Original Article

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Outcomes After Initial Rehydration with Isotonic Saline or Ringer's Lactate in Pediatric Acute Gastroenteritis (1-59 Months): A Single-center Retrospective Cross-sectional Study

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Abstract

Aim: The optimal choice of initial intravenous crystalloid solution in pediatric acute gastroenteritis (AGE) remains a matter of clinical debate due to differing effects on acid–base status and overall recovery. In this context, we aimed to compare clinical recovery and laboratory changes in children with AGE treated with isotonic saline (IS) versus Ringer's lactate (RL) as initial replacement therapy.

Methods: This single-center retrospective cross-sectional study included patients aged 1-59 months who presented with dehydration due to AGE and received initial intravenous rehydration with either IS or RL between January 2022 and February 2023. The primary outcome was the change in a standardized dehydration score from presentation to post-treatment. Secondary outcomes were pre- and post-treatment blood gas and electrolyte values. The comparisons used appropriate statistical tests.

Results: Both groups showed significant clinical improvement after fluid therapy. Dehydration scores decreased, and lactate levels fell significantly from baseline in each group ($p<0.001$ for both). Post-treatment electrolyte and acid-base parameters improved in both arms, with no serious adverse events. Between-group differences favored RL for overall clinical recovery and metabolic profile, although most laboratory changes were similar across groups.

Conclusion: In pediatric AGE, initial isotonic crystalloid replacement rapidly improves clinical and laboratory parameters. Due to its balanced composition and demonstrated benefits in overall recovery, RL may be favored as the primary replacement solution, whereas IS continues to serve as a viable alternative. Prospective randomized trials are warranted to confirm these findings.

Keywords: Child, dehydration, gastroenteritis, fluid therapy, sodium chloride, Ringer's lactate

Introduction

Acute gastroenteritis (AGE) is a significant cause of mortality and morbidity in childhood. Diarrhea, the most prominent symptom of AGE, is the second most common cause of death among children under five, following respiratory tract infections. Each year, 1.7 billion children suffer from AGE, and 525,000 of them lose their lives (1). Dehydration severity can be predicted in patients with significant fluid loss due to acute diarrhea using the clinical dehydration scale (CDS). The severity of dehydration can be determined by physical examination (2).

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Blood gas measurements in children with acute diarrhea can quickly detect acid-base imbalances, including blood potential hydrogen (pH), actual and standard bicarbonate values (HCO_3 act, HCO_3 st), base excess [BE(B), BE(ecf)] in blood and extracellular fluid, and electrolyte levels (3). Metabolic acidosis detected in blood gas can be classified as normal anion gap or increased anion gap metabolic acidosis. Hyperchloremic metabolic acidosis is a typical example of normal anion gap metabolic acidosis. Abnormal gastrointestinal bicarbonate loss in acute diarrhea can lead to hyperchloremic acidosis when large amounts of high-chloride solutions, such as isotonic saline (IS), are given (4). In addition, every 1-unit change in serum pH is associated with an average change in ionized calcium (iCa) by 0.36 and potassium by 0.6 mmol/L (5,6). Isotonic saline is an isotonic sodium chloride solution containing 154 meq of sodium and 154 meq of chloride per liter (7). Ringer's lactate (RL) contains 130 meq of sodium, 109 meq of chloride, 4 meq of potassium, 3 meq of calcium, and 28 meq of lactate per liter, with lactate being metabolized in the liver to become HCO_3 (8).

The aim of our study is to compare changes in laboratory values and clinical conditions of children presenting to the emergency department with acute diarrhea when IS or RL is preferred as the initial rehydration solution. We assessed post-treatment clinical and laboratory changes after initial rehydration with IS vs. RL and determine which solution offers superior overall recovery. We hypothesized that RL, compared with IS, would yield greater clinical recovery, reflected by a larger reduction in a standardized dehydration score and a more favorable metabolic profile (higher pH and lower lactate/chloride) without increasing adverse events.

Materials and Methods

Compliance with Ethical Standards

The study protocol was conducted in accordance with the Helsinki Declaration. Ethical approval to conduct this study was obtained from the University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (approval no.: 74-2023, date: 12.04.2023). Written consent was obtained from the parents of enrolled children.

