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# Effects of normal saline versus isotonic balanced crystalloid on acid–base balance and renal functions in patients undergoing intracranial tumor resection surgeries

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## Abstract

**Background** Normal saline (NS) infusion in large volumes may result in hyperchloremic metabolic acidosis and renal compromise. Balanced crystalloid (BC) with physiochemical composition akin to that of plasma may avoid these problems associated with NS. The present study aimed to evaluate effects of NS versus BC on acid–base balance and renal functions in patients undergoing intracranial tumor resection surgeries.

**Methods** Fifty adult patients scheduled to undergo elective neurosurgery for intracranial tumor resection were randomized to receive either NS or BC as intraoperative or maintenance fluid. Metabolic and renal parameters were estimated prior to induction (baseline), at 1 h and 2 h after induction, at the end of surgery and 4 h after extubation. Serum neutrophil gelatinase-associated lipocalin (NGAL) was measured postoperatively. Brain relaxation score was assessed by the operating surgeon.

**Results** Baseline values of variables were similar between the groups. At rest of the observed time-points, pH was significantly lower, while blood urea, serum creatinine, sodium, chloride, NGAL and plasma osmolality were significantly higher in the NS group as compared to the BC group. Brain relaxation score, serum bicarbonate and base excess were comparable between the two groups.

**Conclusion** Use of balanced crystalloid (plasmalyte) resulted in better metabolic and renal profile as compared to normal saline in neurosurgical patients.

**Keywords** Balanced crystalloid, Plasmalyte, Normal saline, Metabolic, Renal

## Introduction

Normal saline (NS) is frequently used intraoperatively in patients undergoing neurosurgeries. However, it is not a physiological solution. It has a high chloride content, and infusion of large volumes may result in hyperchloremic

metabolic acidosis [1]. It causes afferent arteriolar vasoconstriction, leading to reduced glomerular filtration rate and acute kidney injury [2]. Balanced crystalloid (BC) solutions such as plasmalyte (PL) and kabylyte have come into use as alternatives to NS. The osmolality, pH and electrolyte content of BC is akin to that of plasma [3]. PL has been associated with fewer adverse effects and complications with regard to metabolic and renal system as compared to NS. [4]

Against this background, the present study was designed to evaluate effects of NS versus isotonic BC on acid–base balance and renal functions in patients

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undergoing intracranial tumor resection surgeries. Primary objectives included evaluation of acid–base status (pH, bicarbonates, base excess) and renal functions [blood urea, serum creatinine, serum electrolytes, NGAL (neutrophil gelatinase-associated lipocalin)]. Secondary objectives included brain relaxation score and plasma osmolality.

## Materials and methods

It was a prospective, randomized, double-blinded study carried out in the Anaesthesiology Department of a tertiary care institute. The study was done from October 2020 to November 2021 after obtaining clearance from Institutional Ethics Committee. The trial was registered with the Clinical Trials Registry India (CTRI/2020/09/027507), and informed written consent was obtained from patients. We included 50 patients of age group 18–45 years, of either sex belonging to American Society of Anesthesiologists (ASA) class I–II, scheduled to undergo elective surgery for intracranial tumor resection (CP angle tumor, glioma, meningioma and posterior fossa tumor). Patients having dyselectrolytemia, preoperative Glasgow Coma Scale (GCS) < 13, hemodynamic instability, pre-existing renal failure (serum creatinine > 2), coagulation abnormalities and diabetes mellitus were excluded from the study.

The patients were randomized into two groups on the basis of computer-generated randomization numbers with the help of <https://www.random.org>.

Group NS ( $n = 25$ ): received normal saline.

Group BC ( $n = 25$ ): received balanced crystalloid (plasmalyte).

The selected fluid was infused intraoperatively and postoperatively till 4 h after the surgery.

In the operating room, all routine monitors such as electrocardiography (ECG), noninvasive blood pressure (NIBP) and pulse oximeter ( $SpO_2$ ) were attached. IV access was obtained and a blood sample was sent for complete hemogram, hematocrit, blood urea, serum creatinine and serum electrolytes (sodium, potassium, chloride) to record baseline values. Left radial artery cannulation was done to monitor invasive BP, and a sample for arterial blood gas (ABG) analysis was sent. IV fluid was administered as per group allocation. The operating neurosurgeon was blinded to the infused fluid by covering the label.

