The Effect of Perioperative N-acetylcysteine on the Short and Long Term Outcomes in Pediatrics Undergoing Living-Donor Liver Transplantation

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ABSTRACT

Background: Ischemia-reperfusion injury during transplantation can cause post-operative graft dysfunction.

Objective: To assess the efficacy of N-acetylcysteine in preventing hepatic ischemia-reperfusion injury and post-transplant outcomes.

Methods: In this retrospective study on pediatrics undergoing living-donor (from one of their parents) liver transplantation, N-acetylcysteine was administered to one group (n=20) after induction in the donors until graft harvest, and in the recipients during implantation, which was maintained for 19 hours. The second group (n=20) did not receive NAC. Early allograft dysfunction was determined in the presence of alanine aminotransferase or aspartate aminotransferase ≥2000 IU/L and bilirubin ≥10 mg/dL within the first 7 days, and an international normalized ratio ≥1.6 on day 7. Data were collected from a retrospectively maintained database.

Results: The incidence of post-reperfusion syndrome was lower in N-acetylcysteine group compared with the other group (5% *vs.* 30%, p=0.037). Serum creatinine level was significantly (p=0.04) different in the N-acetylcysteine group during the second post-operative week (0.14 *vs.* 0.15 mg/dL). There was no significant difference in the incidence of early allograft dysfunction (21% *vs.* 14%, p=0.327), and the survival rate (p=0.409).

Conclusion: Peri-operative infusion of N-acetylcysteine in both donor and recipient would effectively prevent post-reperfusion syndrome and renal insufficiency. However, it might not affect the early allograft dysfunction, ICU stay, and mortality. NAC increases the chance of re-operation due to non-surgical bleeding in the first post-operative day.

KEYWORDS: Ischemic-reperfusion injury; Living donor liver transplantation; N-acetylcysteine

INTRODUCTION

Liver transplantation is widely adopted for treating end-stage liver failure in both pediatrics and adults. Several studies have reported the beneficial effects of N-acetylcysteine (NAC) on either donors or recipient in liver transplantation but in our study NAC was administered for both donors and recipients simultaneously [1, 2]. Despite long-term survival, some post-operative adverse events still remain [3, 4]. Hepatic ischemia-reperfusion injury (IRI), occurring mostly during liver resection and transplantation, has been identified as the main cause of graft dysfunction [5]. However, the main pathogenesis of IRI remains questionable. Factors such as oxidative stress, overloading intracellular cal-

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cium, anaerobic metabolic pathways, and hyper-secretion of some inflammatory cytokines are among known contributing factors [6-8]. IRI is the result of hypoxia during ischemic and cytotoxic events in the reperfusion phase [9], when active chemicals such as hydrogen superoxide and hydroxyl radicals cause necrosis and apoptosis in the transplanted organ cells [10]. Several studies showed that the IRI leads to immediate and long-term impairments of the transplanted organ, including primary graft dysfunction (PGD), which can be subdivided into early allograft dysfunction (EAD) and primary graft non-function (PNF) [9, 10]. EAD or DGF is temporary laboratory changes but PNf correlates with clinical and laboratory changes leading to re-transplantation or recipient death within the first 7 days [10, 11]. Oxidative stress is a common reason for PGD, especially in marginal donors.

Many researchers are looking for the most effective method to prevent IRI. NAC, an antioxidant drugs has been studied and might be an effective drug [11, 12]. Some studies showed that NAC is not only an antioxidant and would protect various organs from oxidative stress but it also affects the microcirculation of the connective tissue and prevents the neutrophil assemblages [13].

The liver ischemic phenomenon might begin via clamping hepatic vascular supply in the donor. Meanwhile, the reperfusion phenomenon occurs during the portal vein and hepatic artery de-clamping in the recipient [14]. Therefore, it seems that the protective effects of NAC are necessary in both phases. Several studies reported the role of NAC in liver transplantation, administered prior to ischemia or reperfusion [1, 2]. Herein, we aimed at determining the effects of administration of NAC to both the donor and the recipient prior to ischemic and reperfusion phase on the incidence of post-reperfusion syndrome and renal insufficiency, and also on allograft dysfunction, ICU stay, and mortality in pediatric age group.