Study Design and Patient Selection

We conducted a retrospective cross-sectional study. The study included patients aged 1 month to 5 years who presented to pediatric emergency departments with acute diarrhea and significant dehydration and received initial rehydration treatment between January 01, 2022, and February 28, 2023. The study was conducted in a single academic hospital located in the city center.

Patients receiving RL as replacement therapy (Group 1) and those receiving IS as replacement therapy (Group 2) were categorized accordingly. Patients with venous blood gas results and dehydration scores in their anamnesis, before and after replacement therapy, were included in the study. Patients with chronic causes of diarrhea (short bowel syndrome, malabsorption disease, celiac disease, etc.); those with mild dehydration; those with additional diseases that could cause electrolyte imbalance (renal tubular acidosis, adrenal diseases, cystic fibrosis, diabetic ketoacidosis, etc.); those with less than 30 minutes or more than 90 minutes between pre- and post-replacement blood gas analysis; those who had capillary or arterial blood gases; and those receiving replacement fluids other than IS or RL were excluded. Malnourished [<-2 standard deviation score (SDS) weight] and obese (>+2 SDS weight) patients were also excluded. Patients with life-threatening conditions, patients with elevated acute phase reactants suggestive of bacterial diarrhea, and patients with inflammatory bowel disease were excluded. Patients were selected based on inclusion and exclusion criteria, forming the sample group. The sample size was determined using the G*power Version 3.1.6 program with a standard effect size of 0.63, resulting in a total of 100 cases (50 for each group) for a study power of 90% and a 5% error rate (9).

During system screening, 149 patients were identified for Group 1. Of these, 47 patients were excluded due to deficiencies in blood gas analysis, other diagnostic tests, and/or incomplete medical histories. The remaining 102 patients were further reduced to 55 through random sampling, and these 55 patients were then included in the study. For Group 2, 428 patients were identified through system scans. Of these, 278 patients were excluded from the study due to the absence of pre- and post-replacement treatment blood gases in the system (e.g., delayed blood gas after fluid administration, clotted blood gas results due to treatment refusal, patients leaving without undergoing follow-up tests, etc.). Additionally, 41 patients were excluded from the study in Group 2 due to deficiencies in other blood tests or medical history information, such as dehydration scores and weight. To ensure equality between the groups, 55 patients were selected from the remaining 109 patients using the method of random sampling and included in the study (Figure 1).

Definitions, Scoring, Treatment, and Equality of Tests

Acute diarrhea was defined as lasting no more than seven days with more than three watery stools per day (1). The CDS from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition 2014 guidelines was used to determine dehydration scores.