Standard anesthesia protocol was followed in all patients. Induction was done with injection fentanyl 2 mcg/kg, propofol 2 mg/kg and vecuronium 0.1 mg/kg. Airway was secured with a cuffed endotracheal tube. Volume control mode of ventilation was instituted with tidal volume = 7 ml/kg, flow rate = 2.5 L/min, and I/E ratio = 1:2, and frequency was titrated to keep  $EtCO_2$

between 28 and 32 mmHg. Right subclavian vein was cannulated with a triple lumen central venous cannula. Temperature probe (nasopharyngeal) and Foley's catheter were also secured.

Maintenance of anesthesia was done with oxygen (40%) and nitrous oxide (60%) in isoflurane (0.9–1.2 MAC). Vecuronium topups of 1 mg were given hourly for neuromuscular relaxation. Injection Mannitol 1 gm/kg was administered to all the patients before opening of dura. Brain relaxation score was noted by the operating surgeon (same surgeon for all 50 patients) who was blinded to the fluid infused on a four-point scale (1—perfectly relaxed, 2—satisfactorily relaxed, 3—firm brain, and 4—bulging brain). [5]

Fluid was administered as per CVP guidance which was maintained between 8 and 12  $cmH_2O$ . Blood transfusion was carried out in accordance with ASA guidelines [6]. Following completion of surgery, injection glycopyrrolate 0.01 mg/kg and neostigmine 0.05 mg/kg were administered to reverse neuromuscular blockade. Patients were shifted to post-anesthesia care unit after extubation. The same IV fluid was continued as per group allocation @ 2 ml/kg/hr. ABG analysis (pH, bicarbonate, base excess), blood urea, plasma osmolality, serum sodium, potassium and chloride values were recorded at baseline (prior to induction), 1 h and 2 h after induction, at completion of surgery and 4 h after extubation. Serum creatinine was recorded at baseline and 4 h after extubation. Serum NGAL sample was obtained at 4 h after extubation and was estimated using an ELISA kit (Wuhan Fine Biotech Co., Ltd.). Plasma osmolality was calculated using the following formula: Serum osmolality =  $2(Na + K) + (glucose \div 18) + (Blood \text{ urea} \div 2.8)$ . No nephrotoxic drug was administered during the study period.

## Sample size

Ankita Dey et al. reported the mean change in the pH value was  $0.06 \pm 0.05$  in the normal saline group vs  $0.01 \pm 0.04$  in the plasmalyte group at 6 h post-induction as compared to baseline [7]. Assuming these as reference values, the minimum required sample size at 5% level of significance and 95% power was at least 21 patients in each group. Hence, we enrolled 25 patients in each group to compensate for the dropouts.

## Statistical analysis

The data were entered in an MS Excel file and were analyzed using SPSS version 16.0. The quantitative variables in both groups were expressed as mean  $\pm$  SD and compared using unpaired *t* test between groups and paired *t* test within each group at various follow-ups. The qualitative variables were expressed as frequencies