PATIENTS AND METHODS

This retrospective study was conducted on pediatric recipients scheduled for liver transplantation from living donors (one of their parents) who had referred to the Transplantation Center at Namazi Hospital, affiliated with Shiraz University of Medical Sciences from 2011 to 2013. In our center, there were two protocols for living donors and recipients, but have not been compared so far.

The exclusion criteria for NAC protocol for recipients were age >14 years, body weight <10 kg, a serum creatinine level >1.2 mg/dL or creatinine clearance >140 mL/min. The exclusion criteria for donors are sensitivity to NAC, history of asthma, body mass index (BMI) >35 kg/m^2, a serum creatinine level >1.2 mg/dL or creatinine clearance >140 mL/min. Standard anesthetic and surgical techniques for liver transplantation were considered by the same anesthesiology and surgical team. Donors and recipients were routinely monitored with electrocardiography, pulse oximetry, non-invasive blood pressure measurements, arterial lining, central venous pressure assessment, capnometry, and temperature monitoring. Anesthesia induction in the recipients included midazolam 25–100 μg/kg, fentanyl 1–2 μg/kg, thiopental 3–10 mg/kg, cisatracurium 0.5 mg/kg, and morphine 0.1 mg/kg; the anesthesia was maintained by isoflurane 0.5–1 MAC and oxygen/air. The induction of anesthesia for living donors included midazolam 25–100 μg/kg, fentanyl 1–3 μg/kg, thiopental 3–5 mg/kg, cisatracurium 0.5 mg/kg, and morphine 0.1 mg/kg; maintenance was done with isoflurane and oxygen/air. After the induction of anesthesia, living donors in the treatment group (n=20) received NAC infusion at a dose of 150 mg/kg for one hour followed by a dose of 50 mg/kg until the graft was harvested; the recipients received 150 mg/kg NAC for one hour followed by 50 mg/kg for 4 hours until the end of the operation, and 100 mg/kg for 19 hours. Donors and recipients in the second group (n=20) did not receive the drug.

Demographic data, preop etiology, intraop and post op outcomes were retrieved from our

Table 1: Baseline characteristics in studied groups (n=20 per group)

Parameter	NAC group	Other group	p value
Age (yrs) mean±SD	5.8±3.0	5.5±3.6	0.776
Weight (kg) mean±SD	17.9±1.9	17.3±1.6	0.678
Sex n(%)			
Male	12 (60)	12 (60)	0.999
Female	8 (40)	8 (40)	
Etiology			
Tyrosinemia	2 (10)	3 (15)	0.776
Biliary	1 (5)	3 (15)	
Cryptogenic cirrhosis	2 (10)	3 (15)	
Progressive familial intrahepatic cholestasis	6 (30)	5 (25)	
Other	9 (45)	6 (30)	

database. During the operation, the amount of blood loss, the amount of blood products transfusion, as well as the hemodynamic parameters (HR, MAP) and arterial blood gas analysis, were recorded for both groups at the baseline, after the induction of anesthesia, before the infusion of NAC, during the hepatectomy stage, during portal irrigation at the anhepatic stage, and 30 minutes after finishing the hepatic artery anastomosis and unclamping it or neohepaic stage. At the end of the surgery, all the recipients were transferred to the intensive care unit (ICU). Post-operatively, extubation time, liver functional test (ALT, AST, serum bilirubin, INR), the amount of urinary output (mL/ 24 hrs), serum creatinine level, the amount of blood transfusion required, and re-operation were assessed daily up to the 7th day after revascularization. In addition to the survival rate, liver function tests and serum creatinine level were measured for 2 weeks, 3 months, and 3 years.