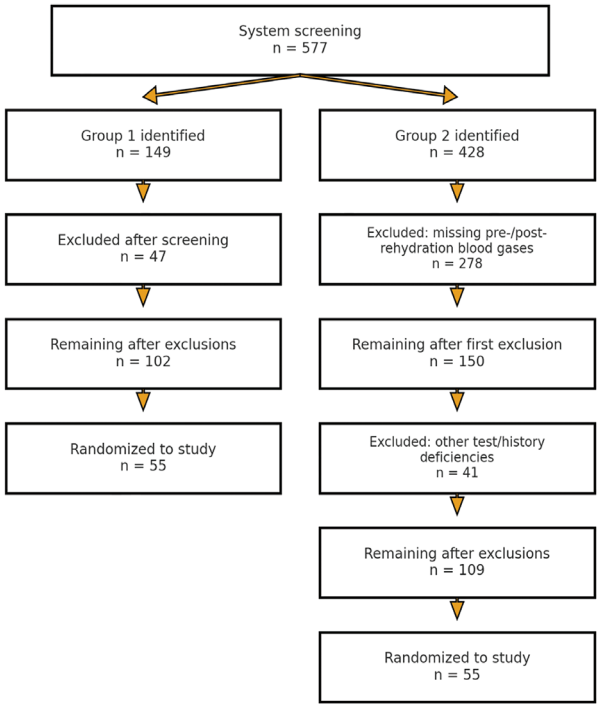

Figure 1. Flowchart of study

The scale evaluates four clinical signs—general condition, eyes, mucous membranes (tongue), and the presence of tears—with a scoring range of 0-2 points for each. A score of 0 indicates no or mild dehydration, 1-4 points indicate moderate dehydration, and 5-8 points indicate severe dehydration (2). Dehydration scores before and after treatment were calculated only for patients assessed by pediatric doctors. The anion gap was calculated as sodium - (bicarbonate + chloride) (10). According to the National Institute for Health and Care Excellence guidelines, glucose-free fluids containing 131-154 mEq/L of sodium are recommended for children with diarrhea and vomiting; hence the comparison between RL and IS (11). Patients who received 20 mL/kg of replacement fluid within 1 hour to ensure treatment protocol uniformity were included in the study. As a hospital policy, we administer initial fluid therapy to all children with moderate to severe dehydration at a dose of 20 mL/kg in 1 hour. All tests and scoring were performed immediately after the end of bolus treatment.

Statistical Analysis

Statistical analysis was conducted using SPSS 28.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The Shapiro-Wilk test was used to assess normal distribution. Descriptive statistics were presented, including numbers and percentages for categorical variables and mean, standard deviation, minimum, maximum, and median values for numerical variables. Categorical variables were compared using the chi-square test. Student's t-test and the Mann-Whitney U test were used to compare mean or median values between the two groups, depending on the sample distribution. The Wilcoxon test was used to determine whether there was a difference between the measurements at two different times or conditions. A paired samples t-test was used to test the significance of the difference between the arithmetic means of two related groups, provided that the condition of normality of distribution was met. The statistical significance level was set at $p<0.05$.

Results

Of the 110 patients included in the study, 51 (46.4%) were male, and 59 (53.6%) were female. Among patients receiving RL (Group 1) as replacement therapy, 26 (47.3%) were male, and 29 (52.7%) were female, with a median age of 22 months (min.: 1 months, max.: 58 months). Patients receiving IS (Group 2) as replacement therapy included 25 (45.5%) males and 30 (54.5%) females, with a median age of 26 months (min: 1 month, max: 59 months). No statistically significant differences were observed between the groups regarding gender and age ($p=1$ and $p=0.974$, respectively).

29% of the patients were severely dehydrated, while 71% had moderate dehydration. The dehydration score at admission in Group 1 was statistically significantly higher than in Group 2 ($p=0.008$). There was no statistically significant difference in the dehydration scores during the first hour between the groups after treatment ($p=0.260$). The change in the dehydration score with treatment in Group 1 was statistically significant and higher ($p<0.001$) (Figure 2).

Comparisons of pre- and post-replacement therapy venous blood gases and blood gas changes between the groups are shown in Table 1. Changes in laboratory values with replacement therapy for patients are presented in Table 2.

Discussion

The most significant outcome of our study is that in moderately to severely dehydrated patients diagnosed with AGE, RL solution provided more effective improvement in clinical and laboratory findings compared to IS as initial replacement therapy. Another important finding is that in children with metabolic acidosis due to diarrhea, replacement therapy with RL corrected acidosis more robustly compared to IS while also lessening the potential electrolyte disturbances associated with pH elevation. Despite the retrospective nature of our study, it facilitated the comparison of two distinct fluid therapies in children by evaluating blood tests and dehydration scores before and after fluid administration. This approach may serve