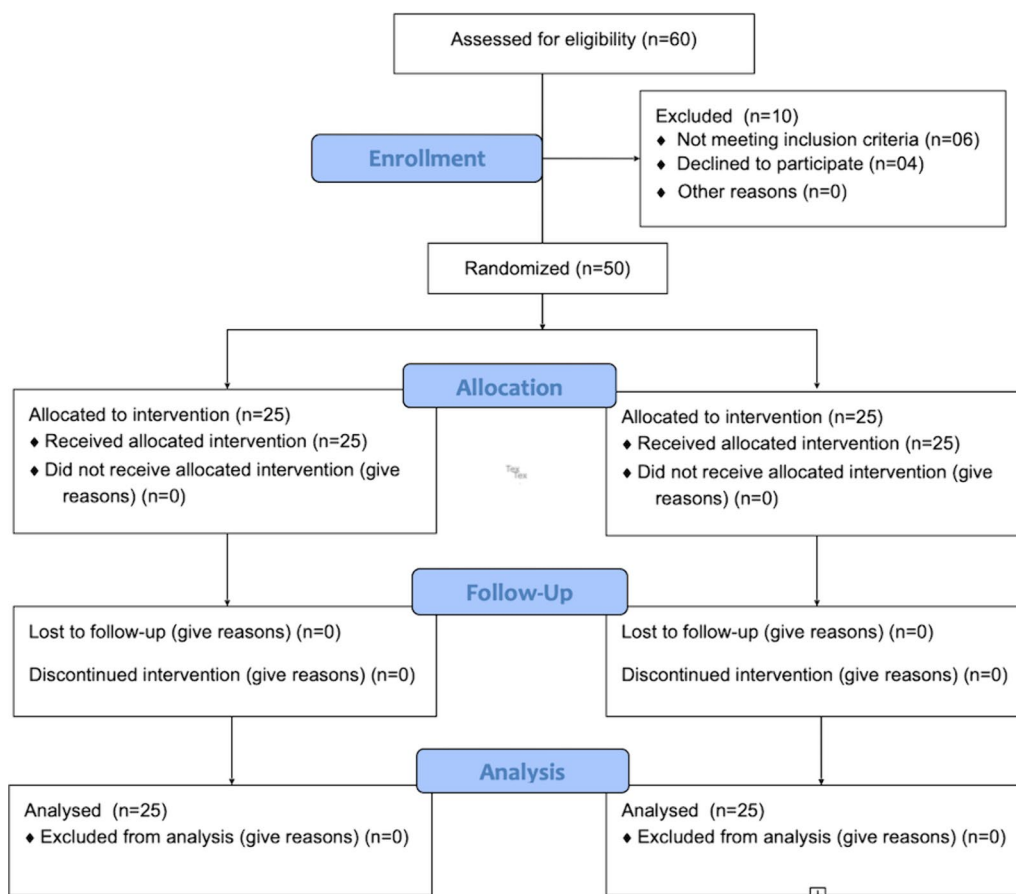
or percentages and were compared using Chi-square test. A *p* value < 0.05 was considered statistically significant.

**Results**

A total of 50 patients were analyzed (Fig. 1). Demographic characteristics were comparable between the two groups (Table 1).

**Metabolic parameters**

The pH was statistically similar and within normal physiological range in both the groups at baseline. However, pH values were significantly lower in the NS group as compared to the BC group at 2 h after induction, at the end of surgery as well as 4 h after surgery (Table 2). Moreover, the number of patients who developed acidemia (pH < 7.35) was also significantly higher in the NS group as compared to the BC group (14 vs. 4, respectively,



**Fig. 1** Consort diagram

**Table 1** Comparison of demographic parameters between the two groups

Parameter	Group BC (n = 25)	Group NS (n = 25)	P value
Age (years)	35.96 ± 8.63	36.44 ± 7.55	0.83
Gender (M/F)	16/11	9/14	0.15
Height (cm)	166.22 ± 8.75	164.60 ± 8.34	0.50
Weight (kg)	65.64 ± 13.35	64.72 ± 11.06	0.79
BMI (kg/m <sup>2</sup> )	22.9 ± 3.32	23.43 ± 2.65	0.54
ASA category (I/II)	15/10	18/7	0.37
Duration of surgery (minutes)	328.40 ± 49.39	335.20 ± 67.15	0.68

M male, F female, NS normal saline, BC balanced crystalloid

**Table 2** Comparison of metabolic parameters between the two groups

Parameter	Group BC (n = 25)	Group NS (n = 25)	P value*
pH-1	7.39±0.07	7.36±0.05	0.14
pH-2	7.43±0.06	7.40±0.08	0.08
pH-3	7.44±0.05	7.37±0.06	0.001*
pH-4	7.46±0.06	7.23±0.59	0.05*
pH-5	7.49±0.04	7.33±0.08	0.001*
PaCO2-1	30.4±2.08	32.04±1.36	0.23
PaCO2-2	34.76±6.39	36.98#±5.41	0.19
PaCO2-3	33.24±2.13	34.58±2.47	0.60
PaCO2-4	35.46±2.58	36.31#±2.39	0.23
PaCO2-5	32.81±2.51	32.97±2.91	0.83
HCO3-1	22.87±4.87	22.74±4.47	0.92
HCO3-2	23.92±4.66	22.42±3.96	0.22
HCO3-3	24.19±3.91	22.86±3.81	0.22
HCO3-4	23.87±4.28	22.58±4.18	0.28
HCO3-5	25.39±4.08	22.87±5.68	0.07
Base excess-1	- 3.72±4.17	- 3.10±3.24	0.55
Base excess-2	- 2.25±4.03	- 2.28±3.35	0.97
Base excess-3	- 0.67±3.53	- 0.72±2.43	0.95
Base excess-4	1.06#±3.24	1.36±2.43	0.71
Base excess-5	2.84±5.98	2.17#±2.13	0.60