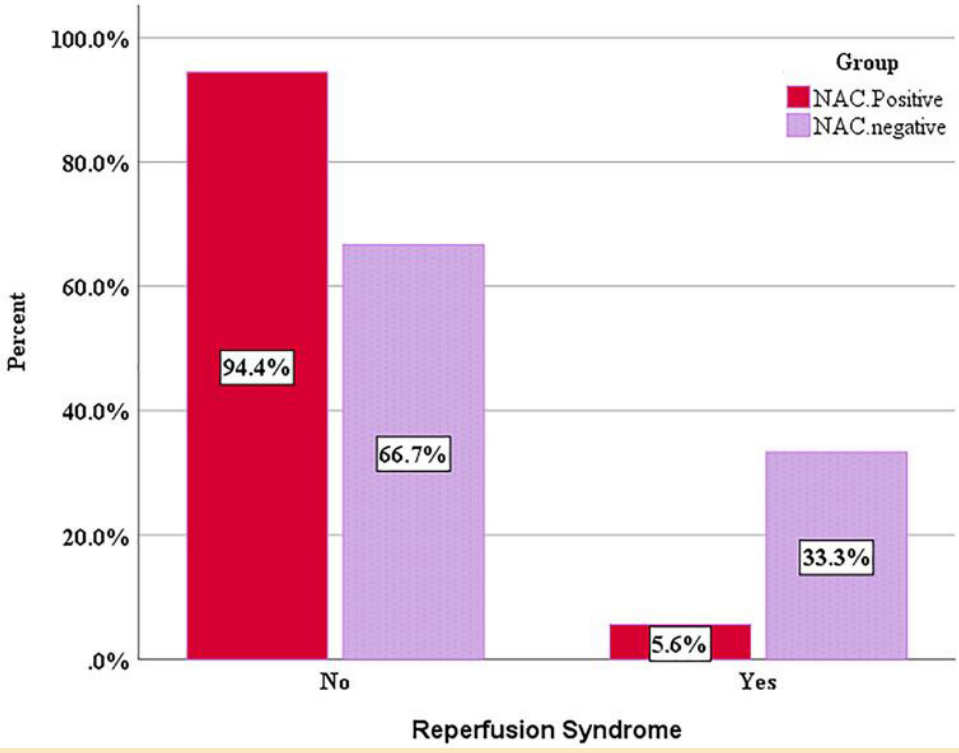

Figure 1: Relative frequency distribution of post-reperfusion syndrome (PRS) between the two studied groups