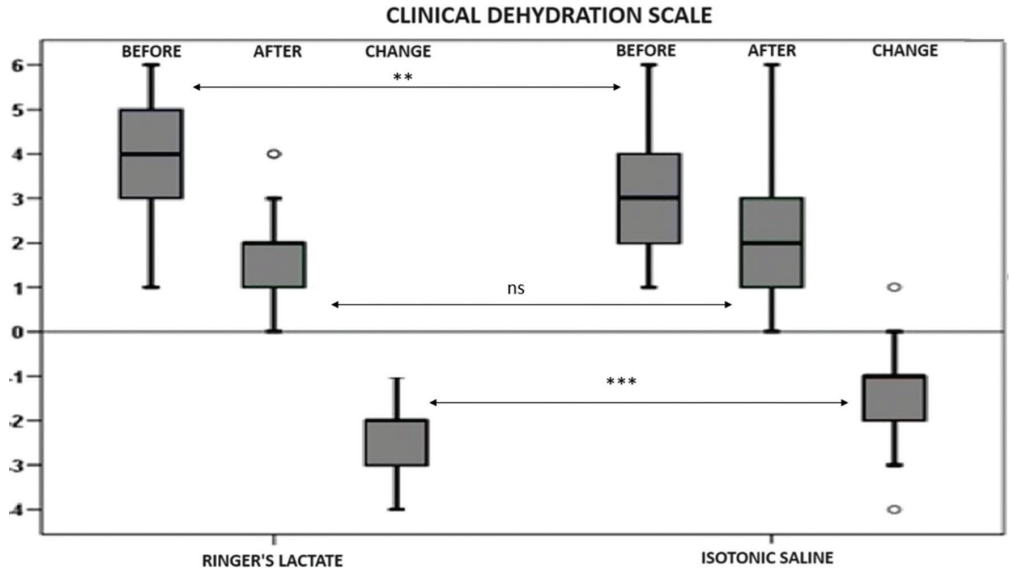

Figure 2. Change in dehydration scores before and after treatment by fluid type (IS vs RL). Error bars show 95% CIs
IS: Isotonic saline, RL: Ringer's lactate, CIs: Confidence intervals

as a guide for future prospective studies. In our cohort, both solutions produced rapid improvement. However, the magnitude of recovery—reflected by the larger reduction in the dehydration score and lactate—was more pronounced with RL after initial rehydration.

In studies, the dehydration rate among hospitalized patients with AGE requiring intravenous replacement therapy is reported to be between 26% and 55% (12,13). Recent studies in age groups similar to our study have shown that the rate of severe dehydration in children with diarrhea varies between 3.3 and 12.5% (14,15). In our study, 29% of the cases were severe, and 71% were moderately dehydrated. Our cases, according to the CDS, included patients with moderate to severe dehydration requiring intravenous replacement therapy. Since mildly dehydrated patients were not included in our study, our rate of severe dehydration may appear higher compared to other studies. Clinical dehydration scale scoring indicates the severity of a patient's dehydration at the time of admission and can also assist physicians in determining the patient's clinical status and the rate of recovery following treatment. In our emergency department cohort, RL produced a greater reduction in both the dehydration score and lactate after initial rehydration, suggesting a more favorable acid-base shift. Across most electrolytes, between-arm differences were small, indicating that both crystalloids effectively restored intravascular volume during the index visit.

In a study by Kartha et al. (9), comparing RL and IS treatments in children with AGE during acute dehydration, no significant difference was observed in the resolution of dehydration symptoms between the groups. In our study, the dehydration score at admission in Group 1 was higher compared to Group 2, and the decrease in dehydration scores with treatment in Group 1 was significantly greater. This difference may be attributed to the fact that our study applied a rapid 20 mL/kg fluid replacement within 1 hour, compared to the 6-hour slow fluid protocol used by Kartha et al. (9). The preference for RL as a replacement solution for AGE patients may contribute to a greater improvement in clinical and dehydration parameters. In both groups of our study, irrespective of the type of replacement solution, a decrease in dehydration scores was observed with treatment. The majority of dehydration was noted to be hypovolemic, and improvement could be achieved with intravenous fluid therapy (16).

In a study of patients hospitalized with a diagnosis of AGE, 84.8% were found to have acidosis in blood gas analysis (17). In our study, this rate was observed to be 49%, and the difference might be attributed to the absence of patients requiring hospitalization on the ward. Another study conducted in India showed no significant difference in changes in blood pH levels between RL and IS treatments in patients diagnosed with AGE; however, there was a tendency for an increase in both groups (18). In a similar study on the same patient group, but with different treatment protocols, where 100 cc/kg of fluid was administered over 3 or 6 hours with simultaneous oral rehydration solution support, the results may differ due to these various factors. In a study by Kartha et al. (9), comparing RL and IS during a 6-hour treatment period, both groups exhibited an increasing trend in pH levels, with no significant difference between the groups.

