantation	1(13)	31(23)	
Spontaneous Survival	6(75)	84(61)	

* 5 participants with unknown encephalopathy in negative group

** 8 participants with unknown maximal encephalopathy in negative group