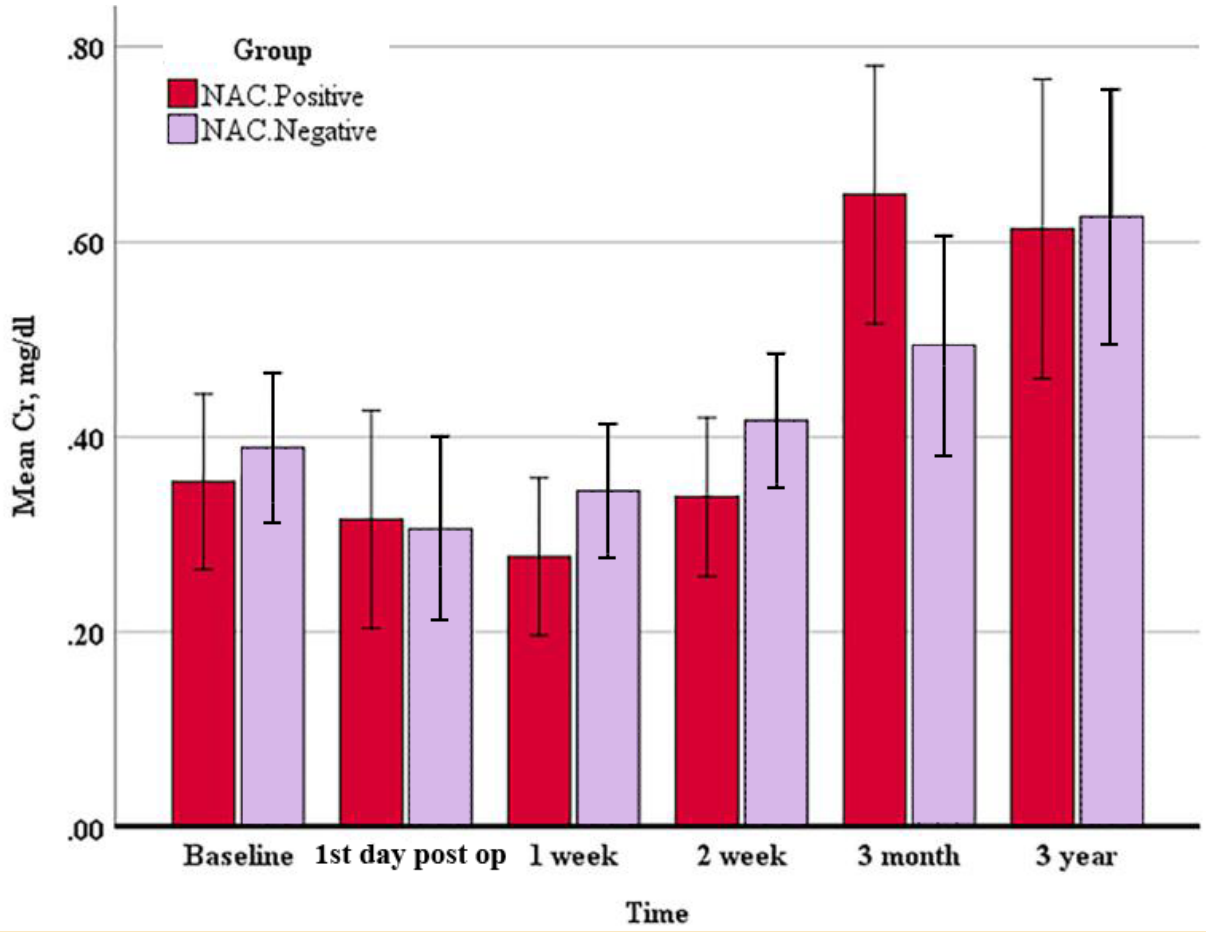

Figure 2: Temporal trend of the mean serum creatinine level in the studied groups. Error bars represent SD.

Our first objective was to reduce post-reperfusion syndrome (PRS), which is defined as the reduction in the mean arterial blood pressure (MAP) by >30% of the baseline value lasting for at least 1 min within 5 min after declamping, asystole or arrhythmias in neohepatic phase, or the need for administration of vasopressor/inotropic agents during the intra-operative period or to be maintained in post-operative period. The second objective was to prevent early allograft dysfunction (EAD), renal dysfunction. The ICU stay and survival rate were also assessed. EAD was determined as the presence of one or more of the following criteria: serum ALT or AST ≥2000 IU/L within the first 7 days post-operatively, bilirubin ≥10 mg/dL on day 7, international normalized ratio (INR) ≥1.6 on day 7 [11, 12].

The data were analyzed by SPSS® for Windows® ver 16.0 (SPSS Inc, Chicago, IL, USA). The results are presented as mean±SD for the quantitative variables and summarized as absolute frequencies and percentages for the categorical ones. Normality of the data was assessed with the Kolmogorov-Smirnov test. The categorical variables were compared with χ^2 test or Fisher's exact test when >20% of the cells had an expected frequency of <5. Quantitative variables were compared with Mann-Whitney U test. Survival analysis was used to compare mortality rates between the groups. A p value <0.05 was considered statistically significant.

RESULTS

We studied 40 patients (24 boys and 16 girls) with mean±SD age of 5.6±3.3 years, weight of 17.6±7.7 kg, and pre-operative serum creatinine level 0.4±0.2 mg/dL. The etiologies of hepatic failure included progressive familial intrahepatic cholestasis (PFIC) in 27.5%, thy-