p < 0.05 is considered significant

\*p ≤ 0.05

NS, Normal saline; BC, balanced crystalloid; PaCO2, partial pressure of carbon dioxide; HCO3, bicarbonate; 1, baseline (pre-induction); 2, 1 h after induction; 3, 2 h after induction; 4, at completion of surgery; 5, 4 h after extubation

p = 0.001). Serum bicarbonate levels increased in the BC group intraoperatively while decreased/slightly increased in the NS group. However, the values were comparable between the two groups at all the observed ‘time-points’ (Table 2). Base excess values were statistically similar between both groups at all observed ‘time-points’ in our

study. Base deficit improved over time with iv fluid therapy in both the groups (Table 2).

**Renal functions**

Blood urea values were comparable between the two groups at baseline as well as at 1 h after induction, but were significantly higher in NS group as compared to BC group at rest of all observed ‘time-points’ (Table 3). Patients in the NS group had significantly higher serum creatinine values 4 h after surgery as compared to the BC group (1.28±0.28 mg/dL vs. 0.85±0.14 mg/dL, respectively; p < 0.001) (Table 3). NGAL values were significantly higher in the NS group as compared to the BC group (236.65±85.62 ng/ml vs. 78.27±13.62 ng/ml, respectively; p = 0.001) (Table 3, Fig. 2).

**Serum electrolytes**

Baseline values of serum electrolytes were statistically similar between the two groups. Significantly higher levels of serum sodium and chloride were observed in the NS group as compared to the BC group at rest of the observed ‘time-points’ (p < 0.05) (Table 4).

**Plasma osmolality**

Plasma osmolality increased with time in both the groups, but values were significantly higher in the NS group as compared to BC group (Table 4).

The observations of our study reflect that both the fluid protocols were associated with acceptable brain relaxation. Brain relaxation score of the patients between the two groups was found to be comparable (p > 0.05). Score 1 was observed in 13 versus 17 patients and score 2 in 12 vs 78 patients in the BC vs NS group, respectively. Score 3 and 4 was not found in any of the study participants.

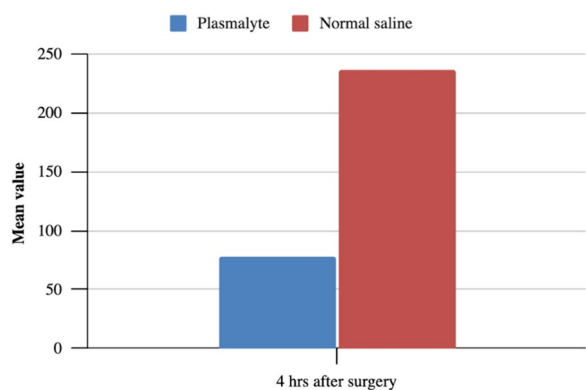
**Table 3** Comparison of renal functions between the two groups

Parameter	Group BC (n = 25)	Group NS (n = 25)	P value
BU-1	29.12±6.71	28.20±5.18	0.58
BU-2	29.88±6.31	32.24±5.03	0.15
BU-3	29.16±5.05	35.12#±6.31	0.001*
BU-4	29.76±6.10	38.80#±7.83	0.001*
BU-5	29.32±7.09	45.48#±12.67	0.001*
Serum creatinine-baseline	0.91±0.17	0.96±0.19	0.40
Serum creatinine-4 h after surgery	0.85±0.14	1.28±0.28	0.001*
NGAL (ng/ml)	78.27±13.62	236.65±85.62	0.001*

p < 0.05 is considered significant

\*p ≤ 0.05

NS, Normal saline; BC, balanced crystalloid; BU, blood urea; 1, baseline (pre-induction); 2, 1 h after induction; 3, 2 h after induction; 4, at completion of surgery; 5, 4 h after extubation