Table 1. Comparison of pre- and post-replacement therapy blood gas results and their changes in the groups

		Group 1	Group 2	p-value
pH	Before replacement	7.33±0.05	7.36±0.05	0.020#
	After replacement	7.38±0.04	7.35±0.05	<0.001#
	Change	0.045 (-0.02; 0.16)	-0.01 (-0.10; 0.07)	<0.001#
	p	**<0.001**ø	**0.013**ø	
pCO_2 (mmHg)	Before replacement	31.05±5.18	30.66±4.95	0.685#
	After replacement	30.40±4.44	31.27±5.39	0.360#
	Change	-0.5 (-10.1; 7.4)	0.5 (-10.1; 18.6)	0.151#
	p	0.266ø	0.356ø	
HCO_3 st (mmol/L)	Before replacement	17.45±2.53	18.29±2.61	0.091#
	After replacement	19.37±2.34	17.92±2.54	0.002#
	Change	1.7 (-1.6; 6.3)	-0.3 (-3.7; -2)	<0.001#
	p	**<0.001**ø	**0.032**ø	
HCO_3 act (mmol/L)	Before replacement	16.29±3.24	16.97±3.27	0.273#
	After replacement	17.96±2.934	16.69±3.23	**0.034**#
	Change	1.2 (-2.4; 5.8)	0 (-5.1; 3.6)	**<0.001**#
	p	**<0.001**ø	0.266ø	
BE (B) (mmol/L)	Before replacement	-8.1 (-16.3; -0.4)	-6.9 (-14.1; 1)	0.140#
	After replacement	-6.3 (-12.4; 0)	-7.5 (-16.4; -0.6)	**0.005**#
	Change	1.8 (-1.9; 7.2)	-0.5 (-5.1; 2.4)	**<0.001**#
	P	**<0.001**ø	**0.020**ø	
BE (ecf) (mmol/L)	Before replacement	-9.2 (-19; -0.13)	-8.1 (-16; 0.5)	0.319#
	After replacement	-7.3 (-14.3; -0.1)	-8.6 (-18.2; -1.2)	**0.012**#
	Change	1.9 (-5.67; 7.5)	-0.3 (-5.1; 4.4)	**<0.001**#
	p	**<0.001**ø	0.153ø	

The values are presented as median (minimum; maximum) and mean ± standard deviation
ø: Paired t-test, #: Student t-test, HCO_3 act: Actual bicarbonate, HCO_3 st: Standard bicarbonate (not affected by respiratory acid-base imbalance), BE(B): Base excess in blood, BE(ecf): Extracellular fluid base excess, pH: Potential of hydrogen, pCO_2: Partial pressure of carbon dioxide

Similarly, in another study in India comparing fluid treatments in 72 children with acute diarrhea, similar to our study, RL solution was found to cause a greater increase in serum pH compared to IS (19). A retrospective analysis of blood gas results in children under 5 years undergoing craniofacial surgery in Uruguay revealed more acidosis in the IS group compared to the RL group (20).

In our study, the group receiving RL treatment showed an increase in pH levels, indicating the correction of acidosis. In contrast, the group receiving IS treatment exhibited a statistically significant decrease in pH levels, suggesting a deepening of acidosis. We believe that the exacerbation of acidosis with IS treatment may be attributed to the increase in chloride ions, a negatively charged anion at high concentrations in the solution, leading to hyperchloremic metabolic acidosis by reducing bicarbonate (21,22). Additionally, we posit that the lactate in the content of RL solution, when metabolized in the liver and converted to bicarbonate, may contribute to an improvement in pH values (23). This signal persisted after the two-step screening and the random selection of cases was balanced to equalize groups, with no signal of harm compared with IS in this dataset. Taken together, these study-specific observations support the pragmatic use of RL when metabolic acidosis predominates at presentation, while acknowledging IS as a reasonable alternative when RL is unavailable.

We observed a significant increase in serum HCO_3 levels in Group 1 while noting a decrease in Group 2 following fluid therapies. Previous studies have demonstrated that balanced solutions result in a greater increase in serum bicarbonate levels compared to IS (18,19,24). In a study involving adult patients, the effects of RL and IS treatments on blood gas were investigated, and similar to our findings, a decrease in bicarbonate levels after treatment was observed in the group receiving IS (25).