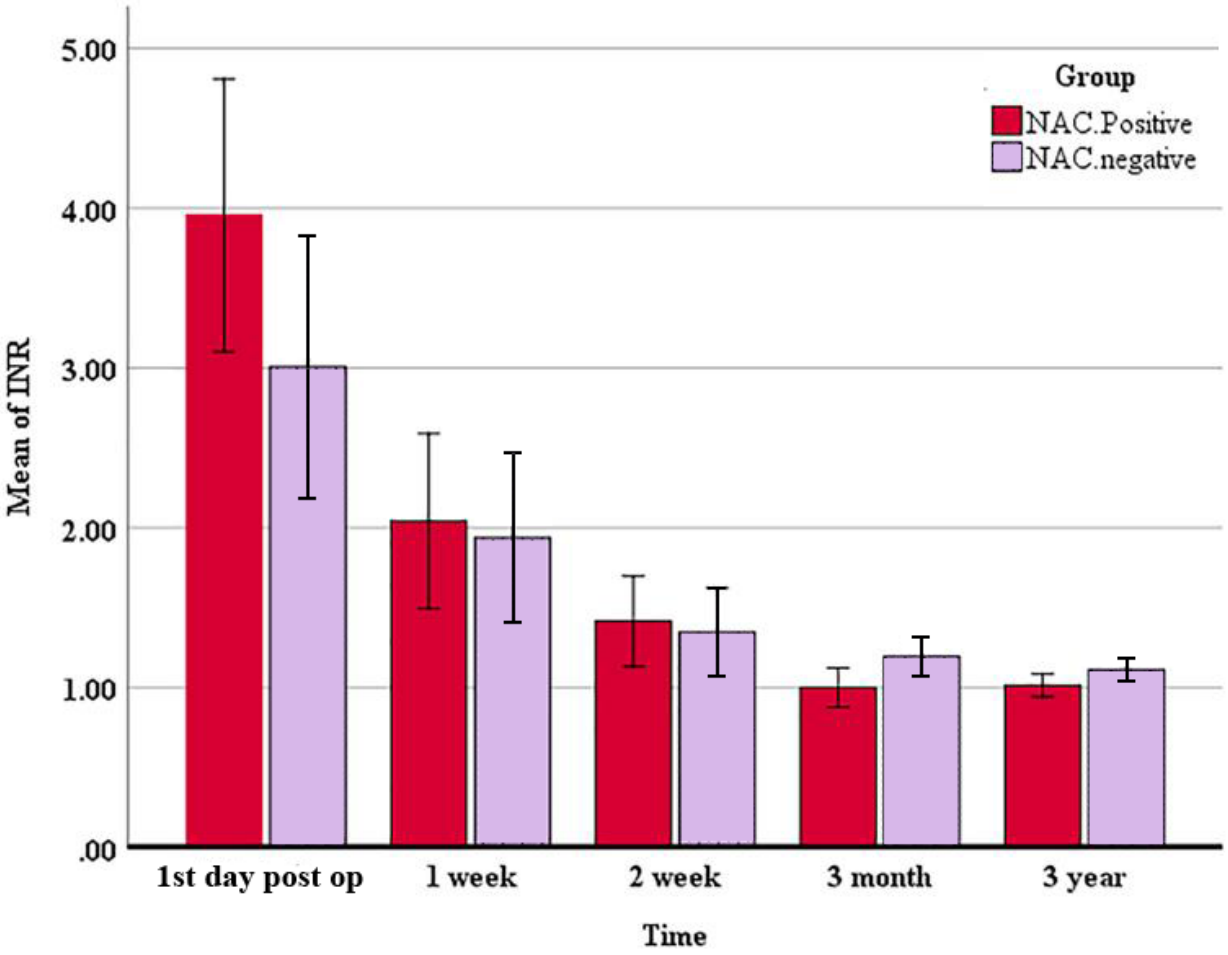

Figure 3: Temporal trend of the mean INR in the studied groups. Error bars represent SD.

rosinemia in 12.5%, cryptogenic cirrhosis in 12.5%, biliary atresia in 10%, and other etiologies in 37.5%.

There were no significant differences between the two groups in terms of age, body weight, sex distribution, baseline serum creatinine level, and the distribution of hepatic failure etiologies (Table 1). The cold ischemic time was similar in both groups—a mean±SD of 30±7 min. The incidence of reperfusion syndrome was significantly (p=0.037) in the second group in comparison to the NAC group (30% *vs.* 5%, Fig 1). The rate of EAD in the NAC group was lower than the other group (55% *vs.* 70%), however, the difference was not significant (p=0.327). Post-operative renal function measured by serum creatinine level, in the NAC group was significantly (p=0.04) lower than the other group after 2 weeks (Fig 2). The rate of re-operation was significantly (p=0.01) higher in the NAC group (65% *vs.* 20%) in the first day postoperation, and the main cause of re-operation was bleeding—a significantly (p=0.03) higher INR was observed on the third post-operative month in the second group (Fig 3). The 3-month, 6-month, 1-year, and 3-year survival rate in the NAC group was 80%, 75%, 70%, and 65%, respectively (Fig 4); in the second group were 90%, 90%, 85%, and 75%, respectively (p=0.409). There were no significant differences between the two groups in terms of the mean intra-operative bleeding (p=0.169), the need for blood transfusion (p=0.144), the rate of acute graft rejection or PNF (p=0.426), the mean dosages of post-operative inotropic agents (p=0.627), the need for re-intubation (p=0.797) and mechanical ventilation days (p=0.627), and the mean total ICU stay (p=0.482). The overall complication rate of post-operative bleeding in donors and the need for re-operation were similar in the