**Fig. 2** Comparison of NGAL between the groups at 4 h after surgery

**Table 4** Comparison of electrolytes and plasma osmolality between the two groups

Parameter	Group BC (n=25)	Group NS (n=25)	P value
Na-1	139±5.03	141.24±5.97	0.15
Na-2	139.44±4.76	143.08±5.94	0.02*
Na-3	138.36±5.08	145.64±6.10	0.0001*
Na-4	138.84±3.76	146.24±6.39	0.0001*
Na-5	140.02±4.78	147.48±7.11	0.0001*
K-1	4±1.03	4.08±0.603	0.745
K-2	3.88±0.55	4.03±0.70	0.410
K-3	3.93±0.58	4.14±0.74	0.282
K-4	3.74±0.46	3.96±0.75	0.219
K-5	3.54±0.73	3.95±1.02	0.08
Cl-1	89.72±4.21	91.4±4.83	0.19
Cl-2	102.60±11.36	112.24±7.51	0.001*
Cl-3	105.92±8.77	120.24±7.96	0.001*
Cl-4	107.60±8.79	132.00±10.73	0.001*
Cl-5	109.76±9.70	140.52±13.55	0.001*
Plasma osmolality-1	288.18±8.72	292.60±10.23	0.10
Plasma osmolality-2	288.54±8.86	296.76±9.48	0.003*
Plasma osmolality-3	287.26±10.95	299.80±13.93	0.001*
Plasma osmolality-4	287.74±8.85	302.40±12.80	0.001*
Plasma osmolality-5	289.78±10.06	303.63±11.39	0.001*

p < 0.05 is considered significant

\*p ≤ 0.05

NS, Normal saline; BC, balanced crystalloid; Na, sodium; K, potassium; Cl, chloride; 1, baseline (pre-induction); 2, 1 h after induction; 3, 2 h after induction; 4, at completion of surgery; 5, 4 h after extubation

### Discussion

Normal saline is a widely used crystalloid in resuscitation, maintenance as well as perioperatively. Of late, trend is shifting toward balanced crystalloid (BC) solutions as infusions of large volumes of NS may result in hyperchloremic acidosis due to a reduction in strong ion difference by an excessive rise in plasma chloride as well

as renal bicarbonate elimination. It may further compromise renal function by causing splanchnic vasoconstriction and reduction in glomerular filtration rate [8], [9]. BC has a lower chloride content which is accomplished by substitution of chloride with an anion such as lactate or acetate. [10]

Neurosurgeries are characterized by major fluid shifts, frequent use of diuretics to reduce cerebral edema and prolonged duration. The intraoperative fluid therapy is critical as hypovolemia may lead to brain hypoperfusion and over-transfusion may lead to increased intracranial tension. The choice of fluid to be used must optimize cerebral brain perfusion, maintain adequate cerebral oxygenation and avoid cerebral edema. However, the optimal fluid therapy for patients undergoing neurosurgery is yet to be determined [11]. Therefore, we planned our study in adult patients undergoing neurosurgeries.

In our study, we observed that NS infusion was associated with deranged metabolic and renal parameters as compared to BC.

### Acid base status

#### pH

The prime goal should be to maintain normal physiological pH since acidosis may lead to hypovolemia, tissue hypoperfusion, lactic acidosis and alterations in myocardial contractility. We observed significantly lower pH values in the NS group as compared to the BC group because of the difference in basic composition of the two fluids; the pH of NS is 5.6, while that of PL is 7.4.

Similar results were observed in the previous studies. Young et al. compared these two crystalloids for the initial resuscitation of academic trauma patients and observed that while pH returned to normal in the BC group after initial resuscitation, patients were still acidemic in the NS group even after 6 h of resuscitation [12]. In a similar study conducted by Kim et al. on renal transplant recipients, pH was significantly lower in those who received NS compared to BC during the post reperfusion period [13]. Similarly, Song et al. conducted a study in patients undergoing lumbar spinal surgery and found that the pH values were significantly lower in the NS group compared with the PL group intraoperatively as well as at the end of surgery (p < 0.05) [14]. Chatrath et al. compared plasmalyte A and NS in patients undergoing elective abdominal surgeries and observed significantly lower pH values at the end of surgery in NS group as compared to PL group (p < 0.001) [15]. Weinberg et al. conducted a study in patients undergoing renal transplant surgery and found that subjects receiving NS were more acidemic (pH 7.32 vs 7.39, p = 0.002) compared to those who received plasmalyte148 at 48 h after surgery. [4]