Table 2. The change in laboratory values with replacement therapy in patients				
		Group 1	**Group 2**	**p-value**
Sodium (mmol/L)	Before replacement	133.92±2.43	133.95±3.51	0.490*
	After replacement	133.51±1.94	134.18±4.90	0.260*
	Change	-0.6 (-4.5; 4.4)	0.4 (-21.4; 6)	**0.005***
	p¥	0.043	0.061	
Potassium (mmol/L)	Before replacement	3.90±0.48	3.98±0.39	0.354#
	After replacement	3.74±0.43	3.82±0.51	0.352#
	Change	-0.09 (-1.28; 0.42)	-0.26 (-1.17; 0.77)	0.921#
	p∅	0.001	**0.008**	
Chloride (mmol/L)	Before replacement	106.04±3.89	106.22±5.41	0.661*
	After replacement	107.04±3.14	107.85±5.57	0.351*
	Change	1 (-10; 6)	2 (-24; 10)	**0.026***
	p¥	<0.001	<0.001	
Ionized calcium (mmol/L)	Before replacement	1.20±0.07	1.20±0.07	0.671*
	After replacement	1.19±0.05	1.17±0.09	0.320#
	Change	-0.02 (-0.14; 0.19)	-0.03 (-0.29; 0.13)	0.304#
	p∅	0.205	0.017	
Glucose (mg/dL)	Before replacement	74.31±18.70	70.85±19.34	0.267*
	After replacement	75.62±17.81	72.60±22.09	0.163*
	Change	1 (-30; 82)	0 (-28; 95)	0.912*
	P¥	0.877	0.866	
Lactat (mmol/L)	Before replacement	2.03±0.75	1.76±0.60	0.083*
	After replacement	1.55±0.42	1.46±0.49	0.283#
	Change	-0.37 (-2.81; 0.54)	-0.27 (-1.43; 0.83)	0.131#
	p∅	<**0.001**	<**0.001**	
Anion GAP	Before replacement	10.43±3.60	9.45±4.48	0.322*
	After replacement	7 (0.1-15.9)	8.1 (-11.9-17.5)	0.090#
	Change	-3.2 (-9.9; 3.2)	-1.4 (-22.7; 23.6)	**0.001***
	p¥	<**0.001**	0.011	

*: p<0.05, **: p<0.01, ***: p<0.001, All values are presented as median (minimum-maximum) and mean ± standard deviation
#: Student t test, *: Mann Whitney U test, ∅: Paired t-test, ¥: Wilcoxon test

Reviewing our study alongside the aforementioned literature, we find that the increase in bicarbonate levels associated with RL use is due to the lactate content in the solution, while the decrease in bicarbonate levels with IS is attributed to the chloride content (26). Therefore, considering our study and the findings of other related studies, we suggest that in dehydrated patients presenting with diarrhea to pediatric emergency clinics, choosing RL solution may lead to a more robust improvement with an effective increase in bicarbonate levels and subsequent improvement in blood pH compared to IS.

Studies have shown that intravenous fluid therapy with balanced solutions such as RL does not lead to a significant change in serum sodium concentration (9,19,27). According to the results of our study, the decrease in serum sodium values in the group receiving RL treatment may be associated with the lower sodium content of the RL solution (≈130 mEq/L), which is slightly lower than typical plasma sodium levels. This association is particularly relevant in pediatric patients with a diagnosis of AGE, dehydration, and hypernatremia. The use of balanced solutions, like RL, in this patient group may contribute to the easier maintenance of serum electrolyte balance. Additionally, hyponatremia is not a contraindication for RL.