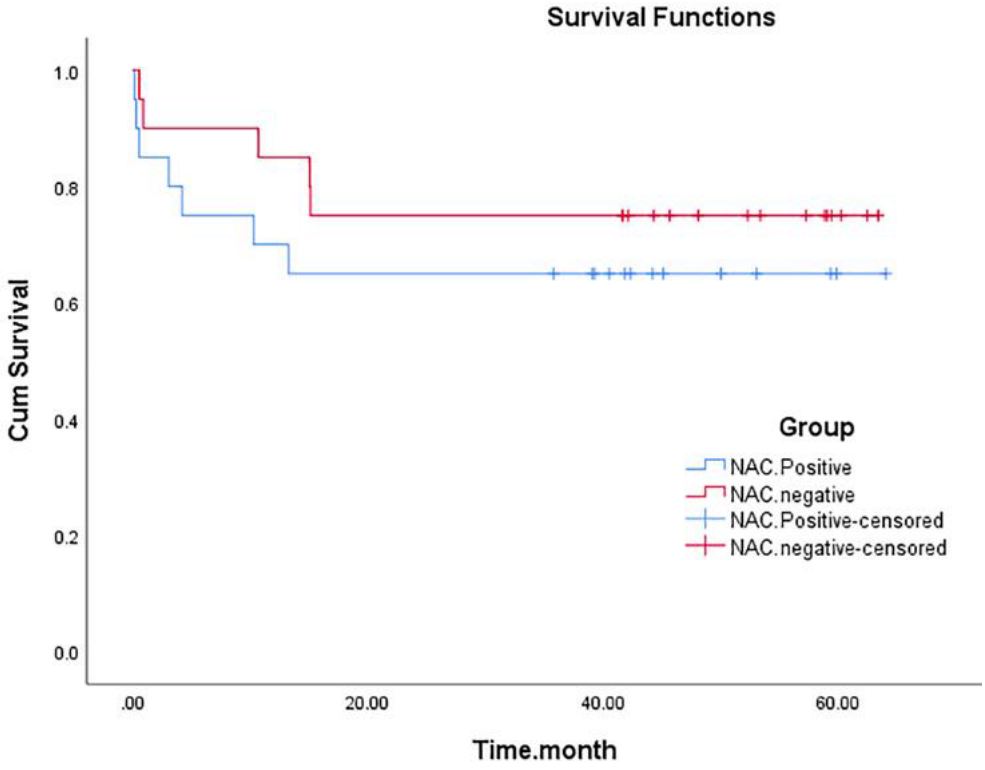

Figure 4: No significant difference was observed with respect to long-term survival of the two groups.

two studied groups.

DISCUSSION

The differentiation between PRS and IRI is imperative. The PRS is a systemic response to reperfusion of an ischemic organ; IRI is a process of paradoxical augmented local damage occurring in a prolonged ischemic cell following restoration of the blood flow [14, 15].

Several studies have reported the beneficial effects of N-acetylcysteine (NAC) on either donors or recipients in liver transplantation [1, 2]. In our study, NAC was administered to both donors and recipients.

Hepatic ischemia followed by reperfusion might be one of the most important adverse consequences of liver transplantation. Occurrence of IRI might affect post-operative liver function and the long-term survival of the recipient. As previously stated, the inflammatory response and over-activity of oxygen-free radicals by mitochondria, which lead to oxidative stress, are known strong evidence for the pathogenesis of IRI in liver transplantation. On one hand, some intracellular mechanisms might be involved in the inhibition of oxidative stress, such as glutathione [16]. Glutathione (GSH) is a major tissue anti-oxidant which acts as a free radical scavenger in the liver, and is depleted after IRI [17]. In the GSH synthesis, the availability of cysteine is generally the limiting factor during ischemia or oxidative stress [17]. On the other hand, NAC is the main cysteine precursor and a rich source of sulfhydryl group (SH), which is significant for restoring the hepatic GSH Reverses [17]. It rapidly diffuses into the cells and hydrolyzes into L-cysteine, which can deactivate intracellular oxidative stress processes [21]. A study by Jegatheeswaran, *et al*, reports that NAC has some advantageous in reducing liver IRI, particularly if the drug is infused before the initiation of the IRI in the recipient [18]. In line with the mentioned study, the present research evaluated the peri-operative use of NAC in a pediatric group undergoing living-related liver transplantation; the drug was administered to both the recipient and the donor before the