### Serum bicarbonate

Bicarbonates constitute an essential buffer system which tends to compensate for any change in pH. Though bicarbonate levels were lower in the NS group, they were comparable between the two groups in our study. Dey et al. noted similar results comparing NS and BC in patients undergoing elective craniotomy for supratentorial brain tumors [7]. Chowdhury et al. compared the effects of NS and plasmalyte148 on renal cortical tissue perfusion in healthy volunteers and observed that the bicarbonate values decreased over time in NS group but were statistically similar between the two groups. [16]

### Base excess

Values were statistically similar at all observed 'time-points' in our study. Base deficit improved over time with fluids in both the groups. Similar results were observed by Dey et al. comparing NS and BC in patients undergoing elective craniotomy for supratentorial brain tumors wherein they did not find any significant difference in base excess intraoperatively between the two groups. [7]

However, few previous studies observed significantly greater improvement in base deficit with the use of BC as compared to NS [16]–[18]. This could be due to different study population, nature of surgery or duration of follow-up.

### Renal functions

#### Blood urea

Changes in renal perfusion owing to the type of iv fluid may have altered clearance of urea leading to significantly higher urea levels in the NS group as compared to BC group in our study. However, objective assessment of renal perfusion using appropriate technique would have been more helpful. Dey et al. compared NS vs BC in patients undergoing neurosurgery and observed significantly higher blood urea levels postoperatively in NS group as compared to BC group ( $p=0.03$ ). [7]

#### Serum creatinine

Serum creatinine is the most widely used index of renal function which starts to rise 24 to 72 h after a clinical insult [19]. In the present study, patients in saline group had significantly higher serum creatinine values at 4 h after surgery as compared to BC group indicating renal compromise. Our results are in corroboration with Dey et al. who observed significantly higher creatinine values in NS group as compared to PL group postoperatively. [7]

Neutrophil gelatinase-associated lipocalin (NGAL) (Table 3, Fig. 2).

It is the novel biomarker for the early detection of AKI. It has been shown to predict the risk of AKI as early as within 6 h of clinical insult [20]. Specificity of NGAL

has been estimated to range between 70 and 80%, while the sensitivity is unpredictable with a varying range of 40–90% [21]. It was observed that postoperative NGAL values were significantly higher in NS group as compared to BC group in our study, thus indicating increased renal compromise with saline. Dey et al. measured serum NGAL 3 h postoperatively and observed significantly higher values in NS group as compared to BC group ( $249.13 \pm 91$  vs  $71.13 \pm 28.7$  ng/ml, respectively;  $p < 0.001$ ) [7]. Funke et al. studied the effects of BC versus NS on urinary NGAL levels in clinically ill adults and observed significantly higher values in NS group than in BC group ( $64.4$  ng/ml vs  $39.4$  ng/ml, respectively;  $p < 0.001$ ). [9]

### Serum electrolytes

#### Serum sodium

The higher sodium content of NS (154 mEq/L) leads to higher serum sodium levels in the NS group as compared to the BC group. Similar results were recorded by Young et al., Chatrath et al. and Hafizah et al. while comparing these fluids. [12], [15], [18]

#### Serum chloride

Significantly higher chloride levels were observed in the NS group as compared to the BC group in our study. This may be due to differences in the fluid composition as the chloride content in NS is 154 mEq/L which is higher compared to 98 mEq/L in BC. Mc Farlane et al. observed that variations in measured chloride values from baseline to the end of surgery were higher in NS group as compared to plasmalyte148 group [ $+6.9$  mmol/L (2.3) vs  $+0.6$  mmol/L (1.2)], respectively;  $p < 0.01$ ] [17]. Chowdhury et al. compared NS and plasmalyte148 in healthy volunteers where after infusion of 2 L of fluid over 1 h, significantly higher levels of serum chloride were observed in NS group than the other group ( $p=0.0001$ ) [16]. Young et al. conducted a study in trauma patients and observed higher serum chloride levels with NS than plasmalyte A throughout 24 h of resuscitation [ $111 \pm 8$  vs  $104 \pm 4$  mEq/L, respectively; RR:  $-7$ ] [12]. Song et al. observed that serum chloride concentrations were significantly higher in the NS group compared with the PL group at 2, 3 and 4 h after anesthetic induction and at the end of surgery ( $p < 0.05$ ) [14]. Chatrath et al. observed significantly higher serum chloride levels in immediate postoperative period in NS group as compared to PL group ( $117.31 \pm 24.84$  mmol/l vs  $96.02 \pm 10.16$  mmol/L, respectively;  $p < 0.001$ ) in patients undergoing abdominal surgeries [15]. Weinberg et al. found that participants receiving NS had higher serum chloride concentration than those who received plasmalyte148 at the end of renal transplant surgery ( $107$  vs  $101$  mmol/L, respectively;  $p < 0.001$ ) [4]. Dey et al.