The increase in chloride levels was observed to be higher in the group receiving IS solution in our study compared to the RL group. Despite being referred to as physiological serum, studies have shown that the excessive

use of IS solution, which is not truly a physiological fluid, can lead to elevated chloride levels (28,29). Our study's findings are consistent with these results, indicating that the use of IS solutions can result in higher chloride levels. In a study by Bampoe et al. (30) the use of RL solution in patients undergoing elective surgery was found to reduce the incidence of hyperchloremia compared to IS. The results obtained in our study seem to be consistent with these findings, suggesting that the lower chloride levels in the content of RL solution compared to IS may have a mitigating effect. This implies that choosing RL over IS may reduce the likelihood of a severe course of the disease, especially in patients at high risk of hyperchloremia and related metabolic acidosis. In cases where acidosis is already present, RL may be the preferred choice over IS in patients diagnosed with AGE.

In our study, we observed a decrease in iCa levels in the group receiving IS treatment, while no significant change was observed in the group receiving RL treatment. Ionized calcium levels respond sensitively to changes in serum pH. An increase of 1 unit in serum pH decreases iCa by an average of 0.36 mmol/L and potassium by 0.6 mmol/L (5,6). Similarly, we observed that potassium levels decreased less in patients receiving RL treatment compared to those receiving IS treatment. In our patients who were dehydrated due to diarrhea and whose existing acidosis was corrected with RL treatment, we noticed that, despite the increase in pH levels, iCa levels remained stable, and potassium levels decreased slightly. However, in patients receiving IS treatment, we observed a decrease in iCa and potassium levels despite no increase in pH. This suggests that the content of calcium and potassium in RL solution prevents electrolyte imbalances that can occur due to pH elevation. In terms of preventing potential complications, the use of RL may be considered safer than IS.

In our study, we observed that in the group treated with RL, post-treatment BE levels approached positive values, indicating an increase. In contrast, in the group receiving IS, we found that BE levels decreased after treatment. A study addressing this topic reported a significant decrease in BE levels with both fluid treatments (20). We observed concurrent changes in BE and pH values in both groups. This could be interpreted as indicating that acidosis may have a primarily metabolic origin. In our study, we identified a significant decrease in lactate levels in both treatment groups. Intravenous hydration with both types of fluid therapy may be associated with reduced lactate levels, facilitating the transport of more oxygen to the tissues. Despite the presence of lactate in RL solution, the stable serum lactate levels can be explained by the metabolism of lactate into bicarbonate, which results in decreased serum lactate levels as acid-base balance is achieved. This signal persisted after the two-step screening and balanced random selection of cases to equalize groups, with no signal of harm compared with IS in this dataset.

Study Limitations

Due to the retrospective design of our study, limitations in data availability prevented the comparison of changes in urea and creatinine levels between the groups. Additionally, information regarding the duration of patients' symptoms, the number of diarrheal episodes, the length of hospital stays, and rates of rehospitalization was not accessible. Although the groups were composed of similar patients without comorbidities, additional treatments or foods (e.g., zinc supplementation, hydration) administered by families during the treatment period without the physician's knowledge may have influenced the results, given the retrospective nature of the study. We believe that prospective studies could contribute new insights and knowledge to the medical field by offering more comprehensive data collection and analysis capabilities.

Conclusion

Our study indicates that for patients diagnosed with AGE, RL fluid therapy provides a more effective improvement in clinical and laboratory findings compared to IS fluid therapy. The high chloride levels in IS may delay the correction of acidosis, and we consider RL solution more advantageous in terms of potential complications such as hypokalemia and hypocalcemia during pH correction. Therefore, in the choice of replacement solution in cases of acute diarrhea, RL might be the preferred option if there are no contraindications.

Ethics

Ethics Committee Approval: Ethical approval to conduct this study was obtained from the University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (approval no.: 74-2023, date: 12.04.2023).

Informed Consent: Written consent was obtained from the parents of enrolled children.

Footnotes

Authorship Contributions

Surgical and Medical Practices: C.Y.D., B.O., Concept: C.Y.D., B.O., K.S., Design: C.Y.D., B.O., H.U.H., Data Collection or Processing: C.Y.D., B.O., Analysis or Interpretation: C.Y.D., B.O., H.U.H., K.S., Literature Search: C.Y.D., B.O., H.U.H., K.S., Writing: C.Y.D., B.O., H.U.H., K.S.

Conflict of Interest: No conflicts of interest were declared by the authors.

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