onset of IRI. A previous study by Bromley, *et al*, reports that intraoperative administration of NAC adjusts hemodynamic variables with a mild vasodilation which diminishes the systemic vascular resistance index (SVRI) and MAP with an incremental cardiac index and oxygen delivery in patients at risk of tissue hypoxia [1]. The present study aimed at testing this hypothesis that if the surgically-induced liver IRI can be successfully controlled with administration of NAC. The drug led to reduced incidence of post-reperfusion syndrome (PRS) ($p=0.037$); consequently, the mean intra-operative norepinephrine dosage was decreased ($p=0.043$). Siniscalchi, *et al*, state that PRS is a significant intra-operative risk factor for the post-operative graft dysfunction, morbidity and mortality in the recipients [19]. They also found that both the cardiac and vascular system were involved in the appearance of this syndrome as well as an increase in the intra-operative blood derivatives transfusions, duration of ICU stay and post-operative kidney injury [19]. Hilmi, *et al*, confirm the relationship between PRS severity and post-transplantation outcomes. They suggested that preventing its occurrence would consequently decrease hemodynamic and coagulation changes and might ameliorate the post-operative outcomes [20].

The present study found that the need for re-operation due to bleeding was significantly higher in the NAC group in the first day post op, which might be due to the effects of NAC on the coagulation pathways. The study by Smyrniotis, *et al*, shows that NAC decreases platelet aggregation and modifies hepatic IRI in rats [21]. Other studies on NAC in healthy population by Knudsen TT, *et al*, and Thorsen S, *et al*, show extended duration of prothrombin time (PT) by direct effect on the vitamin K-dependent clotting factors [22-24]. We did not observe increased mean intraoperative bleeding and the need for blood transfusion in line with findings of Regueira, *et al*, who have reported that the PT was significantly increased 6 hours after transplantation in pigs [28]. We believe that by adjusting the serum NAC level the likelihood of post-operative bleeding, which resulted in interventional procedures, could be minimized. Other beneficial effects of NAC in the present study were the improvement in renal function, reduction of the mean serum creatinine level, both in line with other studies stating the advantageous effects of NAC on improved renal function [2].

Overall, the current study showed that using NAC in both donor and recipient could effectively reduce the risk of renal dysfunction and affect the patients' post-operative short-term outcomes. However, we did not observe the beneficial effects of NAC on EAD, PNF, the mean dosages of inotropic drugs required post operation, mechanical ventilation days, length of ICU stay, and long-term survival. The study by Hanna, *et al* [2], using Nanshimas criteria within the first 72 hours shows that EAD can significantly decline the EAD; however, we did not observe such an effect within the first 7 days post-transplantation. This might be attributed to the time elapsed. In several studies, further beneficial effects of NAC are discussed and evaluated. In a study by Li, *et al*, the prevalence of post-operative pulmonary complications was significantly reduced following the administration of NAC, which was in line with reducing the levels of TNF-α, IL-8, CC16, and ICAM-1 [25]. In the line with our findings, a study by Darweesh, *et al* [26], the use of NAC in patients with non-acetaminophen-induced acute hepatic failure led to a significantly better survival, lower ICU stay, normal creatinine and electrolytes levels, and also regulation of coagulation process. In another study by Squires, *et al* [27], conducted on pediatric patients suffering from non-acetaminophen acute hepatic failure, the 1-year survival did not significantly differ between the NAC- and placebo-treated groups—similar to our findings. There was no significant difference between the treatment and the other groups in terms of the hospital or ICU stay, which was also comparable with our results. In another study by Lee, *et al* [28], using NAC did not affect the graft survival. It seems that the administration of dose-adjusted NAC in a pediatric patient candidate for living donor liver transplantation could effectively prevent PRS in addition to enhancing post-operative renal function. Nonetheless, to minimize the

risk of post-operative bleeding, monitoring of serum NAC level should be considered.

Our study was retrospective and had its inherent shortcomings. Therefore, there might be other overlooked factors affecting non-surgical bleeding post-operation. For instance, bleeding might have been due to other causes such as fibrinolysis or NAC might have been administered in recipients who were more ill.

In conclusion, administration of NAC in living-related donors before clamping the hepatic vasculature and in recipients after the induction of anesthesia could attenuate the incidence of anticipated reperfusion syndrome, which leads to improved creatinine profile in the second week post-operation. It should be noted that NAC increases the chance of re-operation due to non-surgical bleeding in the first post-operative day. In addition, NAC does not affect the early allograft dysfunction and long-term survival.

CONFLICTS OF INTEREST: None declared.

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