studied patients undergoing elective craniotomy and observed higher chloride levels at 2, 4, 6 and 8 h after induction in patients who received NS than those who received BC ( $p=0.03$ ) [7]. Huang et al. concluded that postoperative serum chloride level was significantly higher in the NS group than in the BC group (MD:  $-4.79$ ; 95% CI:  $-8.13-1.45$ ;  $p=0.005$ ;  $I=95\%$ ) [22]. Kang et al. compared NS and BC in patients undergoing surgery for UIA and observed that mean serum chloride values in immediate postoperative period were significantly higher in NS group than in BC group ( $109.1 \pm 2.9$  mmol/L vs  $107.8 \pm 3.1$  mmol/L, respectively;  $p < 0.001$ ). [23]

### Brain relaxation score

There was no significant difference in the brain relaxation score between the two groups in our study. Other authors also noted similar results with respect to brain relaxation score. [1], [7], [23]

### Plasma osmolality

Osmolality values were significantly higher in the NS group as compared to the BC group. This may be attributed to the fact that osmolality of NS (308 mOsm/L) is higher than that of plasmalyte (294 mOsm/L). Furthermore, it was calculated using the values of sodium, glucose and urea. Sodium and urea levels were higher in the NS group. Dey et al. also observed that plasma osmolality values were clinically higher though statistically similar in NS group as compared to BC group. [7]

There were few limitations of our study. The patients with comorbidities such as diabetes mellitus, renal dysfunctions and coagulation abnormalities were excluded from our study in order to avoid the selection bias. Replication of our results in these groups of patients cannot be commented upon. Serum NGAL levels were measured only once postoperatively and trends could not be seen due to economic constraints. Long-term follow-up was not done; thus, the effects of fluids on clinical outcome cannot be opined. Further metacentric randomized trials evaluating effects of these fluids on clinical outcome are warranted.

### Conclusion

As compared to normal saline, use of isotonic balanced crystalloid (plasmalyte) during intraoperative period resulted in better acid–base balance and renal profile in adult patients undergoing elective intracranial tumor resection surgeries.

### Abbreviations

IV	Intravenous
NS	Normal saline
BC	Balanced crystalloid
NGAL	Neutrophil gelatinase-associated lipocalin
KCl	Potassium chloride

PL	Plasmalyte
SpO <sub>2</sub>	Saturation
ECG	Electrocardiography
NIBP	Non-invasive blood pressure
ASA	American Society of Anesthesiologists
GCS	Glasgow Coma Scale
ABG	Arterial blood gas
AKI	Acute kidney injury
SD	Standard deviation

### Author contributions

RB gave ideas and plans for the study. RB and VA put study design and analyzed the data. AK and DY wrote meticulous revision of the paper, and RB and VA approved the final version of the manuscript. All authors read and approved the final manuscript.

### Availability of data and materials

The data supporting our findings can be found with the corresponding author on request and can be contacted through the following emails: drvandna4@gmail.com, akankshakhatri25@gmail.com.

### Declarations

#### Ethics approval and consent to participate

This research was accepted by the Research Ethics Committee (REC) of the Biomedical research ethics committee, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, UHS, Rohtak, Haryana, India. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and its later amendments or comparable ethical standards. Biomedical Research Ethics Committee is organized and operates according to the ICH-GCP/ICMR/New Drugs and Clinical Trial Rules-2019. A written informed consent was obtained from each patient after explaining all steps of this study. The reference number of the ethics approval from the ethics committee: Institutional Ethics Committee Approval No.- IEC/Th/19/Anst17.

#### Consent for publication

Not applicable as there were no identifiable data of patients.

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